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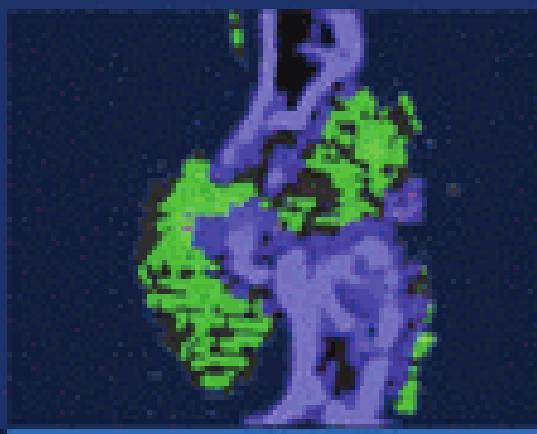

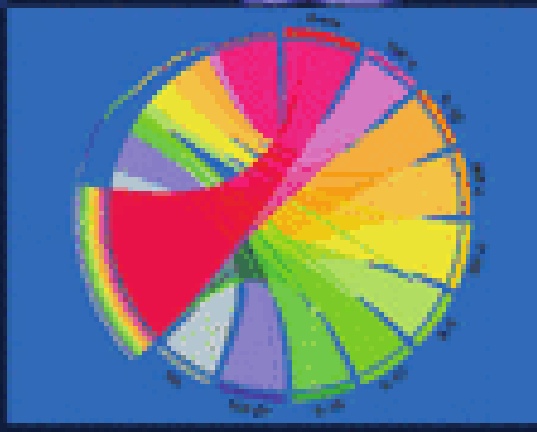

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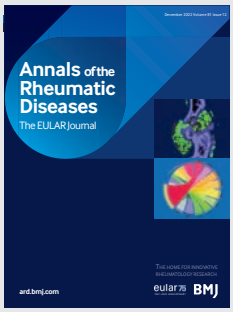

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EULAR 75-year anniversary: commentaries on *ARD* papers from 25 years ago

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1997. An auspicious year which at the time marked the 50th anniversary of the European Alliance of Associations for Rheumatology (EULAR). As we now celebrate the 75th year of EULAR, it is an opportunity to reflect on where we were 25 years ago.

In 1997, many exciting changes were occurring within EULAR to bring the European rheumatology community even closer together (patients, healthcare professionals and rheumatologists). That year, the EULAR standing committee chairs become ex-officio members of the EULAR Executive Committee and a Vice-President for Allied Health Professionals was instituted.

Clinically, the role of EULAR also continued to expand. Just the year before, on behalf of the EULAR Standing Committee for International Clinical Studies including Therapeutic Trials, the EULAR Response Criteria for rheumatoid arthritis (RA), using the Disease Activity Score, had been published, an outcome measure still in wide use today.[1] A EULAR consensus statement, jointly with the European Group for Blood and Marrow Transplantation, on the role of stem cell transplants in autoimmune disease was also published.[2] They were classed as an experimental procedure, to be considered only when an autoimmune disease is severe enough to have an increased risk of mortality. Even now, it remains an exceptionally rare approach to treatment to severe rheumatic diseases.

In 1997, I was an internal medicine trainee in Winnipeg, Canada. I was contemplating my future medical career and having selected rheumatology, was about to accept a training position at the University of Toronto. This was an exciting time for rheumatology as there were discussions of a new treatment, anti-cytokine therapies or specifically, tumour necrosis factor inhibitors (TNFi), which were showing great promise in early clinical trials.[3] Our specialty and importantly, the outcomes for our patients, were about to change in a way we never could have imagined. Indeed, at the time when I shared with a senior rheumatology consultant that I had accepted a training post in rheumatology, they suggested that soon there may not be a role for rheumatologists in the management of RA, as these new drugs would in essence put most patients into remission. As a future epidemiologist with an interest in RA outcomes, never was there a more perfect opportunity for real-world research. Within the next 5 years, biologic registers would be established across many countries and rheumatology would be one of the pioneering specialties in pharmacoepidemiology research.

Reading papers published in the *Annals* in 1997 offers further opportunity to reflect on what rheumatology, and in particular, RA outcomes looked like 'just before' biologics became a common treatment. These papers, while only a selection of the many papers across multiple rheumatic diseases published in the 12 issues of 1997, remind us that RA is an important and severe disease.

Mortality rates in Finnish women aged 15 years and over with inflammatory arthritis (majority RA) were compared with the general population between 1977 and 1993.[4] Across all age groups and all time eras studied, mortality in patients with inflammatory arthritis was higher when compared with an age-matched general population, with little change in most age groups between 1977 and 1993. Amyloidosis was reported as a common cause of death. An extended report from Japan similarly reported on 24 cases of intractable diarrhoea among 179 patients with secondary amyloidosis from RA, with a 5-year survival after the onset of diarrhoea of only 38.9%.[5] There was a further case report of a patient undergoing spinal surgery for severe and deteriorating cervical spine subluxation with vertical axis impaction resulting in significant neck pain and dysphagia. She developed acute and fatal upper airway obstruction post-extubation, thought due to severe cricoarytenoid and temporomandibular joint arthritis preoperatively.[6]

Disease-modifying antirheumatic drug (DMARD) options in 1997 were more limited compared with today and often not very effective. In 1996, there had been a trial of stopping versus continuing DMARDs in patients who had achieved sustained remission (a similar question now explored in trials of TNFi). Disease flare was more common among those patients who stopped.[7] A 1997 paper in the *Annals* reported on the outcomes of 51 patients, all assigned to the stopping arm of the trial, who had resumed their initial DMARDs after flaring.[8] Only 35% regained remission. Most notable were the types of DMARDs used in this population. Only 2 of 51 patients had been receiving methotrexate (MTX), while 25 had received antimalarials as their sole treatment, 10 parenteral gold and 4 d-penicillamine. Gold and penicillamine treatments have been essentially abandoned in rheumatology following the introduction of biologics. I last prescribed gold in 2005, which resulted in the patient developing a terrible rash necessitating treatment discontinuation and a short course of high-dose glucocorticoids.

There were papers published which looked to identify prognostic factors for more severe RA. Cigarette smoking was associated with seropositivity (rheumatoid factor was studied, not

anti-citrullinated protein antibodies (ACPA)) and radiographic erosions.[9] Associations were also found in a second study between presence of antikeratin antibodies or the presence of HLA DRB1*04 or *01 and severe radiographic progression; however, although the title of this paper highlighted the potential role of autoantibodies in disease progression, persistently raised C reactive protein was also an important prognostic factor, confirming the importance of disease control in preventing longer term joint damage.[10]

Two papers caught my attention with their particular significance in terms of clinical and research challenges, both in RA and more widely. The first paper attempted to answer the question of whether MTX increases the risk of cancer in RA. The authors reported on 8 cases of cancer among 426 patients receiving MTX and compared the rate of cancer with 420 RA controls (in this case patients identified through admission to hospital with RA but never treated with MTX) and the general population. In this relatively small study, they could not conclude that MTX was associated with any excess in cancer risk. This study causes one to reflect, however, on whether this is question which can ever be answered satisfactorily. Our biologic registers were established at the outset of biologic therapies to address the question of whether TNFi increases the risk of cancer, among other safety concerns. Almost universally, there has not been convincing evidence that these drugs increase the risk of cancer significantly when compared with MTX or other conventional DMARDs, but no study can answer the question of whether both treatments might be increasing the risk equally.[11] When TNFi are compared with the general population, any observed increase in risk is usually confounded by the fact that RA itself, independent of treatment, is associated with an increased risk of lymphoma and lung cancer,[12] the former elegantly shown to be associated with cumulative disease activity[13] rather than treatment. Therefore, without an appropriate disease or treatment control group, any role played in cancer risk by the treatment itself cannot be disentangled. As the treatment of RA becomes more focused, with recommended early use of MTX and aggressive disease control,[14] we no longer have a relevant non-MTX-treated cohort with which to compare rates of cancer. Whether our newer therapies, such as JAK inhibitors, will have a different profile with respect to malignancy risk waits to be seen, although the results of the recent Oral Surveillance trial[15] remind us that all of our treatments may have unanticipated adverse effects and diligent post-marketing surveillance is still required.

A second paper which looked at functional outcomes in a cohort of patients with RA from Glasgow, Scotland found that those patients living in the most deprived areas had the lowest functional levels, with many, after 5 years, never achieving the initial functional levels seen in the least deprived patients. What makes this paper most notable is that it was published 25 years ago, yet the results are essentially identical to papers published within the last year,[16] showing that health inequities persist among patients with RA. The explanations and potential solutions to eliminate health inequities are complex.[17] At a minimum, we need to ensure that we as a rheumatology community work actively with patients and the public as we plan and undertake research as well as develop our clinical services to ensure that research results and our approaches to healthcare are relevant, valid and acceptable to the whole population.

Although this commentary has largely focused on RA, many other papers across other disease areas were also published in the *Annals* in 1997, reflecting the vast specialty that is rheumatology. While it is impossible to review them all, a few highlights are mentioned here. For systemic lupus erythematosus (SLE), the search for more effective therapies continued with a case series reporting the beneficial effects of MTX for treatment of non-renal SLE,[18] while increases in IgG double-stranded DNA antibodies (dsDNA Ab) but not IgM dsDNA Ab were shown to correlate with disease flares.[19] The prevalence of thrombocytopaenia in antiphospholipid syndrome was quantified in a cohort of 171 patients attending a clinic in London, UK (23.4%),[20] although it did not correlate with any clinical or serological features. For non-inflammatory conditions, a systematic literature review (SLR) of non-steroidal anti-inflammatory drugs (NSAIDs) suggested that NSAIDs may be beneficial for short-term relief of uncomplicated low back pain but not for low back pain with sciatica, although the quality of trials informing the SLR was low to moderate in most cases.[21] Finally, two studies explored risk factors for osteoarthritis (OA) in women. The Chingford Study suggested that there was a protective effect for hormone replacement therapy (HRT) on radiological knee OA, but not hand OA.[22] On the other hand, although recall bias cannot be ruled out, a Swedish study found that high physical workloads in the home before age 50 years were associated with development of severe hip OA in women over age 50 years.[23]

Reflecting on where our specialty was 25 years ago, particularly in relation to where we are now, has been an amazing opportunity. Our specialty, particularly the treatment of inflammatory arthritis, has been transformed over the past 25 years, as we are reminded of where we have been only recently. We have a long way to go and the discussions in this review have only focused on a very small aspect of our specialty, but I am confident that looking back at today in 25 years will similarly show the leaps and bounds that our specialty will continue to make.

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EULAR recommendations for the management and vaccination of people with rheumatic and musculoskeletal diseases in the context of SARS-CoV-2: the November 2021 update

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ABSTRACT

The first EULAR provisional recommendations on the management of rheumatic and musculoskeletal diseases (RMDs) in the context of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), largely based on expert opinion, were published in June 2020. Since then, an unprecedented number of clinical studies have accrued in the literature. Several SARS-CoV-2 vaccines have been approved for population-wide vaccination programmes in EULAR-affiliated countries. Studies regarding vaccination of patients with (inflammatory) RMDs have released their first results or are underway. EULAR found it opportune to carefully review to what extent the initially consensus expert recommendations stood the test of time, by challenging them with the recently accumulated body of scientific evidence, and by incorporating evidence-based advice on SARS-CoV-2 vaccination. EULAR started a formal (first) update in January 2021, performed a systematic literature review according to EULAR's standard operating procedures and completed a set of updated overarching principles and recommendations in July 2021. Two points to consider were added in November 2021, because of recent developments pertaining to additional vaccination doses.

INTRODUCTION

EULAR's first set of provisional recommendations addressing several clinical aspects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease caused by SARS-CoV-2, coronavirus disease 2019 (COVID-19), was published in June 2020.[1] The document addressed the implications of the pandemic for patients with rheumatic and musculoskeletal diseases (RMDs), at a time when very little was known about the epidemiology and the clinical course of patients with RMDs who contracted SARS-CoV-2 infection, and in particular about the risks that patients with RMDs faced, as well as preventive measures that these patients and their caregivers should take. The task force that dealt with the matter was—from a scientific point of view—*flying blindly* and had to rely on sparse clinical experience, a lot of common sense and a paucity of scientific evidence. Two factors may explain the delay in updating the first set of recommendations: (1) While the amount of data about SARS-CoV-2 infection/COVID-19 and RMDs in the literature accrued exponentially, the content of the original EULAR recommendations appeared to remain remarkably current, which in the opinion of the steering committee eliminated the urgency of an immediate update; (2) The advent of SARS-CoV-2 vaccinations by the beginning of 2021 and the initiation of epidemiological vaccination studies in patients with RMDs prompted the steering group to decide to issue an ad hoc advice on vaccination of patients with RMDs in December 2020[2] and to postpone a formal systematic literature review (SLR) until more comprehensive studies had been published.

Finally, EULAR decided to start the update process in January 2021, with a formal two-tier SLR, one covering the preceding year with a deadline of 29 March 2021 and the other covering the remaining months with a deadline of 31 May 2021. The formal SLR was expanded by a post hoc search for additional vaccination studies, on the request of the reviewers of the SLR manuscript, with a deadline of 11 October 2021.

As stated previously,[1] EULAR does not intend to over-rule existing guidelines at the country-level of EULAR member states. EULAR aims to provide a synthesis of the best available evidence ('the SLR') and the aggregated expert opinion, to inform rheumatologists and other healthcare providers (HCPs), as well as patients with RMDs about management decisions to be taken in the context of the global pandemic.

Unlike the unprecedented circumstances and urgency at the beginning of the pandemic, during which the provisional recommendations had to be

developed, the task force has now carefully followed the standard operating procedures (SOPs)[3] for updating management recommendations. As before, the task force was limited by restrictions of social distancing, preventing them from meeting in person and the entire process was conducted remotely by videoconferencing.

PROCEDURES

Focus of recommendations

These recommendations pertain to the management of patients with RMDs as the SARS-CoV-2 pandemic and its consequent COVID-19 disease may interfere with their usual management. The recommendations do not focus on diagnosing or treating COVID-19.

Most focus is on 'inflammatory' RMDs, because most questions from HCPs and patients themselves pertained to systemic autoimmune diseases, in particular to their treatments, as well as to the risks and benefits of vaccination against SARS-CoV-2. Needless to say that these recommendations also include patients with 'non-inflammatory' RMDs.

The task force composition

This EULAR task force consists of 28 members, 26 from 11 EULAR member states and 2 from the USA. Many expert members are internationally recognised rheumatologists and immunologists with many years of clinical and scientific experience, who fulfil or have fulfilled official positions in the EULAR organisation. EULAR's current and past presidents (AI, GRB, IBM, JSS and JWJB), as well as the current chair of EULAR's committee for the quality of care (RBML), the current chair of EULAR's people with arthritis and rheumatism (PARE) committee (SM) and EULAR's past vice-president representing PARE (DW) are members of the task force, among others. Five seats in the task force were reserved for rheumatologists from EULAR countries who could apply for this position and were subsequently selected by the convenor (RBML). Two seats were reserved for members of the emerging EULAR network (EMEUNET) who could apply for this position and were selected by the EMEUNET steering committee (PM and RC). The task force was further completed by an expert in infectious diseases (KW), one nominated representative of the American College of Rheumatology (ACR) (JC), three SLR fellows (FPBK, AA and AN), one senior methodologist and past-chair of the standing committee on epidemiology and health services research (PMM) and one junior methodologist (VN-C). The steering committee was formed by the convenor (RBML), the two methodologists (PMM and VN-C) and the three fellows (FPBK, AA and AN). All taskforce members were informed about, or had prior experience of, the development of EULAR recommendations according to EULAR's SOPs.[3]

Handling potential conflict of interest

In accordance with EULAR's SOPs, task force members are asked on an annual basis to provide and update their interactions with third parties (guideline committees, reimbursement bodies, pharmaceutical industries or other industries) that are not directly related to daily patient care but may give an impression to others of conflict of interest (*potential* COI). The EULAR office keeps record of these declared potential COIs.

The steering committee's workflow and procedures

The steering committee convened several times by videoconference and prepared the task force meetings and the SLR, as well as the draft updates of overarching principles (OPs) and recommendations, all for discussion and decision-making among the entire task force. The steering committee, in particular the convenor and methodologists, supervised the fellows' SLR work, discussed the application of instruments for risk of bias assessment, performed together with the fellows the actual risk of bias assessment and approved the reports of the SLR for dissemination among the task force members. Finally, the steering committee solicited the levels of agreement from task force members (by anonymous online survey), determined levels of evidence per item (according to the 2011 Oxford Centre for Evidence-Based Medicine) and drafted (two) manuscripts that were submitted to the EULAR Council for formal approval.

The task force's workflow and procedures

The task force members reviewed the preparatory work sent to them by email and were given the opportunity to propose changes. The task force convened by videoconference in four separate sessions: the first on 19 January 2021, in which the research questions for SLR were established; a second meeting on 25 May 2021, in which the task force was informed about the results of the first tier of the SLR; a third meeting on 16 July 2021, in which the task force was informed about the results of the second tier of the SLR and in which consensus about updated OPs and recommendations was reached; and a fourth meeting on 16 November 2021, in which the task force was informed about the results of the post hoc SLR limited to vaccination studies, and in which consensus was reached about two additional points to consider pertaining to additional vaccination doses. All task force members reviewed, discussed and agreed to the final version of this manuscript before submission to the EULAR Council.

Target audience

In line with EULAR's SOPs, the task force agreed to target their guidance primarily for rheumatologists, and other HCPs, and for patients with RMDs and their families. Secondarily, these recommendations target public health officials and public health experts by making them aware of particular problems pertaining to patients with RMDs and their treatments, as well as policymakers, who decide about infection prevention and control measures, access to healthcare for patients with RMDs, SARS-CoV-2 vaccination and availability of drugs for patients with RMDs.

Systematic literature research

The procedures, course and results of the SLR are described in detail in an accompanying article.[4]

Formal decision-making

Formal voting was only performed when deemed necessary during the task force meeting on 16 July 2021. Questions for voting were formulated by the meeting chair (RBML) in such a manner that a choice between two options (A and B) remained, and voting took place using the chat function of Microsoft Teams virtual platform. Voting was not blind, results were aggregated by non-voting EULAR staff present at the meeting and EULAR voting rules for making decisions applied (consensus accepted if >75% of the members voted in favour of the recommendation at the first round, ≥67% at the second round and at a third round >50% was accepted). If thresholds were not met, unresolved questions were rediscussed and the voting question was reformulated for subsequent voting. This process was repeated until a formal decision was reached. Each expert's level of

Table 1 EULAR recommendations for the management of rheumatic and musculoskeletal diseases in the context of SARS-CoV-2: the November 2021 update

	Overarching principles	LoA, mean (SD)	% ≥8/10
1.	In general, patients with RMDs do not face higher risk of contracting SARS-CoV-2 than individuals without RMDs, and do not have a worse prognosis when they contract it.	8.8 (1.5)	81
2.	The diagnosis and treatment of COVID-19 in patients with RMDs is the primary responsibility of an expert in treating COVID-19.	9.9 (0.3)	100
3.	Rheumatologists are the leading experts for the immunomodulatory or immunosuppressive treatments of their patients and should be involved in the decision to maintain or discontinue them.	9.9 (0.4)	100
4.	In view of their expertise, rheumatologists should be engaged in local hospital, regional or national guideline committees for COVID-19 management.	9.2 (1.2)	89
5.	The off-label use of immunomodulatory or immunosuppressive drugs for the treatment of COVID-19 outside of established guidelines, protocols or clinical trials should be discouraged.	9.2 (1.2)	93
	Recommendations		
1.	Patients with RMDs should be strongly advised to comply with all infection prevention and control measures prescribed by public health authorities, before and after SARS-CoV-2 vaccination.	9.9 (0.2)	100
2.	Patients with RMDs should be advised to receive SARS-CoV-2 vaccination with any of the vaccines approved in their country.	9.6 (1.6)	96
3.	Patients with RMDs who have been vaccinated against SARS-CoV-2 should be advised to continue their treatment unchanged; those who have not been vaccinated should be advised to continue their treatment, taking into account that certain therapies have been associated with an increased risk of severe COVID-19.	9.5 (0.6)	100
4.	If a patient with RMD receiving long-term glucocorticoid treatment develops suspected or confirmed COVID-19, this treatment should be continued.	9.3 (0.9)	96
5.	If a patient with RMD receiving rituximab treatment contracts SARS-CoV-2, postponing the next cycle of rituximab should be considered.	9.7 (0.6)	100
6.	Patients with RMDs and initially mild symptoms who experience worsening of COVID-19 symptoms should immediately seek further healthcare advice of an expert in treating COVID-19.	9.9 (0.3)	100
7.	Patients with RMDs should be advised to update their general vaccination status in accordance with the EULAR recommendations for the vaccination of patients with RMDs, with a particular focus on pneumococci and influenza.	9.7 (0.6)	100
8.	In patients with RMDs not using immunomodulatory or immunosuppressive treatment, SARS-CoV-2 vaccination should precede a treatment start with such therapy if clinically feasible.	9.6 (1.1)	93
9.	In patients with RMDs using rituximab or another B-cell depleting therapy, SARS-CoV-2 vaccination should be scheduled in a way to optimise vaccine immunogenicity.	9.6 (1.1)	96
	Points to consider		
1.	There are concerns that individuals on certain immunosuppressive or immunomodulatory drugs may not mount an adequate protective response to COVID-19 vaccination. Data are not currently available to reliably identify who might, or might not, benefit from a third primary dose of a SARS-CoV-2 vaccine. Taking a precautionary position, third primary doses are being recommended by some authorities in selected groups of individuals and EULAR supports this approach.	9.7 (0.6)	100
2.	There are concerns that protection provided by vaccines against severe COVID-19 decreases gradually over time. Insufficient time has passed to know what levels of protection might be expected 4–6 months after the primary course. Taking a precautionary position, booster doses are being recommended by several authorities and EULAR supports this approach.	9.4 (1.0)	95

COVID-19, coronavirus disease 2019; LoA, level of agreement (between 1 and 10); Mean (SD), mean level of agreement (SD); RMDs, rheumatic and musculoskeletal diseases; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

agreement (from 0 (no agreement at all) to 10 (full agreement)) with the statement was solicited after the final task force meeting by anonymous online survey for each OP and recommendation. The mean level of agreement, as well as the proportion of experts with a level of agreement of at least 8, was calculated.

RESULTS

The previous version of the recommendations contained 5 OP and 13 recommendations.[1] In the update process, the task force agreed on 5 OP, 9 recommendations and 2 additional points to consider (table 1). The bullet-text of these OP, recommendations and points to consider can be read in table 1. Below, an item-by-item discussion serves to give insight into the reasoning of task force members, focuses on how previous items and new items relate to each other and provides a justification for amendments and additions.

Old OP 1: To date, there is no evidence that patients with RMDs face more risk of contracting SARS-CoV-2 than individuals without RMDs, nor that they have a worse prognosis when they contract it.

New OP 1: In general, patients with RMDs do not face more risk of contracting SARS-CoV-2 than individuals without RMDs, and do not have a worse prognosis when they contract it.

New OP 1 is almost unchanged, but its evidence base has considerably improved, as the results of the SLR demonstrate. While the old OP 1 was preceded by the words *To date,* in order to reflect the scarcity of reliable data, many studies have been published thereafter and testify to the credibility of the statement. This statement pertains to the incidence of COVID-19 among patients with RMDs, as well as to the risk factors for contracting COVID-19 and for an unfavourable clinical course of COVID-19: while patients with RMDs may generally face worse outcomes and increased mortality, the incidence, risk and course of COVID-19 are globally the same as in the general population.

The words *In general* have been added to the new OP 1 to refer to a few situations in which the accuracy of the global statement can be disputed. Examples are patients with some rare and severe systemic autoimmune or autoinflammatory diseases.[4] Obviously, as a consequence of their scarcity, these exceptional cases have not yet been studied well. The same reservation pertains to certain treatments that have been associated with a worse COVID-19 course, such as rituximab, mycophenolic acid/mycophenolate mofetil (MMF), glucocorticoids (discussed under new recommendation (RC) 4) and potentially Janus kinase inhibitors (JAKi) (discussed under new RC 3).[5–9] The taskforce discussed that either methodological considerations preclude a firm(er) stand, or that the drug in question was too infrequently investigated in studies to base a general statement on. While these examples are more explicitly addressed in the SLR for reference,[4] they were kept out of the realm of the OP and recommendations (the exception to the rule being rituximab, as further outlined below).

Level of agreement: 8.8/10.

Old OP 2: The diagnosis and treatment of COVID-19 in patients with RMDs is the primary responsibility of an expert in treating COVID-19, such as a pulmonologist, an internist or a specialist in infectious diseases, dependent on local circumstances.

New OP 2: The diagnosis and treatment of COVID-19 in patients with RMDs is the primary responsibility of an expert in treating COVID-19.

This OP did not change significantly. It was considered more clear now than it was in the past that other medical experts than rheumatologists are primarily responsible for the treatment of

COVID-19. The task force felt that further specification of those experts was redundant and beyond the scope of this task force, especially since the situation may vary per country, per region and per hospital.

Level of agreement: 9.9/10.

Old OP 3: Rheumatologists are the leading experts for the immunosuppressive treatments of their patients and should be involved in the decision to maintain or discontinue them.
New OP 3: Rheumatologists are the leading experts for the immunomodulatory or immunosuppressive treatments of their patients and should be involved in the decision to maintain or discontinue them.

While this OP has not substantially changed, the term 'immunomodulatory or immunosuppressive treatment' is introduced here for the first time, and will be used throughout the entire document. Already from the beginning (in April 2020) there was dissent about using the term 'immunosuppressive' versus 'immunomodulatory', which led to an explanatory Viewpoint by Isaacs and Burmester,[10] who argued that some of the drugs used in rheumatology are 'immunomodulatory' (eg, targeted therapies), while others are 'immunosuppressive' (eg, glucocorticoids, azathioprine and MMF), and that the 'immunosuppressive' designation should not be used to cover all these drugs. Therefore, the task force decided to use the terminology 'immunomodulatory or immunosuppressive' throughout the document.

Level of agreement: 9.9/10.

Old OP 4: The knowledge about immunosuppressive treatments, including synthetic DMARDs and biological DMARDs, for the treatment of severe COVID-19 is rapidly evolving. In view of their expertise, rheumatologists should make themselves available for local-hospital, regional or national guideline committees for COVID-19. The use of immunosuppressive drugs for the treatment of COVID-19 should be a multidisciplinary decision.
New OP 4: In view of their expertise, rheumatologists should be engaged in local-hospital, regional or national guideline committees for COVID-19 management.

This OP has been condensed by virtue of evolving evidence. During the pandemic it has become clear that some of the treatments often used by rheumatologists have gained a prominent position in the management of patients with a hyperinflammatory state due to COVID-19 (eg, Kineret and tocilizumab, recently approved by the European Medicines Agency (EMA) for the treatment of adults with severe COVID-19 who are receiving systemic treatment with corticosteroids and require supplemental oxygen or mechanical ventilation), since randomised controlled trials (RCTs) have proven their efficacy and several are currently under review for marketing authorisation by the EMA, while a drug like hydroxychloroquine, promoted as a potentially life-saving compound in the beginning of the pandemic, has clearly been discredited after the results of several RCTs were published. Given that rheumatologists are the experts with the most experience in the benefits and risks, pharmacokinetics and pharmacodynamics of glucocorticoids and targeted therapies, such as interleukin-6-receptor (IL-6R) inhibitors and JAKi, rheumatologists are well placed to be involved in guideline developments that include such treatments for COVID-19.

Level of agreement: 9.2/10.

Old OP 5: Availability and distribution of, and access to, synthetic DMARDs and biological DMARDs for the treatment of patients with RMDs as well as for patients with COVID-19 (but without RMDs) is a delicate societal responsibility. Therefore, the off-label use of DMARDs in COVID-19 outside the context of clinical trials should be discouraged.
New OP 5: The off-label use of immunomodulatory or immunosuppressive drugs for the treatment of COVID-19 outside of established guidelines, protocols or clinical trials should be discouraged.

The initial fear for a shortage of certain disease-modifying antirheumatic drugs (DMARDs) for patients with RMD (with or without COVID-19), due to overuse for the treatment of patients with COVID-19, which formed an important element of the previous OP 5, has not materialised. As said, hydroxychloroquine is ineffective in COVID-19, and should not be used for that indication anymore. Glucocorticoids (including dexamethasone) are now part of most COVID-19 treatment protocols worldwide and are widely available and shortages are not expected.

After a long period of uncertainty, invoked by RCT with varying results, finally the IL-6R-inhibitor tocilizumab has been proclaimed an effective treatment for COVID-19, in particular for those with severe COVID-19 and largely restricted to the (short) hyperinflammatory phase. The drug has now been included in treatment protocols worldwide, as recommended by the WHO,[11] which has led to an increased demand for tocilizumab. Still, this increase should be manageable in light of the fact that patients with severe COVID-19 need only one or two intravenous doses and the manufacturer of tocilizumab has had ample time to adapt its production facilities. Therefore, the manufacturer's announcement of global supply constraints of tocilizumab has surprised the professional rheumatological community. EULAR, ACR and the WHO, among others, acted promptly with press-releases,[12–14] expressing concerns and calling on the company to ensure equitable allocation of current stocks of tocilizumab, and EULAR continues to monitor the availability, distribution and access to this and other medicines. The situation also led to the release of guiding principles by several professional organisations with considerations about the possibility of replacing tocilizumab by compounds with similar mechanism of action, starting new patients on alternative medications or switching intravenous tocilizumab to subcutaneous tocilizumab.[15 16]

In view of recent positive delivery developments, this task force decided to suspend the explicit warning about shortage of conventional synthetic DMARDs, but to maintain a general warning against the off-label use of immunomodulatory or immunosuppressive treatments.

Level of agreement: 9.2/10.

GENERAL MEASURES AND PREVENTION OF SARS-COV-2 INFECTION

The old RCs 1–3 included general public health measures and precautions, meant for patients with RMD without symptoms of SARS-CoV-2 infection, who had not been in contact with SARS-CoV-2 infected patients. By the end of 2020, SARS-CoV-2 vaccination became available and nowadays arguably forms the key measure of prevention of COVID-19 for patients with RMD and beyond.

Old RC 1: Patients with RMDs should be strongly advised to comply with all preventive and control measures prescribed by the health authorities in their countries.
New RC 1: Patients with RMDs should be strongly advised to comply with all infection prevention and control measures

prescribed by public health authorities, before and after SARS-CoV-2 vaccination.

This recommendation remains largely unchanged, but wording is added to reiterate that preventive measures remain important even after (full) vaccination, in order to prevent asymptomatic but infected patients with RMD from unknowingly spreading the virus, even though they may themselves be well protected against severe COVID-19 (hospitalisation, mechanical ventilation and death). Ongoing studies will hopefully reveal to what extent spreading of virus by asymptomatic individuals, as well as mild COVID-19 itself, is prevented by full SARS-CoV-2 vaccination.

Level of agreement: 9.9/10. Level of evidence: 5.

Old RC 2: Patients with RMDs should in general be advised to comply with the same preventive and control measures as patients without RMDs.

The task force felt that, in analogy with new OP 1, and by virtue of evolving evidence supportive of new OP 1, this recommendation had become redundant.

New RC 2: Patients with RMDs should be strongly advised to receive a SARS-CoV-2 vaccination with any of the vaccines approved in their country.

In line with previous EULAR recommendations, issued in December 2020,[2] as well as with evolving evidence outlined in the SLR,[4] the task force keenly felt that patients with RMDs should be strongly encouraged to receive full SARS-CoV-2 vaccination with one of the approved vaccines. On the basis of the available evidence, the task force was of the opinion that there are no compelling arguments to prioritise or dismiss particular approved vaccines for reasons of less efficacy or increased adverse events, in line with EMA guidance,[17] even though the two messenger RNA COVID-19 vaccines have been most thoroughly investigated in this regard. However, the task force stipulates that patients and HCP must follow national guidelines that are in place, which may sometimes deviate from EULAR's general principle of equal advisability.

Given that EULAR's remit extends beyond the European Union, and even beyond Europe (as a minority of EULAR countries are not geographically located in Europe), the task force acknowledged that limiting this recommendation to EMA-approved vaccines would not be in the best interest of patients with RMDs living in countries outside the European Union. Therefore, while EULAR encourages vaccine manufacturers to subject not-yet-EMA-approved vaccines to EMA scrutiny and procedures, this recommendation pertains to any vaccine approved in the respective EULAR-affiliated country.

The task force was of the opinion that in the realm of suboptimal SARS-CoV-2 vaccination status worldwide—due to scarcity of vaccines, non-equitable distribution, fear of vaccination or inappropriate vaccination information—it is important to improve vaccination status among the still unvaccinated patients with RMD. Arguably, this is more relevant than administering an additional vaccine dose to those that have already been fully vaccinated and—with exceptions (*vide infra*)—can be assumed to have a basic level of protection against SARS-CoV-2. In line with this position, and in light of the worldwide reach of EULAR recommendations, the task force encourages rheumatology societies of EULAR-affiliated countries to motivate their governments to facilitate the distribution of vaccines from high-income countries to medium-income and low-income countries, so that patients with RMD worldwide can better be protected. The failure of wealthy nations to distribute vaccines to the developing world is likely to result in serious global consequences for the pandemic, promoting the spread and mutation of SARS-CoV-2 among unvaccinated people and the emergence of new and potentially more transmissible and virulent SARS-CoV-2 variants.

Level of agreement: 9.6/10. Level of evidence: 3/4.

Old RC 3: Patients with RMDs who do not have suspected or confirmed COVID-19 should be advised to continue their treatment unchanged, namely non-steroidal anti-inflammatory drugs, glucocorticoids, synthetic DMARDs, biological DMARDs, osteoporosis medications and analgesics, among others.

New RC 3: Patients with RMDs who have been vaccinated against SARS-CoV-2 should be advised to continue their treatment unchanged; those who have not been vaccinated should be advised to continue their treatment, taking into account that certain therapies have been associated with an increased risk of severe COVID-19.

The old set of recommendations made a distinction between patients with RMDs (and treatment) at risk of COVID-19 and those who had (already) contracted COVID-19. The somewhat premature advice (old RC 3) to continue drug treatment in patients with symptomless RMD at risk of COVID-19 has proven its validity by evolving evidence, but has also gained dimension by the advent of SARS-CoV-2 vaccines. The new RC 3 makes a distinction between those who have been vaccinated against SARS-CoV-2, and those who have not yet received the vaccine.

The vaccinated patients may, in the opinion of the task force members and based on evolving evidence, safely continue their immunomodulatory or immunosuppressive treatment unchanged, even though an optimal humoral immune response may not occur under treatment. The task force was of the opinion that *any protection* is better than *no protection* and that temporarily discontinuing treatment of RMDs bears the risk of flare, and also points to the fact that an optimal immune response against SARS-CoV-2 is not unambiguously defined.

The not (yet) vaccinated patients should realise that the likelihood of severe COVID-19 is increased with certain immunomodulatory or immunosuppressive treatments, as outlined in the SLR,[4] in particular those who are treated with rituximab, MMF, glucocorticoids (discussed under new RC 4) and potentially JAKi. This recommendation should be read as an encouragement to patients and HCP to optimise vaccination status for SARS-CoV-2, taking certain precautions into account (as further outlined below).

Level of agreement: 9.5/10. Level of evidence: 3/4.

MANAGEMENT RMDS WHEN LOCAL MEASURES OF SOCIAL DISTANCING ARE IN EFFECT

Old RC 4 If the RMD and its drug treatment are stable, and signs or symptoms of drug toxicity are absent, regular blood monitoring and face-to-face rheumatology consultations can be postponed temporarily. If necessary, consultation can take place remotely.

Old RC 5: If the RMD is active, if drug therapy has recently been started or needs adjustment, or if signs or symptoms of drug toxicity emerge, patient and rheumatologist should liaise, weigh the risks of a visit to the clinic against the limitations of remote advice and decide together.

Old RC 6: If a patient with RMD is offered an outpatient, day care or other type of hospital appointment, patients and members of the rheumatology team should follow local guidance for

infection prevention and control, including the use of personal protection equipment if indicated.

The old recommendations 4–6 advised patients with RMDs on how to act when official restrictions in the freedom of movement apply. They referred to social distancing, varying from keeping 1, 1.5 or 2 metre distance for subpopulations to a complete country-lockdown. When discussing the advisability of these three recommendations, the task force agreed that their content was overtaken by reality and evolving evidence. This does not mean that the recommendations were wrong, or have become obsolete, but rather that the professional rheumatological community and patients with RMD have become accustomed to remote monitoring (old RC 4), initiating DMARD treatment during the pandemic (old RC 5) and triaging those who need a face-to-face consultation (old RC 6). Therefore, the task force decided to remove these three previous recommendations and further refer for this matter to EULAR guidance about remote monitoring in development.

MANAGEMENT OF COVID-19 IN THE CONTEXT OF RMDS

Old recommendations 7–10 referred to scenarios in which a patient with RMD had been in contact with a SARS-CoV-2 infected patient or had become infected themselves, with a focus on the use of immunomodulatory or immunosuppressive drugs.

Old RC 7: Patients with RMDs without COVID-19 symptoms who have been in contact with a SARS-CoV-2-positive person should be tested for SARS-CoV-2 themselves.

While in April 2020 this recommendation still raised dissent among task force members, due to the scarcity of SARS-CoV-2 tests and uncertainty about the potential consequences of a positive test result (eg, the need to pause drugs), this was no longer a source of discussion anymore in July 2021. SARS-CoV-2 testing has become ubiquitous and part of usual clinical care. The old RC 7 was considered redundant by the task force and therefore removed.

Old RC 8: If a patient with RMD and symptoms of COVID-19 is chronically treated with glucocorticoids, this treatment should be continued.

New RC 4: If a patient with RMD receiving long-term glucocorticoid treatment develops suspected or confirmed COVID-19, this treatment should be continued.

In spite of several studies pointing to an association between glucocorticoid use and worse COVID-19 prognosis, extensively outlined in the SLR,[4] old RC 8 (renumbered as new RC 4) has stood the test of time. After studying the results of the SLR, the task force came to the conclusion that the observed association between glucocorticoid-exposure and severe COVID-19 could well be explained by *confounding by indication*, with the confounder being disease activity, which has also been associated with a worse COVID-19 prognosis.[7 8] The suggestion of a glucocorticoid dose response that was seen in a few studies may reinforce this conclusion. While an adverse effect of glucocorticoids themselves cannot be entirely excluded, there is also sparse indirect evidence in the literature that pausing or discontinuing glucocorticoids for reasons of safety is associated with disease flaring, which in itself may contribute to an adverse outcome of COVID-19. Finally, it should also be noted that patients on long-term glucocorticoid therapy are at risk of glucocorticoid-induced adrenal suppression and may therefore require glucocorticoid supplementation in the context of major trauma, surgery or significant intercurrent infection, including COVID-19.[18]

The advice to continue glucocorticoids in patients with RMD *without* symptoms of COVID-19 is now covered by the generic new RC 3; the advice to continue glucocorticoids in patients with RMD *with* suspected or proven COVID-19 is covered by new RC 4. The task force remains of the opinion that the principle of 'lowest possible dose' as per existing EULAR-recommendations for the management of medium-dose to high-dose glucocorticoids therapy is part of good clinical practice and valid under all circumstances.[19]

Level of agreement: 9.3/10. Level of evidence: 3/4.

New RC 5: If a patient with RMD receiving rituximab treatment contracts SARS-CoV-2, postponing the next cycle of rituximab should be considered

This new recommendation without precedent in the first set was included because of evolving evidence that patients who use B-cell depleting therapy (in particular anti-CD20 therapy with rituximab) for their RMD have a higher risk of developing severe COVID-19 and an inferior antibody response to SARS-CoV-2 vaccination.[4] The task force realised that there are many practical questions around the best possible management of patients with RMD treated with B-cell-depleting therapy. Other professional organisations than EULAR have sometimes provided more granular recommendations about B-cell-depleting therapy in association with COVID-19.[20] This task force was of the opinion that an evidence-based recommendation on how to act in specific circumstances is not opportune, since the data proving that specific measures are indeed effective and safe are currently lacking. Still, the task force felt some pressure of sister organisations to make recommendations regarding rituximab, administered in cycles with intervals ranging from 1 to 12 months. This recommendation, as well as the ones pertaining to vaccination that follow below, is based on expected effects of rituximab and clinical feasibility, rather than on solid evidence. In general, for patients on rituximab, the task force found it reasonable to postpone a next cycle of rituximab (or, alternatively, to replace rituximab by an equally effective drug) in a patient with stable RMD as long as the clinical situation allows a delay. While the task force recognises some excess risk of rituximab in such circumstances, a contraindication for rituximab is relative, not absolute.

Level of agreement: 9.7/10. Level of evidence: 3/4.

Old RC 9: If patients with RMDs experience mild symptoms of COVID-19, potential treatment changes in DMARDs should be discussed on a case-by-case basis.

This old recommendation reflected a compromise between task force members who considered the continuation of DMARDs in a patient with RMD with symptoms of COVID-19 undesirable, and those who agreed with the argument that more than 90% of patients with COVID-19 usually experience a mild and self-limiting course, and that early data did not point to a significantly increased risk of severe COVID-19 in patients with RMD on DMARD treatment. Since then, the ever-increasing body of evidence has tipped the balance towards a more moderate and lenient attitude of continuing DMARDs in case of mild COVID-19 symptoms. Herewith, this old RC 9 has become redundant, and its content is now entirely covered by new RC 3.

Old RC 10: Patients with RMDs and initially mild symptoms who experience worsening of COVID-19 symptoms should immediately seek further healthcare advice of an expert in treating COVID-19, such as a pulmonologist, an internist or a specialist in infectious diseases, dependent on local circumstances.

New RC 6: Patients with RMDs and initially mild symptoms who experience worsening of COVID-19 symptoms should immediately seek further healthcare advice from an expert in treating COVID-19.

While consensus has now been obtained regarding the continuation of DMARDs in a patient with mild COVID-19, it is still opportune to advise on patients with RMD with worsening of COVID-19. They should be referred to an expert in treating COVID-19, not being the rheumatologist, as per new RC 6.

It has become clear during the pandemic that a small minority of patients with COVID-19 will experience a more severe course. Patients with severe COVID-19, with or without RMDs, may require ventilatory support, antibiotic treatment, anticoagulation and temporary immunomodulatory or immunosuppressive treatment. While some of these treatments involve medications with which rheumatologists are considered broadly familiar, the task force is (still) of the opinion that the diagnosis of severe COVID-19, the indication to start adjunctive therapy and the monitoring of the course of severe COVID-19 belong to the realm of an expert in COVID-19 (new OP 2). This does not mean that rheumatologists should not be involved in the design of—and discussion about—protocols and guidelines, as per new OP 4. For more details about the immunomodulatory treatment of (severe) COVID-19 per se, the task force refers to the EULAR's points to consider on the use of immunomodulatory therapies in COVID-19.[21–24]

Level of agreement: 9.9/10. Level of evidence: 3/4.

Old RC 11: Patients with RMDs who are admitted to the hospital because of significant COVID-19 should follow local treatment recommendations for COVID-19 as applied by the treating expert.

This recommendation dates back to the time at which task force members made a deliberate distinction between patients with mild COVID-19, those with worsening of once mild COVID-19 and those with significant or severe COVID-19. This distinction has gradually become outdated and redundant for the advice of how to manage patients with RMD with symptoms of COVID-19 today. Those with mild symptoms may continue their treatment unchanged and followed up until recovery, as per new RC 3. Those with worsening symptoms should be referred to an expert in COVID-19 without exception, as outlined in new RC 6. Herewith, old RC 11 has become redundant.

PREVENTION OF OTHER INFECTIONS THAN SARS-COV-2

Old recommendations 12 and 13 intended to remind the rheumatologist of potentially coexisting comorbid infections for which regular vaccinations exist (old RC 12), and of other important infectious diseases that could phenotypically mimic COVID-19 (old RC 13).

Old RC 12: Patients with RMDs without symptoms of COVID-19 should be advised to update their vaccination status in accordance with the EULAR-recommendations for the vaccination of patients with RMDs, with a particular focus on pneumococci and influenza.

New RC 7: Patients with RMDs should be advised to update their general vaccination status in accordance with the EULAR-recommendations for the vaccination of patients with RMDs, with a particular focus on pneumococci and influenza.

This recommendation was essentially unchanged. The update of EULAR recommendations for vaccination in adult patients with autoimmune inflammatory rheumatic diseases was published in 2019 and should be consulted for further information.[25]

Level of agreement: 9.7/10. Level of evidence: 5.

Old RC 13: In patients with RMDs treated with cyclophosphamide or glucocorticoids, pneumocystis Jiroveci pneumonia-prophylaxis should be considered

This recommendation pertaining only to a small minority of patients with RMDs, particularly those with intensive immunosuppressive therapy, served to alert the rheumatologist's attention to a phenotypical mimic of COVID-19 at a time at when confirmatory COVID-19 testing was not self-evident. The task force was of the opinion that clinical confusion between *Pneumocystis jiroveci pneumonia* (PJP) and COVID-19-pneumonia has become unlikely. While PJP-prophylaxis remains highly topical for those at risk of PJP due to (severe) immunosuppression, the task force was of the opinion that this is out of the scope of the current manuscript and the old RC 13 could be deleted.

NEW RECOMMENDATIONS

The task force added two recommendations referring to SARS-CoV-2 vaccination that had no precedent in the old set of recommendations.

New RC 8: In patients with RMDs not using immunomodulatory or immunosuppressive treatment, SARS-CoV-2 vaccination should precede a treatment start with such therapy if clinically feasible.

This recommendation finds its justification in recent evidence, summarised in the SLR,[4] pointing to an impaired humoral immune response in patients with RMD treated with particular immunomodulatory or immunosuppressive treatments. The level of impairment varies by compound: from the suppression of humoral immune response in case of B-cell-depleting therapy and MMF, to the generally mild-to-moderate impairment in case of methotrexate, glucocorticoids and JAKi, to no distinguishable impairment for tumour necrosis factor (TNF)-inhibitors and IL-17-inhibitors, as well as for most conventional synthetic DMARDs. While the task force agreed that the clinical significance of an impaired level of antibodies to SARS-CoV-2 (humoral immune response) is still unclear, it also argued that it makes sense to first vaccinate and then start with immunomodulatory or immunosuppressive therapy, unless the delay of treatment is damaging or life threatening, a consideration that is left at the discretion of the rheumatologist and the patient in shared decision-making.

Level of agreement: 9.6/10. Level of evidence: 3/4.

New RC 9: In patients with RMDs using rituximab or another B-cell depleting therapy, SARS-CoV-2 vaccination should be scheduled in a way to optimise vaccine immunogenicity.

This new recommendation serves to bring the EULAR recommendations in sync with guideline documents of professional sister organisations that have recommended explicitly on this matter.[26] It draws the rheumatologist's, HCP's and patient's attention to the fact that—as outlined above several times—B-cell depleting therapy may compromise the development of an appropriate (humoral) defence against SARS-CoV-2 on vaccination. While new RC 8 points to postponement of the start of immunomodulatory or immunosuppressive treatment when clinically feasible, it does not suffice for patients who have already been treated with cycles of rituximab, which may surely cause a long-lasting and not immediately reversible functional

suppression of B-cell activity. The task force acknowledged that patients and HCP may ask for more specific guidance in terms of a minimal duration between the last cycle of rituximab and the vaccination, but had to conclude that such a time frame does not logically follow from the currently available data; the highly variable B-cell repopulation kinetics may in fact be a more important factor to take into account when deciding when to vaccinate rather than a specific timeframe. The task force acknowledges that the advice to *optimize vaccine immunogenicity* [sic] without further explanation may not fully cover patients' and HCP's expectations. However, in the absence of evidence, although in spite of existing guidance from other organisations, the task force feels they could not be more specific at this point in time.

Level of agreement: 9.6/10. Level of evidence: 3/4.

NEW POINTS TO CONSIDER

At the same time as real-world effectiveness data of SARS-CoV-2 vaccination emerged over the last few months, pre-emptive action was undertaken by governments of many countries, fuelled by public opinion and anxiety among health experts regarding waning vaccine effectiveness. These countries implemented two types of strategies, namely:

1. Administering an additional dose of the vaccine to individuals who had received their primary course of vaccination while on immunomodulatory or immunosuppressive drugs, or to individuals with an underlying health condition causing a primary or acquired immunodeficiency state (*third primary dose* (or: *second primary dose* if the initial vaccine administered was a single-dose vaccine)—for simplicity this manuscript will consistently refer to the term *third primary dose*); and:
2. Reinforcing immunisation of the vaccinated population with a *booster* vaccine dose, usually starting with priority groups (such as: older and more vulnerable individuals) and potentially expanding this strategy to the entire vaccinated population.

Based on these developments, EULAR updated its SLR with the most recent evidence on vaccination of patients with RMDs, and re-opened the discussion about the desirability of recommendations pertaining to revaccination of previously vaccinated patients with RMD.

Two additional statements emerged from this discussion, informed by the post hoc data of the SLR.

It was decided that these statements did not deserve a status of recommendation, since supportive evidence was fragmentary, often unconfirmed and methodologically not robust enough. However, the task force was also of the opinion that EULAR cannot ignore public health advice by authorities at the countries' level, and that patients with RMDs and the HCPs taking care of them value the opinion and guidance of EULAR. Therefore, the task force chose the formulation of *points to consider (PtC)*, in order to convey the message of immaturity of the evidence regarding revaccination, on the one hand, and the appreciation of—and compliance to—precautionary public health measures issued by authorities under conditions of uncertainty, on the other hand. The two points to consider are read as follows:

New PtC 1: There are concerns that individuals on certain immunosuppressive or immunomodulatory drugs may not mount an adequate protective response to COVID-19 vaccination. Data are not currently available to reliably identify who might, or might not, benefit from a third primary dose of a SARS-CoV-2 vaccine. Taking a precautionary position, third primary doses are being recommended by some authorities in selected groups of individuals and EULAR supports this approach.

Here the task force elaborated on evidence that some patients with RMD may not mount a full immune response to COVID-19 vaccination. This has been well-documented for patients exposed to anti-CD20-therapy during vaccination, who have been shown to have impaired (or even absent) humoral response to the vaccine, and on accruing evidence that this latter might convey an increased risk of (severe) COVID-19, as per RCs 8 and 9. However, many uncertainties remain. First, impaired humoral immunity is not the same as *no protection*, and for example studies looking at cellular responses have largely been reassuring, even in the absence of a humoral response (there is no proper *correlate of protection*). Second, proving an association between the level of humoral immunity and the risk of (severe) COVID-19 neither means that re-vaccination will improve humoral immunity in these patients, nor that improving humoral immunity by re-vaccination will reduce the risk of (severe) COVID-19. Epidemiological studies that allow such a causal chain of argumentation are lacking so far and the potential for additional protection from a *third primary dose* is unknown at an individual level.

As argued before, however, authorities in several (high-income) countries have already issued guidance that *immunosuppressed patients* (among which patients with RMD who use certain immunomodulatory or immunosuppressive drugs) should receive a *third primary dose*. This approach is based on the assumption that a *third primary dose* is unlikely to confer significant harms or disadvantages, but may offer the possibility of benefit. So far, there is no unanimity about which patients and which drugs are critical in this regard. Following the information from the SLR, the task force is of the opinion that the data on anti-CD20 therapy are most compelling, followed by data on MMF and glucocorticoids (potentially at higher dosages, but a dose-dependent effect and potential dosage cut-off are still unclear). Data on methotrexate, JAKi and abatacept are not (yet) consistent/robust. Reassuringly, the use of hydroxychloroquine and some targeted therapies (eg, TNF-inhibitors, IL-17-inhibitors, IL-6R blockers, belimumab) have not been associated with lower antibody responses. Data are scarce (or lacking at all) for other conventional synthetic/targeted synthetic DMARDs (eg, sulfasalazine, leflunomide, apremilast), other biological DMARDs (eg, IL-12/23-inhibitors, IL-1-inhibitors) and other immunosuppressive drugs (eg, cyclophosphamide, cyclosporine, azathioprine and tacrolimus).

The task force recognises the positions taken by authorities in several countries, usually fueled by expert committees in those countries and largely based on expert-opinion or expert-suspicion, even though different authorities may delineate different groups of individuals to whom the guidance should refer. The task force stipulates that support of a third primary dose vaccination policy does not mean that EULAR recommends this approach on the basis of firm scientific evidence at this point in time. The decision to administer third primary doses (or not) is the outcome of shared decision-making by the physician and the well-informed patient.

Level of agreement: 9.7/10. Level of evidence: 5.

New PtC 2: There are concerns that protection provided by vaccines against severe COVID-19 decreases gradually over time. Insufficient time has passed to know what levels of protection might be expected beyond 4–6 months after the primary course. Taking a precautionary position, booster doses are being recommended by several authorities and EULAR supports this approach.

Table 2 Research agenda

General measures and prevention of SARS-CoV-2 infection	
1.	Large unselected registry studies to assess the course of COVID-19 in patients with rare autoimmune diseases compared with the general population.
Management of immunosuppressive or immunomodulatory drugs in patients with RMD with COVID-19	
1.	Large unselected registry studies to assess the risk of Janus kinase inhibitors and immunosuppressants (glucocorticoids, azathioprine, cyclosporine, cyclophosphamide, mycophenolate and tacrolimus) on a worse course of COVID-19.
2.	Studies to assess the impact of other B-cell depleting strategies (eg, belimumab) on the outcome of SARS-CoV-2 infection and COVID-19 diseases course.
3.	Studies to compare different disease-modifying antirheumatic drugs management strategies in the context of SARS-CoV-2 infection: unchanged, versus dose reduction versus interruption in patients with RMDs.
Vaccination of the patients with RMD	
1.	Studies to assess the impact of temporarily stopping medications 'of concern' before or after SARS-CoV-2 vaccination and supplemental (booster) dosing, in order to improve immunogenicity, and the impact of such strategies on disease activity and need of additional treatments, for example, glucocorticoids.
2.	Studies to assess the impact of an additional dose as part of the initial primary SARS-CoV-2 vaccination in selected subsets of patients with RMDs, in order to improve the humoral and/or cell-mediated immunity to SARS-CoV-2 vaccines.

COVID-19, coronavirus disease 2019; RMDs, rheumatic and musculoskeletal diseases; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Here, the task force took the *better safe than sorry* approach. The argument of waning protection is based on waning humoral immunity against SARS-CoV-2 over time, which is an 'intermediate outcome'. Robust epidemiological evidence that waning immunity is a particular problem in patients with RMD (and if yes, how long after completion of a primary vaccine series?) is lacking so far, but this is conceivable from a theoretical perspective. Some evidence that a *booster* vaccine in such circumstances improves protection in the general population now also starts to accrue.[27 28] Following guidance issued by an increasing number of governments, especially in high-income countries, and knowing that *booster* vaccination is likely a relatively safe medical intervention, also in patients with RMD, the task force decided to take a passive but supportive stand. It means that the task force has understanding of the authorities' approach (and its potential benefit in fighting the pandemic), rather than that the task force is of the opinion that *booster* vaccination has undeniably proven its merits (yet).

Level of agreement: 9.4. Level of evidence: 4.

DISCUSSION

This set of five overarching principles, nine recommendations and two points to consider forms the first update of the original EULAR provisional recommendations for the management of patients with RMDs during the SARS-CoV-2 pandemic. The scientific status of the first set was meagre, but the level of evidence of the updated recommendations has significantly improved, in accordance with evolving knowledge. However, there are still many unknowns. Despite an exponential and likely unprecedented explosion of scientific studies, many critical clinical questions, some of which are mentioned in the research agenda (table 2), have not yet been fully addressed in clinical studies and remain largely unanswered. While the overall impression is not a negative one, the majority of available studies still received the predicate of *unclear* or *high* risk of bias. Those few studies with *low* risk of bias, the best ones so to say, have had a significant impact on the reformulation of the old recommendations into new ones.

Old versus new recommendations

When comparing the old and new recommendations, a few observations stand out.

The first is that the number of recommendations has reduced from 13 to 9 and the length of each recommendation has importantly reduced too. This may seem a trivial observation without scientific meaning, but may also testify to an increased maturity of the field and (consequently) more unanimity among task force members. That levels of agreement were (even) higher than in the previous set, adds to the credibility of the latter. In April 2020 diverging opinions, due to lack of available evidence and a necessary reliance on (sparse) experience (not to say: beliefs), had materialised into a rather high number of rather verbose recommendations, in order to better reflect different, sometimes even opposing opinions. In July 2021, after properly being informed by the SLR-committee, the task force reached consensus within 3 hours of discussion, and delivered nine concise and structured recommendations.

The second observation is that the content of the updated set is dominated by SARS-CoV-2 vaccination. SARS-CoV-2 vaccination is indisputably an example of unprecedented medical progress. While in April 2020 the prospect of SARS-CoV-2 vaccination was still uncertain, in July 2021 a significant proportion of the population in many EULAR countries were already vaccinated and discussions about (and implementation of) a *third primary dose* had started, although confirmatory evidence for that policy is still lacking.

More focus on vaccination also illustrates the progress that has been made in understanding the hazards that patients with RMD face in the context of COVID-19. Because the risk of poor outcomes in general is increased in several RMDs, many had feared that patients with RMDs were not only at higher risk of contracting COVID-19, but would also experience a worse course when having COVID-19. In spite of a couple of exceptions and uncertainties, amply described in the accompanying SLR,[4] and some disagreement among task force members, this fear has not become manifest and the updated set of recommendations is a good reflection of that appreciation; patients with RMD are not very different from unaffected individuals in the general population (even though they may have a higher comorbidity burden), most treatments can be safely continued and special precautions for patients with RMD (beyond those advised for the general population) are in general not necessary.

This does not mean that there are no outstanding questions. JAKi and (even) sulfasalazine have recently been associated with an increased risk of severe COVID-19, rituximab is a notoriously difficult therapy to manage in the context of COVID-19 and vaccination and there are also question marks about some truly immunosuppressive drugs such as MMF, a drug prescribed for several systemic autoimmune diseases, about which the first impressions were slightly worrisome. Still, the make-up of the studies that released these associations preclude a causal interpretation; selection bias and confounding-by-indication, rather than the drug itself, may be responsible for the reported excess risk in many studies.

New EULAR recommendations in context

Comparing these EULAR recommendations with other recent recommendations, such as the latest version of the ACR recommendations,[26 29] reveals, as expected, high levels of similarity. Issues of controversy are of relatively minor importance. The ACR has released guidance documents that have been more frequently updated than EULAR's, and are far more detailed, since they deal with several scenarios and drugs separately.[26 29] A main discrepancy pertains to ACR's recommendation of a drug-pause for most DMARDs in case of known or suspected SARS-CoV-2 exposure. ACR also advises to pause DMARDs in case of active or presumptive COVID-19 (exceptions are sulfasalazine and, conditionally, IL-6 inhibitors). Reinitiating treatment should, according to the ACR, depend on COVID-19 symptom resolution (after at least 7–14 days, or more for certain DMARDs). The British Society of Rheumatology[16] and the UK's National Institute of Clinical Excellence[30] also advise to pause DMARDs for a while in case of manifest COVID-19. The EULAR task force is more lenient in this regard, since it does neither recommend to pause in case of exposure to SARS-CoV-2, nor in case of mild symptomatic COVID-19 (ie, the large majority of patients with COVID-19 that do *not* require oxygen supplementation or hospitalisation). In case of more severe (eg, hospitalised) COVID-19, EULAR leaves the decision about pausing or stopping DMARDs at the discretion of the treating physician for COVID-19 (new OP 2), in consultation with the treating rheumatologist (new OP 3). Whether this discrepancy in policies results from a different interpretation of the available literature, from different local circumstances or from differences in medicolegal context between Europe and the USA, is unclear.

Regarding SARS-CoV-2 vaccination, EULAR has aggregated management recommendations and vaccination recommendations into one document. The ACR has recently released a guidance document entirely dedicated to SARS-CoV-2 vaccination in patients with RMDs.[31] The ACR has provided no less than 76 guidance statements to cover all possible scenarios that patients with RMDs may encounter. Basically, these ACR-statements are in line with EULAR's simple and concise recommendation that all patients with RMDs, without exception, should be fully vaccinated as soon as possible (new RC 2). The ACR provides more detailed guidance on how to manage patients with RMD in specific scenarios (the ACR, for instance, advises to pause certain DMARDs around vaccination, gives specific advice per DMARD and provides timelines). The EULAR task force was aware of the ACR document, and discussed these matters, but was essentially of the opinion that the available scientific evidence precluded such a detailed level of advice. The task force decided that a more generic advice was opportune (new RC 2), which could rely on a very high level of agreement among task force members.

Addition of points-to-consider into context

Mixing recommendations and points-to-consider in one EULAR manuscript is unprecedented, but likely justifiable in the context of the unprecedented pandemic with rapidly evolving evidence and changing scenarios. What remains to be discussed is the realisation that public health advice (including medical interventions) by governments in different countries is not always driven by solid scientific evidence, but also by public (and experts') beliefs and perceptions, and by emotions. The EULAR task force struggled with this issue, for which current SOPs do not provide resolution. The task force finally compromised that EULAR will not publicly contest official guidance issued by individual EULAR member states, but will report their conclusions based on their interpretation of the evidence and according to the rules laid down in their SOP.

The final outcome, in clinical practice, is always the result of the process of shared decision-making between the patient, who is optimally informed about facts and residual uncertainties, and the HCP.

A critical appraisal of evolving epidemiological evidence on SARS-CoV-2/COVID-19

Translating scientific evidence, stemming from high-profile epidemiological surveys, RCTs or high-quality observational studies, to the situation of the individual patient in daily clinical practice is not an easy task. Communicating such information accurately to patients is even more difficult. Big-data studies and multicountry epidemiological registries will often attract most attention from physicians and lay press, because of the high numbers of patients involved in such studies. Not infrequently do these studies report small but statistically significant excess risks for patients with RMD in comparison to the general population. It is of utmost importance for HCP, who have to deal with individual patients rather than an entire population of patients, to realise that a small excess risk (risk estimates arbitrarily between 1 and 2–3) is often irrelevant if the base case risk for that patient is low (eg, less than 1 in 100), even if the small excess risk is highly statistically significant. The anticipated consequence (eg, lower risk of severe COVID-19) of a certain interventional recommendation (eg, DMARD pausing), seemingly justified by an excess risk at the group level, should always be weighed against unwarranted and often unforeseen consequences of that interventional recommendation (eg, relapse of disease activity). In addition, the task force realised that the technical demonstration of an observational association between an exposure (eg, the use of a DMARD) and an outcome (eg, hospitalisation for COVID-19) alone does not constitute sufficient evidence to recommend an intervention (eg, pausing the DMARD, revaccination) if the proof that such an intervention really works is lacking.

On the other hand, the task force sometimes expressed support for proposed interventions with potential but largely theoretical benefit and little harm to expect (a good example is revaccination of patients with RMD), but reiterated in such cases that the scientific evidence was lacking.

CONCLUSION

The task force hopes that these updated, now more evidence-based, recommendations on how to manage patients with RMDs in the context of SARS-CoV-2 and COVID-19 give HCPs the tools to make clinical decisions about SARS-CoV-2 prevention, DMARD management and SARS-CoV-2 (re)vaccination. More importantly, it hopes that it will help build confidence among patients with RMDs that, (in general), their risk of severe COVID-19 is not importantly increased and that SARS-CoV-2 (re)vaccination, crucial to finally contain the pandemic, can safely take place.

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EULAR points to consider for minimal reporting requirements in synovial tissue research in rheumatology

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ABSTRACT

Background Synovial tissue research has become widely developed in several rheumatology centres, however, large discrepancies exist in the way synovial tissue is handled and, more specifically, how data pertaining to biopsy procedure, quality check and experimental results are reported in the literature. This heterogeneity hampers the progress of research in this rapidly expanding field. In that context, under the umbrella of European Alliance of Associations for Rheumatology, we aimed at proposing points to consider (PtC) for minimal reporting requirements in synovial tissue research.

Methods Twenty-five members from 10 countries across Europe and USA met virtually to define the key areas needing evaluation and formulating the research questions to inform a systematic literature review (SLR). The results were presented during a second virtual meeting where PtC were formulated and agreed.

Results Study design, biopsy procedures, tissue handling, tissue quality control and tissue outcomes (imaging, DNA/RNA analysis and disaggregation) were identified as important aspects for the quality of synovial tissue research. The SLR interrogated four databases, retrieved 7654 abstracts and included 26 manuscripts. Three OPs and nine PtC were formulated covering the following areas: description of biopsy procedure, overarching clinical design, patient characteristics, tissue handling and processing, quality control, histopathology, transcriptomic analyses and single-cell technologies.

Conclusions These PtC provide guidance on how research involving synovial tissue should be reported to ensure a better evaluation of results by readers, reviewers and the broader scientific community. We anticipate that these PtC will enable the field to progress in a robust and transparent manner over the coming years.

INTRODUCTION

Analyses of synovial tissue (ST) at both cellular and molecular levels offer a promising approach for personalised therapy in rheumatic diseases. ST analysis may also advance understanding of disease pathophysiological mechanisms and permit identification of potential therapeutic targets.[1–3] Moreover, new developments in single cell methodologies are driving innovation and demand for ST-based studies.[4–6] Methods to obtain ST, namely synovial biopsy (SB) procedures, are becoming more acceptable to patients and have been performed with increasing frequency over recent years for both clinical and translational research purposes. This is due in part to the introduction of ultrasound (US) guidance enabling minimally invasive approaches that have now been extensively validated in terms of safety, tolerability and tissue yield.[7–11]

However, the recent increase in numbers of studies using ST as a source of scientific material also raises questions in terms of interpretability and generalisability. Previous efforts have been initiated by the Outcome Measures in Rheumatology (OMERACT) group and the European Alliance of Associations for Rheumatology (EULAR) Synovitis Study Group (ESSG) in providing guidance on harmonisation of ST analysis procedures across centres for both clinical practice and research.[12–16] Nevertheless, minimal requirements for reporting of SB procedures and handling methods of ST remain to be defined. Both reliability and validity of results in the field rely critically on tissue quality and processing. Moreover, selection of patients, methods of retrieval (as well as location within the joint), experience of the operator, handling and analysis methods and quality of the tissue have potential to affect the final research outcome. Therefore, there is an unmet need for evidence and consensus-based points to consider (PtC) defining minimum reporting requirements that could ensure interpretability of the research. Complete and accurate reporting will allow the reader to detect potential biases in the study (internal validity) and to assess the generalisability and applicability of the results (external validity). In this context, the aim of this work was to formulate the EULAR PtC for minimal reporting requirements in ST clinical practice and research in rheumatology.

METHODS

From December 2020 to May 2021, a steering committee composed of the conveners (AN—also fellow—and AF) and the senior and junior

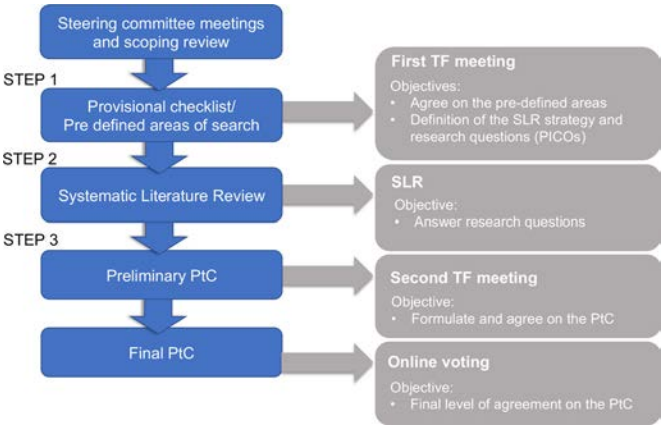

Figure 1 Project framework. PICO, population, intervention, comparison and outcomes; PtC, points to consider; SLR, systematic literature review; TF, task force.

methodologists (M-AD'A and FC) led a multidisciplinary task force following the 2014 updated EULAR standardised operating procedures.[17] The task force included in total 25 members (including the steering committee) from 10 countries, composed of 19 rheumatologists (2 of them also representing the Emerging EULAR Network), 1 translational immunologist and 2 pathologists alongside one allied health professional and two patient representatives. Two virtual meetings of the task force were held, one in December 2020 and one in May 2021. During the first task force meeting, research questions pertaining to the project were formulated. The fellow (AN), guided by the methodologists, performed an systematic literature review (SLR), gathering articles on ST biopsy procedures, their tolerance and outcomes, tissue handling and randomisation, tissue quality control and tissue outcomes. The SLR is published separately, and it forms an integral part of the project.

During the second task force meeting, the results of the SLR were presented and discussed, leading to the formulation of PtC based on evidence and expert opinion. Every statement was presented, iteratively discussed and voted on (informal voting). The level of evidence (LoE) supporting each statement was assigned according to the Oxford Centre for Evidence Based Medicine 2011 Levels of Evidence.[18] Of note, an LoE of five corresponds to expert opinion and an LoE of four corresponds to case–control studies. Finally, each task force member anonymously indicated their level of agreement (LoA) with each PtC online (Numerical Rating Scale ranging from 0='completely disagree' to 10='completely agree'). The aspects emerging during discussion that required further evidence were integrated in the research agenda. All these steps are summarised in figure 1. The final manuscript was reviewed and approved by all task force members, followed by verification by the EULAR Executive Committee.

RESULTS

Three overarching principles (OP) and nine PtC were formulated (table 1). All OP and PtC were approved after one round of hand raised voting during the task force meeting and one round of online voting after the task force meeting. The mean LoAs were higher or equal to 9, with a percentage of votes above 8/10 of 100% for most of the OP and PtC. LoA was reported in table 1. The LoE was 4 or 5 for all PtCs. The PtC is intended to provide guidance on how research involving ST should be reported in the following areas: biopsy procedures, study design, patients and disease characteristics, handling and processing methods of tissue, quality control, histological analysis, molecular analysis and single cell technologies. The target population was identified as rheumatologists, pathologists and scientists (eg, computational biologists, translational immunologists, molecular scientists), using or involved in research on ST. The target users were defined as physicians and allied health professionals (eg, physiotherapists and specialist nurses), patient research partners and patient charities and organisations, reviewers, journal editors, scientific societies and OMERACT, pharmaceutical industry, biopsy device manufacturers, and the enhancing the quality and transparency of health research network.

Overarching principles

OP-1: Synovial biopsies (single and sequential), performed in aseptic conditions, are safe, well tolerated and can be performed for both clinical and research purposes

SB is performed in both clinical and research settings across numerous centres in Europe. The body of evidence suggesting that the technique is safe and well tolerated has grown over the years, and the safety of the procedure is now well established.[7 8 10 11 19–23] The task force emphasised that this applies to both single and sequential biopsies.[11 20]

OP-2: In both clinical and research settings, synovial biopsies should be guided by imaging techniques. Arthroscopy and ultrasonography are the preferred techniques to guide synovial biopsies

The task force strongly felt that SB should no longer be performed without imaging guidance. This is justified by the fact that blind needle biopsy (NB) procedures retrieve less graded tissue than guided techniques.[24] The most commonly used imaging techniques to guide synovial biopsies are US-guided NB, US-guided portal and forceps and arthroscopy. CT and MRI guidance are not used commonly and therefore cannot be recommended.

OP-3: US or arthroscopy can be used to guide the SB without affecting the tolerability of the procedure or the minimal required tissue for meaningful analysis

While the number of graded ST fragments/total number of ST fragments does not differ with US and arthroscopic guided biopsy, the quantity and quality of RNA retrieved was superior with arthroscopy in a study comparing the tissue outputs of different techniques.[24] Nevertheless, all techniques allow retrieval of a sufficient quantity of ST for meaningful analysis. Short-term and long-term tolerance is satisfactory with all guided techniques in terms of Visual Analogue Scale pain, swelling and stiffness for both small and large joints, with no difference reported between techniques in a study of over 500 procedures.[11]

Points to consider

PtC-1: The details of the biopsy procedure should be reported in every study. This should include non-exhaustively:

- Exclusion criteria for biopsy.
- Target joint(s) and recess.
- Intra-articular steroids in the previous 4 weeks or during the procedure.
- Technique used (type and size of biopsy retrieval device).
- Machine/probe for US-guided biopsies, arthroscopic equipment.
- Adverse events.
- Operator's experience and training.

Among the 26 manuscripts retrieved by the supporting SLR reporting on biopsy procedures, details of the procedure were very heterogeneously reported. For instance, exclusion criteria

Table 1 Overarching principles and points to consider for minimal reporting requirements in synovial tissue clinical practice and research in rheumatology, with levels of evidence (LoE) and levels of agreement (LoA)

Overarching principles	LoA mean (SD); % of votes ≥8/10
1. Synovial biopsies (single and sequential), performed in aseptic conditions, are safe, well-tolerated and can be performed for both clinical and research purposes.	**9.77 (0.53), 100%**
2. In both clinical and research settings, synovial biopsies should be guided by imaging techniques. Arthroscopy and ultrasound are the preferred techniques to guide synovial biopsies.	**9.71 (0.56), 100%**
3. Ultrasound or arthroscopy can be used to guide the synovial biopsy without affecting the tolerability of the procedure or the minimal required tissue for meaningful analysis.	**9.14 (0.96), 83.6%**
Points to consider	
1. The details of the biopsy procedure should be reported in every study. This should include non-exhaustively: ► Exclusion criteria for biopsy ► Target joint(s) and recess ► Intra-articular steroids in the previous 4 weeks or during the procedure ► Technique used (type and size of biopsy retrieval device) ► Machine/probe for ultrasound guided biopsies and arthroscopic equipment ► Adverse events ► Operator's experience and training (noting that no minimal training requirements are yet defined). (LoE 5)	**9.38 (0.80), 100%**
2. Overarching clinical study design, including aspects related to participant disease characteristics and treatments, must be defined in order to evaluate the generalisability and validity of the outcome. (LoE 5)	**9.81 (0.51), 100%**
3. Conventional patient disease activity measures, disease stage and treatment should be described in order to evaluate the generalisability and validity of the outcome. (LoE 5)	**9.45 (1.19), 95%**
4. Clinical and contemporary imaging characteristics of the biopsied joints should be described in order to evaluate the generalisability and validity of the outcome. (LoE 4)	**8.95 (1.28), 90.5%**
5. Tissue handling and processing methods must be described in order to ensure reproducibility, including numbers and size of fragments allocated randomly to each analytic. (LoE 4)	**9.10 (1.64), 90.5%**
6. Method and results of tissue quality assessment should be reported, including the percentage of graded tissue. (LoE 5)	**9.33 (1.06), 90.5%**
1. When histological or immunohistological analysis is performed, the scoring or analysis system should be defined including: ► Representative images ► Reference to original publication for validated scoring systems only ► Digital analysis software used, including version numbers of platforms ► Immunohistological staining protocol, including antibody sources and clones ► Area assessed and sampling strategy ► Numbers of observers and intra- and inter-observer variability. (LoE 5)	**9.48 (0.75), 100%**
8. Methods of extraction and quantification should be defined, and purity, quantity and quality of DNA/RNA should be reported (LoE 5).	**9.67 (0.58), 100%**
1. In case of single cell analysis, methods used and quality outcomes should be detailed, including: ► Methods of tissue or cell preservation ► Methods of tissue dissociation ► Percentage of viable cells ► Percentage of mitochondrial gene expression seen in the sequenced cells and the threshold chosen for analysis ► If sorting is used, the strategy used and purity of sorted cells. (LoE 4)	**9.71 (0.56), 100%**

LoA, Level of agreement; LoE, Level of Evidence; SD, Standard deviation.

for biopsy and intra-articular treatments in the previous 4 weeks or during the procedure (intra-articular steroids) were reported in less than 10% of the manuscripts, while the target joint(s) and recess, technique used, and equipment were more frequently reported (>75% of included studies). Adverse events were reported in only 20% of the manuscripts and none reported operator's experience.

Based on these results, the task force developed a non-exhaustive list of elements pertaining to the procedure itself that should be mentioned in every study involving SB. Although minimal training requirements for SB are not yet defined, operator's experience and training should be reported in every study. Of note, a standardised training model for US-guided, minimally invasive SB procedures in large and small joints constitutes another EULAR task force initiative.[25] In addition, depending on the study design, a description of patient tolerability of the procedure is desirable.

PtC-2: Overarching clinical study design, including aspects related to participant disease characteristics and treatments, must be defined in order to evaluate the generalisability and validity of the outcome

This point refers to the study design, defined a priori when the study framework is elaborated by authors. It is known that treatments and disease phenotype can affect ST outcomes in terms of histopathology and transcriptomics especially in inflammatory arthritis.[1 4 26–32] Therefore, the task force recommends that aspects pertaining to study design, including participants, disease characteristics (including fulfilment of classification criteria) and treatments, should always be reported in manuscripts.

PtC-3: Conventional patient disease activity measures, disease stage and treatment should be described in order to evaluate the generalisability and validity of the outcome

This point refers specifically to outcome measures and characteristics of the patients included in the study that should always be reported. In the SLR, 100% of studies reported patient

demographics and diagnosis, but only 62% reported clinical data, such as disease activity and current therapy. More specifically, disease activity measures should be outlined, including Disease Activity Score 28 C reactive protein or erythrocyte sedimentation rate (ESR), Clinical Disease Activity Index or Simple Disease Activity Index for rheumatoid arthritis or other measures depending on the rheumatic disease under evaluation. Disease duration and conventional synthetic, targeted synthetic or biologic disease-modifying antirheumatic drugs should be reported.

PtC-4: Clinical and contemporary imaging characteristics of the biopsied joints should be described in order to evaluate the generalisability and validity of the outcome

First, the task force felt that clinical assessment of the biopsied joint including swelling should be reported. In the context of US guided SB, the US synovitis grade of the target joint is typically assessed. Surprisingly, these data were described in only 36% of the manuscripts describing US-guided SB in the SLR. The task force emphasised that the US grade of the synovitis in B mode and Doppler can affect tissue quality and outcomes,[7 15 33] and therefore, recommends that contemporary imaging characteristics of the biopsied joint are described. In addition, when available, radiographic aspects should be described and when the erosive status of the biopsied joint is known, this information should be provided.

PtC-5: Tissue handling and processing methods must be described in order to ensure reproducibility, including numbers and size of fragments allocated to each analytic.

Our SLR retrieved numerous studies looking into intra-articular variability of tissue outcomes and sampling error.[15 34–39] More specifically, immune cell infiltrate, immunohistochemistry, cytokine mRNA and T cell repertoire displayed little or no difference when retrieved in different parts of large joints.[36–38]

Of interest, studies assessing sampling error showed that a minimum of 4 tissue fragments provided a reliable sample with 10% sampling error for small joint histopathological analysis,[15] while 4–7 tissue fragments were required to detect a twofold change with a 25% sampling error in PCR in large joints[34] and the percentage mean difference for the staining of immunohistochemical cellular markers decreases below ±10% when a minimum of eight samples are considered in the evaluation.[39] In addition, a minimum of 6 fragments and the assessment of an area of tissue of minimum 2.5 mm^2 were deemed necessary to ensure representativity of histological analysis.[40 41] In this context, the task force recommends that authors report data pertaining to tissue handling and processing, including numbers and size of fragments allocated to each analytic.

PtC-6: Method and results of tissue quality assessment should be reported, including the percentage of graded tissue

Among the 26 studies included in the SLR, only 17 reported having controlled the tissue quality during their study (65%). The task force felt it was absolutely necessary for a quality control to be performed and reported by authors in manuscripts in order to ensure reliability and reproducibility of the results. When histopathological analysis is performed, the percentage of tissue presenting with a typical ST structure and an intact lining layer or positive CD68 staining should be reported.[7 15 16]

PtC-7: When histological or immunohistological analysis is performed, the scoring or analysis system should be defined including

- Representative images.
- Reference to original publication for validated scoring systems only.
- Digital analysis software, including version numbers of platforms used.
- Immunohistological staining protocol, including antibody sources and clones.
- Area assessed and sampling strategy.
- Number of observers and intraobserver and interobserver variability.

Since numerous studies assess histological aspects of the tissue, it was felt important by the task force to formulate a PtC related to histological scoring. More specifically, several aspects were deemed mandatory by the task force, such as describing staining protocols and antibodies sources and clones, providing representative images illustrating the findings and describing area assessed and sampling strategy. In the manuscripts included in the SLR, scoring systems were rarely described and chains of references to previous publications, but not the original scoring system, were often observed. Subsequently, the task force recommended that only the original publication describing the scoring system should be cited. The interobserver and intraobserver variability for histological analysis was similarly rarely described (n=4/26 publications, 15%) and should always be reported for studies using scoring by observers.

PtC-8: Methods of extraction and quantification should be defined and purity, quantity and quality of DNA/RNA should be reported

Although no study specifically assessed the difference in outcomes arising from tissues yielding DNA or RNA of high versus poor quality (measured by the RNA integrity number for RNA), it was considered important by the task force members that such information should be reported in every manuscript. It is indeed anticipated that poor quality RNA, if used for RNA sequencing, will provide unreliable results. In addition, it has been noted in the SLR that such information was very rarely reported in the analysed publications (two out eight publications looking at molecular aspects of ST (25%)).

PtC-9: In case of single cell analysis, methods used and quality outcomes should be detailed, including

- Methods of tissue or cell preservation.
- Methods of tissue dissociation.
- Percentage of viable cells recovered or analysed.
- Percentage of mitochondrial gene expression seen in the sequenced cells and the threshold chosen for analysis.
- If sorting is used, the strategy used and purity of sorted cells.

The recent development of single cell technologies has also raised methodological challenges. Of interest, the methods of tissue conservation or dissociation can influence the tissue outcome. In a study from Donlin *et al*, mechanical versus mechanical and enzymatic ST dissociation methods have been compared, showing that the latter retrieved a higher total cell count per gram of tissue, a higher viable cell count and a more representative number of cell subpopulations. In addition, they compared methods of tissue preservation, showing that cryopreserved samples retrieved similar numbers of viable cells and a similar variety of cell subpopulations to fresh samples.[35] Therefore, the task force recommends that these elements are reported in every publication, in addition to other aspects related to

quality, such as the percentage of viable cells and percentage of mitochondrial gene expression seen in the sequenced cells alongside parameters related to cell sorting methods, including flow cytometric staining protocols.

DISCUSSION

These are the first EULAR-endorsed PtC on ST research in rheumatology with the aim of them serving as a reference and checklist for clinicians and scientists involved in publishing, reviewing and reading manuscripts reporting ST research. They have been proposed by a multidisciplinary team of international experts in the field involving rheumatologists, translational researchers, methodologists and pathologists.

Our SLR retrieved several manuscripts, which were analysed from different perspectives. With respect to the OPs, we emphasised that the body of evidence on SB tolerability and safety is very reassuring for both single and sequential biopsies. In addition, the task force stated that SB should no longer be performed without imaging guidance, more specifically blind needle biopsies are no longer recommended. There is no preferred guiding technique between US or arthroscopy, since both allow retrieval of a sufficient quantity of tissue for meaningful analysis and are well tolerated.[11 24] Of interest, we assessed how comprehensively data relevant to study design, patients' characteristics, biopsy procedures, tissue handling, quality control and tissue outcomes were reported in the publications. In this regard, these PtC focus on specific areas requiring attention from authors when reporting their study results in order to ensure internal and external validity of the studies and generalisability. These PtCs are also proposed in an editor friendly document appearing as a checklist and provided in online supplemental material 1.

While conducting this work, we realised that the paucity of literature on clinical applications of tissue analysis did not allow the formulation of PtC dedicated to the clinical aspects. Indeed, although recent publications propose encouraging data for the use of SB for diagnosis, outcome prediction or disease management in clinical practice, the task force felt that these aspects should be included in the research agenda.[2 4 42–44] One major limitation encountered in the development of these PtC was the scarcity of literature appraising the practical aspects of tissue retrieval, handling and analysis. Due to the paucity of evidence comparing methods or outcomes based on tissue handling, quality or analysis, most of these PtC rely on expert opinion. In this respect, it is noteworthy that the members of this task force acted as representatives of the most prominent centres working in the field of translational research in ST, including EULAR centres of excellence. Based on the SLR results and the inputs and discussion arising from the second task force meeting, other relevant items were incorporated in the research agenda (box 1).

In conclusion, these EULAR PtCs provide relevant guidance on minimal reporting requirements in ST research in Rheumatology. These first EULAR PtCs are intended to be disseminated and used by the broad research community, adding to previous initiatives from OMERACT and ESSG in order to allow the field of ST research to evolve in a robust and transparent manner in the future.

Box 1 Research agenda

Research agenda

⇒ Minimal training requirement for ultrasound guided SB for physicians and allied health professionals.
⇒ Impact of training on patient tolerability; tissue yield/quality/outcomes.
⇒ Influence of tissue handling and processing (fixation, standardised operating procedures for freezing in optimal cutting temperature (OCT), fixation, and freezing (and time to freezing) for subsequent live tissue/cell analysis) on tissue quality/outcome.
⇒ Clinical practice: supportive data for diagnosis or prognosis or disease management.
⇒ Risk of Bias tools for translational research.
⇒ Tissues considered as best 'non-inflammatory' controls (eg, healthy subjects, meniscectomy surgery, traumatic arthritis, cadavers).

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Correction notice This article has been corrected since it published Online First. The affiliation and name details for Juan D Cañete have been updated.

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2022 American College of Rheumatology/EULAR classification criteria for giant cell arteritis

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ABSTRACT

Objective To develop and validate updated classification criteria for giant cell arteritis (GCA).

Methods Patients with vasculitis or comparator diseases were recruited into an international cohort. The study proceeded in six phases: (1) identification of candidate items, (2) prospective collection of candidate items present at the time of diagnosis, (3) expert panel review of cases, (4) data-driven reduction of candidate items, (5) derivation of a points-based risk classification score in a development data set and (6) validation in an independent data set.

Results The development data set consisted of 518 cases of GCA and 536 comparators. The validation data set consisted of 238 cases of GCA and 213 comparators. Age ≥50 years at diagnosis was an absolute requirement for classification. The final criteria items and weights were as follows: positive temporal artery biopsy or temporal artery halo sign on ultrasound (+5); erythrocyte sedimentation rate ≥50 mm/hour or C reactive protein ≥10 mg/L (+3); sudden visual loss (+3); morning stiffness in shoulders or neck, jaw or tongue claudication, new temporal headache, scalp tenderness, temporal artery abnormality on vascular examination, bilateral axillary involvement on imaging and fluorodeoxyglucose–positron emission tomography activity throughout the aorta (+2 each). A patient could be classified as having GCA with a cumulative score of ≥6 points. When these criteria were tested in the validation data set, the model area under the curve was 0.91 (95% CI 0.88 to 0.94) with a sensitivity of 87.0% (95% CI 82.0% to 91.0%) and specificity of 94.8% (95% CI 91.0% to 97.4%).

Conclusion The 2022 American College of Rheumatology/EULAR GCA classification criteria are now validated for use in clinical research.

INTRODUCTION

Giant cell arteritis (GCA), formerly known as temporal arteritis, is the most common form of systemic vasculitis in patients aged ≥50 years.[1] GCA is defined by granulomatous arteritis that affects large-sized and medium-sized blood vessels with a predisposition to affect the cranial arteries.[2] Common presenting features of the disease include headache, constitutional symptoms, jaw claudication, scalp tenderness, visual disturbances and elevated inflammatory markers.[3]

In 1990, the American College of Rheumatology (ACR) endorsed classification criteria for GCA.[4] These criteria were established before the widespread use of non-invasive and advanced vascular imaging modalities, which have become increasingly incorporated in the clinical assessment of GCA. Vascular ultrasound can be used to diagnose GCA, and depending on the clinical setting, a non-compressible 'halo' sign of a temporal±axillary artery may replace the need for temporal artery biopsy (TAB).[5–8] Moreover, vascular imaging has demonstrated that arterial involvement in GCA is not exclusively confined to the cranial arteries[9 10] and can commonly affect the aorta and primary branches in a pattern similar to Takayasu arteritis (TAK).[11 12]

The limitations of the ACR 1990 criteria for GCA have become more apparent in the conduct of recent clinical trials and other research studies, in which investigators typically modify the 1990 ACR criteria to reflect modern practice.[6 13 14] Notably, the 1990 ACR criteria focus mostly on cranial features of GCA and do not perform well in classifying patients with disease predominantly affecting the larger arteries. The 1990 ACR criteria were derived by using comparator populations, which included many patients with small-vessel vasculitis, a form of vasculitis that is not typically difficult to differentiate from GCA. In addition, the 1990 ACR criteria for GCA followed the 'number of criteria' rule, which considered each criterion to have equal weight as a classifier for the disease. Since then, methodologic advances in classification criteria have allowed movement towards weighted criteria with threshold scores that improve performance characteristics.[15 16]

This article outlines the development and validation of the revised ACR/EULAR-endorsed classification criteria for GCA.

METHODS

An international Steering Committee comprising clinician investigators with expertise in vasculitis, statisticians and data managers was assembled to oversee the overall development of classification criteria for primary vasculitis.[17] A detailed and complete description of the methods involved in the development and validation of the classification criteria for GCA is located in online supplemental appendix 1. Briefly, the Steering Committee implemented a six-stage plan using data-driven and consensus methodology to develop the following criteria.

Stage 1: generation of candidate classification items for the systemic vasculitides

Candidate classification items were generated by expert opinion and reviewed by a group of vasculitis experts across a range of specialties using nominal group technique.

Stage 2: Diagnostic and Classification Criteria for Vasculitis prospective observational study

A prospective, international, multisite observational study was conducted (see online supplemental file 3 for study investigators and sites). Consecutive patients representing the full spectrum of vasculitides were recruited from academic and community practices. Patients were included if they were 18 years or older and had a diagnosis of vasculitis or a condition that mimics vasculitis (eg, infection, malignancy, atherosclerosis). Patients with GCA could only be enrolled within 2 years of diagnosis. Only data present at diagnosis were used to develop the classification criteria.

Stage 3: expert review to derive a gold standard-defined set of cases of large-vessel vasculitis

Experts in vasculitis from a wide range of geographic locations and specialties (see online supplemental file 3) reviewed all submitted cases of vasculitis and a random selection of vasculitis mimics. Each reviewer was asked to review ~50 submitted cases to confirm the diagnosis and to specify the degree of certainty of their diagnosis as follows: very certain, moderately certain, uncertain or very uncertain. Only cases agreed on with at least moderate certainty by two reviewers were retained for further analysis.

Stage 4: refinement of candidate items specifically for large-vessel vasculitis

The Steering Committee conducted a data-driven process to reduce the number of candidate items of relevance to cases and comparators for large-vessel vasculitis (LVV). Density plots were assessed to study age distribution at diagnosis and symptom onset for GCA and TAK. Absolute age requirements vs incorporation of age as a candidate criteria item was considered. Items related to the vascular physical examination, vascular imaging, arterial biopsy and laboratory values were combined or eliminated based on consensus review. Items were selected for exclusion if they had a prevalence of <5% within the data set, and/or they were not clinically relevant for classification criteria (eg, related to infection, malignancy or demography). Low-frequency items of clinical importance could be combined, when appropriate. Patterns of vascular imaging findings detected by vascular ultrasound, angiography or positron emission tomography (PET) were defined by K-means clustering.[18]

Stage 5: derivation of the final classification criteria for GCA

The Diagnostic and Classification Criteria for Vasculitis (DCVAS) data set was split into development (70%) and validation (30%) sets. Comparisons were performed between cases of GCA and a randomly selected comparator group in the following proportions: TAK, 33.5%; other vasculitides that mimic GCA and TAK (isolated aortitis, primary central nervous system vasculitis, polyarteritis nodosa, Behçet's disease and other LVV), 33.4%; and other diagnoses that mimic LVV (eg, atherosclerosis, unspecific headache), 33.1%. Least absolute shrinkage and selection operator (lasso) logistic regression was used to identify predictors from the data set and create a parsimonious model including only the most important predictors.[19] The final items in the model were formulated into a clinical risk-scoring tool, with each factor assigned a weight based on its respective regression coefficient. A threshold that best balanced sensitivity and specificity was identified for classification.

Stage 6: validation of the final classification criteria for GCA

Performance of the new criteria was validated in an independent set of cases and comparators. Performance of the final classification criteria was examined in specific subsets of patients with GCA using data from the combined development and validation sets to maximise sample sizes for the subgroups. Patients were studied according to different disease subtypes (biopsy-proven GCA and large-vessel GCA) and regions of the world (North America, Europe) where clinical strategies to assess GCA are known to differ.[20] Biopsy-proven GCA was defined as definite vasculitis on TAB reported by the submitting physician, and large-vessel GCA was defined as vasculitic involvement of the aorta or its branch arteries on either angiography (computed tomography, magnetic resonance imaging, or catheter-based angiography), ultrasound or PET, without vasculitis on TAB. Comparison was made between the measurement properties of the new classification criteria for GCA and the 1990 ACR classification criteria in the validation data set. Performance characteristics of the criteria were also tested in patients with TAK and compared with those with GCA diagnosed between the ages of 50 and 60 years.

RESULTS

Generation of candidate classification items for the systemic vasculitides

The Steering Committee identified >1000 candidate items for the DCVAS Case Report Form (see online supplemental appendix 2).

DCVAS prospective observational study

Between January 2011 and December 2017, the DCVAS study recruited 6991 participants from 136 sites in 32 countries. Information on the DCVAS sites, investigators and study participants is listed in online supplemental appendices 3, 4 and 5.

Expert review methodology to derive a gold standard-defined final set of cases of LVV

The LVV expert panel review process included 56 experts who reviewed vignettes derived from the Case Report Forms for 2131 cases submitted with a diagnosis of LVV (1608 (75.5% of Case Report Forms)), another type of vasculitis (118 (5.5% of Case Report Forms)) or a mimic of vasculitis (405 (19.0% of Case Report Forms)). Characteristics and the list of expert reviewers are shown in online supplemental appendices 6 and 7. A sample vignette and the LVV expert panel review flow chart are shown in online supplemental appendices 8 and 9. A total of 1695 cases (80%) passed the main LVV process. An additional 373 cases of LVV and comparators, confirmed during a previous review process to derive the classification criteria for antineutrophil cytoplasmic antibody-associated vasculitis, were also included. In total, after both review processes, 2068 cases were available for the stages 4 and 5 analyses.

The submitting physician diagnosis of GCA was confirmed in 913 of 1137 cases (80.3%) after both expert panel reviews. The reasons for exclusion were diagnosis of GCA categorised as 'uncertain' or 'very uncertain' during panel review (n=187) or change in diagnosis during panel review to another type of vasculitis (isolated aortitis, TAK, other vasculitides) (n=11) or

to a comparator disease (n=26). An additional 29 patients who were not initially diagnosed as having GCA by the submitting physician were diagnosed as having GCA after panel review and DCVAS Steering Committee member adjudication. In total, 942 cases of confirmed GCA were available for analysis. To balance the number of cases of GCA with the number of available comparators, 756 cases of GCA were randomly selected for subsequent analysis.

Refinement of candidate items specifically for GCA

Only 7 of 942 patients with GCA (<1%) were diagnosed at age <50 years (see online supplemental appendix 10 for the distribution of 'age at diagnosis' in patients with LVV, and the similar distribution of 'age at symptom onset,'). Therefore, an age of ≥50 years at diagnosis was considered an absolute requirement to classify GCA. Cluster analyses of vascular imaging data identified bilateral axillary involvement and diffuse fluorodeoxyglucose uptake throughout the aorta on PET as specific imaging patterns for GCA (see online supplemental appendices 11 and 12). These imaging patterns were tested as potential classifiers.

Following a data-driven and expert consensus process, 72 items of the DCVAS Case Report Form were retained for regression analysis, including 32 demographic and clinical items, 14 laboratory items (including values of C reactive protein (CRP) level and erythrocyte sedimentation rate (ESR), each divided into 5 categories), 14 imaging items (13 composite), 11 vascular examination items (5 composite and upper extremity blood pressure divided into 6 categories) and 1 biopsy item (online supplemental appendix 13).

Derivation of the final classification criteria for GCA

A total of 1505 patients were selected for analysis (756 GCA and 749 comparators), of which 1054 (70%) were in the development data set (518 GCA and 536 comparators) and 451 (30%) in the validation data set (238 GCA and 213 comparators). Table 1 describes the demographic and clinical features of the patients with GCA and the comparators. The patients with GCA were recruited from Europe (n=796), North America (n=112), Oceania (n=18) and Asia (n=16). Clinical diagnoses assigned to patients in the comparator group are detailed in online supplemental appendix 14.

Lasso regression of the previously selected 72 items yielded 27 independent predictor variables for GCA (online supplemental appendix 15A). Each predictor variable was then reviewed for inclusion by the DCVAS Steering Committee, based on their ORs and specificity to GCA, to ensure face validity. The variables 'definitive vasculitis on TAB' and 'halo sign on temporal artery ultrasound' were found to dominate the model as quite strong predictors of GCA (see online supplemental appendix 16A for cluster plots showing almost a perfect overlap between the diagnosis of GCA and positive TAB or halo sign on temporal artery ultrasound). Therefore, for the remaining variables to have discriminatory value, both of these items were removed from the model, combined into one composite item 'vasculitis on TAB or halo sign on temporal artery ultrasound' and given a risk score of one point below the final threshold set to classify GCA to maintain face validity. The variables 'jaw claudication' and 'tongue claudication' were combined into one item, as were the variables 'maximum ESR (>50 mm/hour)' and 'maximum CRP (>10 mg/L).' Although the variable 'new persistent headache, occipital or cervical' showed important statistical significance, it decreased the overall specificity of the model when testing their final performance characteristics (patients vs comparators) and was, therefore, also removed. Weighting of the individual criterion included in the model was based on logistic regression fitted to the remaining nine selected predictors (online supplemental appendix 17A).

Table 1 Demographic and disease features of the patients with giant cell arteritis and the comparators*

	GCA (n=756)	Comparators (n=749)†	P value
Age, mean±SD years	72.2±8.5	44.6±18.0	<0.001
Female sex	511 (67.6)	447 (59.7)	0.001
Clinical features			
Morning stiffness, neck/torso	88 (11.6)	15 (2.0)	<0.001
Morning stiffness, shoulders/arms	174 (23.0)	23 (3.1)	<0.001
Sudden visual loss	102 (13.5)	29 (3.9)	<0.001
Jaw claudication	356 (47.1)	19 (2.5)	<0.001
Tongue claudication	21 (2.8)	1 (0.1)	<0.001
New persistent temporal headache	475 (62.8)	90 (12.0)	<0.001
Scalp tenderness	260 (34.4)	25 (3.3)	<0.001
Temporal artery abnormality on vascular examination‡	354 (46.8)	35 (4.7)	<0.001
Investigations			
Maximum ESR ≥50 mm/hour	558 (73.8)	291 (38.9)	<0.001
Maximum CRP ≥10 mg/L	683 (90.3)	445 (59.4)	<0.001
Definitive vasculitis on temporal artery biopsy	335 (44.3)	1 (0.1)	<0.001
Halo sign on temporal artery ultrasound	211 (27.9)	1 (0.1)	<0.001
Bilateral axillary involvement on imaging§	57 (7.5)	12 (1.6)	<0.001
FDG-PET activity throughout aorta¶	52 (6.9)	9 (1.2)	<0.001

*Except where indicated otherwise, values are the number (%).
†Diagnoses of comparators for the classification criteria for giant cell arteritis (GCA) included Takayasu arteritis (n=251), Behçet's disease (n=133), polyarteritis nodosa (n=74), isolated aortitis (n=16), primary central nervous system vasculitis (n=16), large-vessel vasculitis (LVV) that could not be subtyped (n=9), other diseases that mimic LVV (n=250).
‡Absent or diminished pulse, tenderness or hard 'cord-like' appearance.
§Defined as damage (ie, stenosis, occlusion or aneurysm) on angiography (CT, MR or catheter based) or ultrasound, halo sign on ultrasound or abnormal FDG uptake on PET.
¶Descending thoracic and abdominal aorta.
CRP, C reactive protein; ESR, erythrocyte sedimentation rate; FDG-PET, ^{18}F-fluorodeoxyglucose–positron emission tomography; GCA, giant cell arteritis.

Validation of the final classification criteria for GCA

Using a cut-off of ≥6 in total risk score in the validation data set (see online supplemental appendix 18A for different cut-off points), the sensitivity was 87.0% (95% CI 82.0% to 91.0%) and specificity was 94.8% (95% CI 91.0% to 97.4%). The area under the curve for the model was 0.91 (95% CI 0.88 to 0.94) (online supplemental appendix 19A). The final 2022 ACR/EULAR classification criteria for GCA are presented in figure 1 (for the slide presentation versions, see online supplemental figure 1).

The performance characteristics of the criteria in different subsets of patients with GCA are shown in table 2 and online supplemental appendix 20A. Biopsy-proven GCA showed a sensitivity of 100% (95% CI 99.0% to 100.0%) and a specificity of 94.9% (95% CI 93.1% to 96.4%) and large-vessel GCA showed a sensitivity of 55.7% (95% CI 46.5% to 64.6%) and a specificity of 94.9% (95% CI 93.1% to 96.4%). Sensitivity of the

2022 AMERICAN COLLEGE OF RHEUMATOLOGY / EULAR

CLASSIFICATION CRITERIA FOR **GIANT CELL ARTERITIS**

CONSIDERATIONS WHEN APPLYING THESE CRITERIA

- These classification criteria should be applied to classify the patient as having giant cell arteritis when a diagnosis of medium-vessel or large-vessel vasculitis has been made
- Alternate diagnoses mimicking vasculitis should be excluded prior to applying the criteria

ABSOLUTE REQUIREMENT

Age ≥ 50 years at time of diagnosis	

ADDITIONAL CLINICAL CRITERIA

Morning stiffness in shoulders/neck	+2
Sudden visual loss	+3
Jaw or tongue claudication	+2
New temporal headache	+2
Scalp tenderness	+2
Abnormal examination of the temporal artery[1]	+2

LABORATORY, IMAGING, AND BIOPSY CRITERIA

Maximum ESR ≥ 50 mm/hour or maximum CRP ≥ 10 mg/liter[2]	+2
Positive temporal artery biopsy or halo sign on temporal artery ultrasound[3]	+5
Bilateral axillary involvement[4]	+2
FDG-PET activity throughout aorta[5]	+2

Sum the scores for 10 items, if present. A score of ≥ 6 points is needed for the classification of GIANT CELL ARTERITIS.

1. Examination of the temporal artery showing absent or diminished pulse, tenderness, or hard 'cord-like' appearance.
2. Maximum erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) values prior to initiation of treatment for vasculitis.
3. Presence of either definitive vasculitis on temporal artery biopsy or halo sign on temporal artery ultrasound. There are no specific histopathologic criteria to define definitive vasculitis on temporal artery biopsy. Presence of giant cells, mononuclear leukocyte infiltration, and fragmentation of the internal elastic lamina were independently associated with histopathologic interpretation of definite vasculitis in the DCVAS cohort[24]. Halo sign is defined by the presence of an homogenous, hypoechoic wall thickening on ultrasound [25].
4. Bilateral axillary involvement is defined as luminal damage (stenosis, occlusion, or aneurysm) on angiography (computed tomography, magnetic resonance, or catheter-based) or ultrasound, halo sign on ultrasound, or fluorodeoxyglucose uptake on positron emission tomography.
5. Abnormal fluorodeoxyglucose (FDG) uptake in the arterial wall (e.g., greater than liver uptake by visual inspection) throughout the descending thoracic and abdominal aorta on positron emission tomography (PET).

Figure 1 The final 2022 American College of Rheumatology/EULAR Classification Criteria for Giant Cell Arteritis.

Table 2 Performance characteristics of the 2022 ACR/EULAR classification criteria for giant cell arteritis*

Patient subset	Total no patients (no GCA patients)	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)
Development data set	1054 (518)	84.8 (81.4 to 87.7)	95.0 (92.8 to 96.7)	0.90 (0.88 to 0.92)
Validation data set	451 (238)	87.0 (82.0 to 91.0)	94.8 (91.0 to 97.4)	0.91 (0.88 to 0.94)
Biopsy-proven GCA†	1104 (355)	100.0 (99.0 to 100.0)	94.9 (93.1 to 96.4)	0.97 (0.97 to 0.98)
Large-vessel GCA‡	873 (124)	55.7 (46.5 to 64.6)	94.9 (93.1 to 96.4)	0.75 (0.71 to 0.80)

*Performance characteristics were tested in the subsets using the combined development and validation data sets to maximise sample size.
†Definite vasculitis on temporal artery biopsy (TAB).
‡Involvement of the aorta or its branch arteries on imaging, without vasculitis on TAB.
ACR, American College of Rheumatology; AUC, area under the curve; GCA, giant cell arteritis.

criteria in North America was 77.8% (95% CI 67.8% to 85.9%) and in Europe was 87.2% (95% CI 84.4% to 89.7%). Specificity in North America was 95.6% (95% CI 90.6% to 98.4%) and in Europe was 88.8% (95% CI 84.9% to 92.0%).

When the 1990 ACR classification criteria for GCA were applied to the DCVAS validation data set, the criteria performed poorly due to low sensitivity (80.3% (95% CI 74.6% to 85.1%)) but retained good specificity (92.5% (95% CI 88.1% to 95.7%)). In particular, the 1990 ACR criteria had poor sensitivity for patients with large-vessel GCA (37.1% (95% CI 28.6% to 46.2%)).

Age restrictions are absolute requirements for the 2022 ACR/EULAR classification criteria for GCA (≥50 years at diagnosis) and TAK (≤60 years at diagnosis). However, of the 70 patients with GCA diagnosed between the ages of 50 and 60 years, 44 (62.9%) met the new GCA classification criteria, 9 (12.9%) met the new TAK classification criteria, and only 2 (2.9%) met both the new GCA and TAK classification criteria (online supplemental appendix 21).

DISCUSSION

Presented here are the final 2022 ACR/EULAR GCA classification criteria. A six-stage approach was used, underpinned by data from the multinational, prospective DCVAS study and informed by expert review and consensus at each stage. The comparator group for developing and validating the criteria were other vasculitides and conditions that mimic GCA, where discrimination from GCA is difficult but important. In the validation set, the new criteria had a sensitivity of 87.0% (95% CI 82.0% to 91.0%) and a specificity of 94.8% (95% CI 91.0% to 97.4%). These are the official final values that should be quoted when referring to the criteria. The sensitivity and specificity values calculated in the development set were very similar, providing reassurance that the statistical methods avoided overfitting of models. The new criteria incorporate modern imaging techniques and have excellent specificity and sensitivity within a large, international cohort of patients with GCA. The criteria were designed to have face and content validity for use in clinical trials and other research studies.

These criteria are validated and intended for the purpose of *classification* of vasculitis and are not appropriate for use to establish a *diagnosis* of vasculitis. The aim of the classification criteria is to differentiate cases of GCA from similar types of vasculitis in research settings.[21] *Therefore, the criteria should only be applied when a diagnosis of LVV or medium-vessel vasculitis has been made and all potential "vasculitis mimics" have been excluded.* The exclusion of mimics is a key aspect of many classification criteria including those for Sjögren's syndrome[22] and rheumatoid arthritis.[16] The 1990 ACR classification criteria for vasculitis perform poorly when used for diagnosis (ie, when used to differentiate between cases of vasculitis vs mimics without vasculitis),[23] and it is expected that the 2022 criteria would also perform poorly if used inappropriately as diagnostic criteria in people for whom alternative diagnoses, such as infection or other non-vasculitis inflammatory diseases, are still being considered.

The 2022 ACR/EULAR GCA classification criteria are the result of an incredibly large worldwide effort, in which an extensive set of data was collected from >1000 patients with the submitted diagnosis of GCA. These criteria reflect current clinical practice, integrating different investigative methods (eg, TAB, ultrasound, angiography, PET) from various countries and medical specialties. Real cases of GCA and comparators were reviewed by a wide range of experts in vasculitis to establish an unbiased diagnostic reference to derive the criteria. Advanced statistical methods including lasso logistic regression and cluster analyses were applied, which facilitated testing for different covariates of interest, namely specific patterns of vasculitic involvement in imaging. Modern classification techniques with weighted criterion with threshold scores were used, allowing for more discriminatory items to factor more heavily in disease classification.

When compared with the original 1990 ACR classification criteria for GCA, the 2022 ACR/EULAR GCA classification criteria demonstrated greater sensitivity while maintaining similar specificity to the 1990 criteria. In particular, the new criteria were able to correctly classify more patients with the large-vessel GCA subtype, in whom the sensitivity of the 1990 ACR criteria was only 37.1%. Unlike the 1990 ACR criteria, an age of ≥50 years at diagnosis is a mandatory requirement to classify GCA in the 2022 ACR/EULAR criteria. This age threshold included>99% of patients with the reference diagnosis of GCA. The new criteria maintain good discriminative ability for patients diagnosed between the ages of 50 and 60 years, the interval where the absolute age requirements for the 2022 ACR/EULAR criteria for GCA and for TAK can overlap.

A potential limitation of these criteria was the non-standardised acquisition of clinical and imaging data among patients with LVV and comparators (eg, not all patients underwent vascular examination of the temporal arteries, PET was not available in many centres treating patients with LVV, and TAB and/or ultrasound was not performed in all patients with suspected GCA, etc). However, this reflects existing differences in clinical practice, and the 11 items included in the criteria allow for a feasible evaluation of patients in any clinical setting. These criteria also provide flexibility for classifying a patient, regardless of the diagnostic assessment strategy employed by physicians. Definite vasculitis on TAB was defined by the submitting physician and did not undergo central review; ~20% of cases did not have specific histopathologic findings but were reported as 'definitive vasculitis on TAB' alone. Most patients were recruited from Europe and North America, with fewer patients from Asia and Oceania. The performance characteristics of the criteria should be further tested in other populations that were underrepresented in the DCVAS cohort and may have different clinical presentations of GCA.

The 2022 ACR/EULAR classification criteria for GCA are the product of a rigorous methodologic process that utilised an extensive data set generated by the work of a remarkable international group of collaborators. These criteria have been endorsed by the ACR and EULAR and are now ready for use in clinical research.

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2022 American College of Rheumatology/EULAR classification criteria for Takayasu arteritis

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ABSTRACT

Objective To develop and validate new classification criteria for Takayasu arteritis (TAK).

Methods Patients with vasculitis or comparator diseases were recruited into an international cohort. The study proceeded in six phases: (1) identification of candidate criteria items, (2) collection of candidate items present at diagnosis, (3) expert panel review of cases, (4) data-driven reduction of candidate items, (5) derivation of a points-based classification score in a development data set and (6) validation in an independent data set.

Results The development data set consisted of 316 cases of TAK and 323 comparators. The validation data set consisted of an additional 146 cases of TAK and 127 comparators. Age ≤60 years at diagnosis and imaging evidence of large-vessel vasculitis were absolute requirements to classify a patient as having TAK. The final criteria items and weights were as follows: female sex (+1), angina (+2), limb claudication (+2), arterial bruit (+2), reduced upper extremity pulse (+2), reduced pulse or tenderness of a carotid artery (+2), blood pressure difference between arms of ≥20 mm Hg (+1), number of affected arterial territories (+1 to +3), paired artery involvement (+1) and abdominal aorta plus renal or mesenteric involvement (+3). A patient could be classified as having TAK with a cumulative score of ≥5 points. When these criteria were tested in the validation data set, the model area under the curve was 0.97 (95% CI 0.94 to 0.99) with a sensitivity of 93.8% (95% CI 88.6% to 97.1%) and specificity of 99.2% (95% CI 96.7% to 100.0%).

Conclusion The 2022 American College of Rheumatology/EULAR classification criteria for TAK are now validated for use in research.

INTRODUCTION

Takayasu arteritis (TAK) is one of the major forms of large-vessel vasculitis (LVV).[1] TAK is a chronic disease defined by granulomatous inflammation affecting the aorta and its primary branches. Complications from vascular damage can result in substantial morbidity including stroke, myocardial infarction, mesenteric ischaemia and limb claudication.

Unlike diagnostic criteria, the purpose of classification criteria is to ensure that a homogeneous population is selected for inclusion into clinical trials and other research studies.[2] In 1990, the American College of Rheumatology (ACR) endorsed classification criteria for TAK.[3] These criteria were developed using data from only 63 patients with TAK and have never been independently validated. In addition, these criteria were derived using data from patients exclusively from North America without representation from Europe or Asia, where clinical patterns of disease may differ,[4] limiting the generalisability of results. Given these constraints, the 1990 ACR criteria for TAK no longer satisfy accepted current standards[5] for classification criteria development, and updated criteria are warranted. Further highlighting a need for uniform, revised criteria in TAK is the use of divergent eligibility criteria to define study populations in two recent randomised clinical trials conducted in North America and Japan, making comparisons between the trial findings difficult.[6 7]

Advancements in imaging techniques and the ongoing adoption of noninvasive vascular imaging approaches in clinical practice have broadened understanding of the clinical heterogeneity in LVV.[8] Disease of the extracranial arteries is increasingly recognised in patients with giant cell arteritis (GCA), making the distinction between TAK and GCA more challenging.[9] Age is typically used as a primary classifier to differentiate between TAK and GCA; however, specific age thresholds to define each disease have not been standardised. Therefore, in addition to incorporating data from a larger patient population from a wider geographical spectrum, the updated TAK classification criteria should reflect modern clinical practice, including current imaging techniques, and also define specific age thresholds.

This article outlines the development and validation of the new ACR/EULAR-endorsed classification criteria for TAK.

METHODS

An international Steering Committee comprising clinician investigators with expertise in vasculitis, statisticians and data managers was established to oversee the overall development of classification criteria for primary vasculitis.[10] A detailed and complete description of the methods involved in the development and validation of the classification criteria for TAK is located in online supplemental appendix 1. Briefly, the Steering Committee implemented a six-stage plan using data-driven and consensus methodology to develop the following criteria.

Stage 1: generation of candidate classification items for the systemic vasculitides

Candidate classification items were generated by expert opinion and reviewed by a group of vasculitis experts across a range of specialties using nominal group technique.

Stage 2: Diagnostic and Classification Criteria for Vasculitis (DCVAS) prospective observational study

A prospective, international, multisite observational study was conducted (see Appendix A for study investigators and sites). Consecutive patients representing the full spectrum of vasculitides were recruited from academic and community practices. Patients were included if they were 18 years or older and had a diagnosis of vasculitis or a condition that mimics vasculitis (eg, infection, malignancy and atherosclerosis). Patients with TAK could only be enrolled within 5 years of diagnosis. Only data present at diagnosis were used to develop the classification criteria.

Stage 3: expert review to derive a gold standard defined set of cases of large-vessel vasculitis

Experts in vasculitis from a wide range of geographical locations and specialties (see Appendix A) reviewed all submitted cases of vasculitis and a random selection of vasculitis mimics. Each reviewer was asked to review ~50 submitted cases to confirm the diagnosis and to specify the degree of certainty of their diagnosis as follows: very certain, moderately certain, uncertain or very uncertain. Only cases agreed on with at least moderate certainty by two reviewers were retained for further analysis.

Stage 4: refinement of candidate items specifically for large-vessel vasculitis

The Steering Committee conducted a data-driven process to reduce the number of candidate items of relevance to cases and comparators for LVV. Density plots were assessed to study age distribution at diagnosis and symptom onset for TAK and GCA. Absolute age requirements vs incorporation of age as a candidate criteria item were considered. Items related to the vascular physical examination, vascular imaging, arterial biopsy and laboratory values were combined or eliminated based on consensus review. Items were selected for exclusion if they had a prevalence of <5% within the data set, and/or they were not clinically relevant for classification criteria (eg, related to infection, malignancy or demography). Low-frequency items of clinical importance could be combined, when appropriate. Patterns of vascular imaging findings detected by vascular ultrasound, angiography, or positron emission tomography were defined by K-means clustering.[11]

Stage 5: derivation of the final classification criteria for TAK

The DCVAS data set was split into development (70%) and validation (30%) sets. Comparisons were performed between cases of TAK and a randomly selected comparator group in the following proportions: GCA, 33.6%; other vasculitides that mimic GCA and TAK (isolated aortitis, primary central nervous system vasculitis, polyarteritis nodosa, Behçet's disease and other LVV), 33.1%; a comparator mimic of LVV (eg, headache syndrome or atherosclerosis), 33.3%. Least absolute shrinkage and selection operator (lasso) logistic regression was used to identify predictors from the data set and create a parsimonious model including only the most important predictors. The final items in the model were formulated into a clinical risk-scoring tool, with each factor assigned a weight based on its respective regression coefficient. A threshold that best balanced sensitivity and specificity was identified for classification.

Stage 6: validation of the final classification criteria for TAK

Performance of the new criteria was validated in an independent set of cases and comparators. Performance of the final classification criteria was examined in specific subsets of patients with TAK using data from the combined development and validation sets, to maximise sample sizes for the subgroups. Patients were studied according to different intervals of age at diagnosis to determine if the criteria performed well across the age spectrum of TAK. Performance characteristics of the new criteria were also tested in patients recruited into the DCVAS study from different regions of the world where prevalence of TAK and clinical assessment approaches may differ. Comparison was made between the measurement properties of the new 2022 ACR/EULAR classification criteria for TAK and the 1990 ACR classification criteria.

RESULTS

Generation of candidate classification items for the systemic vasculitides

The Steering Committee identified >1000 candidate items for the DCVAS Case Report Form (online supplemental appendix 2).

DCVAS prospective observational study

Between January 2011 and December 2017, the DCVAS study recruited 6991 participants from 136 sites in 32 countries. Information on the DCVAS sites, investigators and study participants is listed in online supplemental appendices 3, 4 and 5.

Expert review methodology to derive a gold standard-defined final set of cases of LVV

The LVV expert panel review process included 56 experts who reviewed vignettes derived from the Case Report Forms for 2131 cases submitted with a diagnosis of LVV (1608 [75.5% of Case Report Forms]), another type of vasculitis (118 [5.5% of Case Report Forms]) or a mimic of vasculitis (405 [19.0% of Case Report Forms]). Characteristics and the list of expert reviewers are shown in online supplemental appendices 6 and 7. A sample vignette and the LVV expert panel review flow chart are shown in online supplemental appendices 8 and 9. A total of 1695 cases (80%) passed the main LVV process. An additional 373 cases of LVV and comparators, confirmed during a previous review process to derive the classification criteria for antineutrophil cytoplasmic antibody-associated vasculitis, were also included. In total, after both review processes, 2068 cases were available for the stages 4 and 5 analyses.

The submitting physician diagnosis of TAK was confirmed in 500 of 610 cases (82.0%) after both expert panel reviews. The reasons for exclusion were diagnosis of TAK categorised as 'uncertain' or 'very uncertain' during panel review (n=95) or change in diagnosis during panel review to another type of vasculitis (eg, GCA, isolated aortitis, LVV that could not be subtyped) (n=10) or to a comparator disease (n=5). An additional 9 patients who were not initially diagnosed as having TAK by the submitting physician were diagnosed as having TAK after panel review and DCVAS Steering Committee member adjudication. Per Steering Committee consensus, imaging evidence of LVV was considered an absolute requirement to classify TAK. Of 509 cases confirmed by expert panel review, 47 patients with TAK did not have documented disease

according to a vascular imaging study and were excluded from further analysis, leaving a total of 462 patients with TAK for subsequent analysis.

Refinement of candidate items specifically for TAK.

Patients with TAK were diagnosed in the following age groups: 18–39 years (n=355; 77%); 40–60 years (n=104; 23%); and >60 years (n=3; <1%) (see online supplemental appendix 10 for the distribution of 'age at diagnosis' in patients with LVV, and the similar distribution of 'age at symptom onset,'). Therefore, an age of ≤60 years at diagnosis was considered an absolute requirement to classify a patient as having TAK.

Prevalence of arterial damage (stenosis, occlusion or aneurysm) was greater in TAK compared with GCA in the following nine arterial territories: thoracic aorta, abdominal aorta, left and right carotid, left and right subclavian, mesenteric and left and right renal arteries (online supplemental appendix 11). Therefore, a composite variable representing the number of affected arteries was created based on luminal damage in those nine territories. As previously reported, cluster analyses identified vascular damage in the abdominal aorta and the renal or mesenteric arteries as a specific imaging pattern for TAK in the DCVAS cohort[11]; thus, this arterial pattern was tested as a potential classifier of TAK (online supplemental appendix 12). Symmetric disease in branch arteries (carotid, subclavian and renal arteries) was seen in 30.3% patients with TAK compared with 2.7% of the comparators (p<0.01), and therefore, was included as a potential classifier. A systolic blood pressure difference of ≥20 mm Hg between upper extremities optimised specificity to differentiate TAK from other forms of LVV.

Following a data-driven and expert consensus process, 72 items from the DCVAS Case Report Form were retained for lasso regression analysis, including 32 demographic and clinical items, 14 laboratory items (including values of C reactive protein level and erythrocyte sedimentation rate, each divided into 5 categories), 14 imaging items (13 composite), 11 vascular examination items (5 composite and upper extremity blood pressure divided into 6 categories) and 1 arterial biopsy item (online supplemental appendix 13). Criteria for TAK and GCA were independently derived from this common set of 72 items.

Derivation of the final classification criteria for TAK.

Table 1 lists the demographic and disease features of the 462 patients with TAK and 450 comparators used to develop and validate the criteria, of which 316 patients with TAK and 323 comparators were in the development data set and 146 patients with TAK and 127 comparators were in the validation data set. The patients with TAK were recruited from Asia (n=298), Europe (n=130), North America (n=28), Africa (n=3) and Oceania (n=3). Clinical diagnoses assigned to patients in the comparator group are detailed in online supplemental appendix 14.

Lasso logistic regression analysis using all 72 items resulted in a model of 9 independent items (online supplemental appendix 15B). Weighting of individual criterion was based on logistic regression fitted to the nine selected predictors. The number of affected arterial territories functioned as an almost perfect classifier (online supplemental appendix 16B) and was thus also included in the final model, with criterion weighting determined by consensus of the Steering Committee (online supplemental appendix 17B).

Table 1 Demographic and disease features of the patients with Takayasu arteritis and the comparators*

	TAK (n=462)	Comparators (n=450)†	P value
Age, mean±SD years	32.3±10.4	58.6±18.0	<0.001
Female sex	391 (84.6)	246 (54.7)	<0.001
Clinical features			
Angina	56 (12.1)	7 (1.6)	<0.001
Arm claudication	233 (50.4)	11 (2.4)	<0.001
Leg claudication	88 (19.0)	17 (3.8)	<0.001
Vascular examination findings			
Arterial bruit	263 (56.9)	32 (7.1)	<0.001
Reduced or absent pulse in upper extremity	309 (66.9)	309 (66.9)	<0.001
Carotid artery with reduced pulse or tenderness	171 (37.0)	16 (3.6)	<0.001
Difference in systolic blood pressure ≥20 mm Hg between arms	190 (41.1)	16 (3.6)	<0.001
Imaging findings			
1 affected arterial territory	76 (16.5)	36 (8.0)	<0.001
2 affected arterial territories	114 (24.7)	12 (2.7)	<0.001
≥3 affected arterial territories	89 (19.2)	5 (1.1)	<0.001
Vasculitis affecting paired branch arteries	140 (30.3)	12 (2.7)	<0.001
Abdominal aorta involvement with renal or mesenteric artery involvement	83 (18.0)	5 (1.1)	<0.001

*Except where indicated otherwise, values are the number (%).
†Diagnoses of comparators for the classification criteria for TAK included giant cell arteritis (n=151), Behçet's disease (n=80), polyarteritis nodosa (n=39), clinically isolated aortitis (n=12), primary central nervous system vasculitis (n=11), large-vessel vasculitis (LVV) that could not be subtyped (n=7) and other diseases that mimic LVV (n=150).
TAK, Takayasu arteritis.

Validation of the final classification criteria for TAK

Using a cut-off of ≥5 in total risk score in the validation data set (see online supplemental appendix 18B for cut-off points), the sensitivity was 93.8% (95% CI 88.6% to 97.1%), and the specificity was 99.2% (95% CI 96.7% to 100.0%). The area under the curve for the model was 0.97 (95% CI 0.94 to 0.99) (online supplemental appendix 19B). The final classification criteria for TAK are shown in figure 1 (for the slide presentation versions, see online supplemental figure 1).

The performance characteristics of the criteria in different subsets of patients with TAK are shown in table 2 and online supplemental appendix 20B. For patients who were diagnosed between 18 and 39 years of age, the sensitivity of the criteria was 94.0% (95% CI 91.0% to 96.3%), and the specificity was 97.7% (95% CI 91.9% to 99.7%). For patients who were diagnosed between 40 and 60 years of age, the sensitivity of the criteria was 83.7% (95% CI 75.1% to 90.2%) and the specificity was 91.8% (95% CI 85.4% to 96.0%). Because age restrictions are absolute requirements for the 2022 ACR/EULAR classification criteria for TAK (≤60 years at diagnosis) and GCA (≥50 years at diagnosis), patients with LVV between the ages of 50 and 60 years are potentially eligible to fulfil criteria for TAK and GCA. Of the 26 patients with TAK diagnosed between the ages of 50 and 60 years, 23 (88.5%) were classified correctly as having TAK, 1 (3.9%) was incorrectly classified as having GCA, and 1 (3.9%) fulfilled criteria for both TAK and GCA (online supplemental appendix 21). The criteria performed well in both

2022 AMERICAN COLLEGE OF RHEUMATOLOGY / EULAR

CLASSIFICATION CRITERIA FOR **TAKAYASU ARTERITIS**

CONSIDERATIONS WHEN APPLYING THESE CRITERIA

- **These classification criteria should be applied to classify the patient as having Takayasu arteritis when a diagnosis of medium-vessel or large-vessel vasculitis has been made**
- **Alternate diagnoses mimicking vasculitis should be excluded prior to applying the criteria**

ABSOLUTE REQUIREMENTS

Age ≤ 60 years at time of diagnosis	
Evidence of vasculitis on imaging[1]	

ADDITIONAL CLINICAL CRITERIA

Female sex	+1
Angina or ischemic cardiac pain	+2
Arm or leg claudication	+2
Vascular bruit[2]	+2
Reduced pulse in upper extremity[3]	+2
Carotid artery abnormality[4]	+2
Systolic blood pressure difference in arms ≥ 20 mm Hg	+1

ADDITIONAL IMAGING CRITERIA

Number of affected arterial territories (select one)[5]	
One arterial territory	+1
Two arterial territories	+2
Three or more arterial territories	+3
Symmetric involvement of paired arteries[6]	+1
Abdominal aorta involvement with renal or mesenteric involvement[7]	+3

Sum the scores for 10 items, if present. A score of ≥ 5 points is needed for the classification of TAKAYASU ARTERITIS.

1. Evidence of vasculitis in the aorta or branch arteries must be confirmed by vascular imaging (e.g., computed tomographic/catheter-based/magnetic resonance angiography, ultrasound, positron emission tomography).
2. Bruit detected by auscultation of a large artery, including the aorta, carotid, subclavian, axillary, brachial, renal, or iliofemoral arteries.
3. Reduction or absence of pulse by physical examination of the axillary, brachial, or radial arteries.
4. Reduction or absence of pulse of the carotid artery or tenderness of the carotid artery.
5. Number of arterial territories with luminal damage (e.g., stenosis, occlusion, or aneurysm) detected by angiography or ultrasonography from the following nine territories: thoracic aorta, abdominal aorta, mesenteric, left or right carotid, left or right subclavian, left or right renal arteries.
6. Bilateral luminal damage (stenosis, occlusion, or aneurysm) detected by angiography or ultrasonography in any of the following paired vascular territories: carotid, subclavian, or renal arteries.
7. Luminal damage (stenosis, occlusion, aneurysm) detected by angiography or ultrasonography involving the abdominal aorta and either the renal or mesenteric arteries.

Figure 1 The final 2022 American College of Rheumatology/EULAR classification criteria for Takayasu arteritis.

Asia (sensitivity 92.0%, specificity 93.2%) and Europe/North America (sensitivity 90.5%, specificity 94.4%).

When the 1990 ACR classification criteria for TAK were applied to the DCVAS validation data set, the criteria performed poorly due to low sensitivity (84.3% (95% CI 77.3% to 89.7%)) but retained excellent specificity (99.2% (95% CI 95.7% to 100.0%)). In particular, the 1990 criteria had poor sensitivity for patients who were diagnosed as having TAK between 40 and 60 years of age (62.5% (95% CI 52.5% to 71.8%)).

DISCUSSION

Presented here are the final 2022 ACR/EULAR TAK classification criteria. A six-stage approach was used, underpinned by data from the multinational, prospective DCVAS study and informed by expert review and consensus at each stage. The comparator group for developing and validating the criteria were other vasculitides and conditions that mimic TAK, where discrimination from TAK is difficult but important. In the validation

Table 2 Performance characteristics of the 2022 ACR/EULAR classification criteria for Takayasu arteritis*

Patient subset	Total no patients (no TAK patients)	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)
Development data set	639 (316)	89.9 (86.0 to 93.0)	96.6 (94.0 to 98.3)	0.93 (0.91 to 0.95)
Validation data set	273 (146)	93.8 (88.6 to 97.1)	99.2 (96.7 to 100.0)	0.97 (0.94 to 0.99)
Age intervals				
18–39 years	437 (351)	94.0 (91.0 to 96.3)	97.7 (91.9 to 99.7)	0.96 (0.94 to 0.98)
40–60 years	226 (104)	83.7 (75.1 to 90.2)	91.8 (85.4 to 96.0)	0.88 (0.83 to 0.92)
World regions				
North America	127 (28)	85.7 (67.3 to 96.0)	92.9 (86.0 to 97.1)	0.89 (0.82 to 0.96)
Europe	422 (130)	91.5 (85.4 to 95.7)	94.9 (91.7 to 97.1)	0.93 (0.90 to 0.96)
North America/Europe combined	549 (158)	90.5 (84.8 to 94.6)	94.4 (91.6 to 96.4)	0.92 (0.90 to 0.95)
Asia	357 (298)	92.0 (88.3 to 94.8)	93.2 (83.5 to 98.1)	0.94 (0.89 to 0.96)

*Performance characteristics for the age and regional subsets were reported using data from the combined development and validation data sets to maximise sample size.
ACR, American College of Rheumatology; AUC, area under the curve; TAK, Takayasu arteritis.

data set, the new criteria had a sensitivity of 93.8% (95% CI 88.6% to 97.1%) and a specificity of 99.2% (95% CI 96.7% to 100.0%). These are the official final values that should be quoted when referring to the criteria. The sensitivity and specificity values calculated in the development data set were very similar, providing reassurance that the statistical methods avoided overfitting of models. Calculations of sensitivity and specificity for patient subgroups were made in the combined development and validation data sets to maximise sample sizes for the subgroups. Reassuringly, the new criteria for TAK have excellent sensitivity and specificity across different regions of the world. The criteria also incorporate modern imaging techniques, which are useful both to diagnose LVV and to differentiate among different types of vasculitis. The criteria were designed to have face and content validity for use in clinical trials and other research studies.

These criteria are validated and intended for the purpose of *classification* of vasculitis and are not appropriate for use to establish a *diagnosis* of vasculitis.[2] The aim of the classification criteria is to differentiate cases of TAK from similar types of vasculitis in research settings.[5] *Therefore, the criteria should only be applied when a diagnosis of LVV or medium-vessel vasculitis has been made and all potential 'vasculitis mimics' have been excluded.* For example, the criteria were not developed to differentiate patients with TAK from patients with atherosclerosis or noninflammatory genetic diseases that damage the large arteries. The 1990 ACR classification criteria for vasculitis perform poorly when used for diagnosis (ie, when used to differentiate between cases of vasculitis vs mimics without vasculitis), and it is expected that the 2022 criteria would also perform poorly if used inappropriately as diagnostic criteria.[12]

The 2022 ACR/EULAR TAK classification criteria reflect the collaborative effort of the international vasculitis community to delineate the salient clinical features that differentiate TAK from other forms of vasculitis, most notably GCA. The final criteria include 10 clinical items that are routinely assessed during clinical evaluation of patients with TAK. The criteria highlight the importance of clinical symptoms, vascular physical examination and vascular imaging as important disease classifiers. Features of TAK may differ in patients from different parts of the world.[13] The 2022 ACR/EULAR TAK classification criteria retained excellent performance characteristics when tested in patients from different regions, including Asia where the disease is most prevalent.[14] While TAK is often considered a disease of the young, 25% of the patients with TAK in the DCVAS cohort were older than 40 years at the time of diagnosis. Therefore, an age at diagnosis of ≤60 years, rather than a lower age threshold, was set as an absolute requirement for disease classification. The 2022 ACR/EULAR TAK classification criteria performed well when applied to patients ages 18–60 years and outperformed the 1990 ACR Classification Criteria for TAK in the subset of patients diagnosed as having TAK ages 40–60 years.

There are several strengths of the new 2022 ACR/EULAR TAK classification criteria. The criteria were developed by a large group of international experts in systemic vasculitis, with guidance from the ACR regarding modern methods of classification criteria development. The criteria represent several important methodologic advancements compared with the original 1990 ACR classification criteria for TAK. First, expert review rather than submitting physician diagnosis was used as the diagnostic reference standard to minimise investigator bias. Second, while the 1990 ACR criteria were entirely derived using data from 63 North American patients with TAK and not validated in a separate data set, the new criteria were developed in 316 patients with TAK and validated in an independent data set which contained an additional 146 patients with TAK from an international cohort. Third, unlike the 1990 ACR criteria, the new ACR/EULAR TAK criteria are weighted to reflect the relative importance of specific items (eg, number of affected arterial territories). Finally, when both criteria sets were tested within the DCVAS cohort, the performance characteristics of the 2022 ACR/EULAR TAK criteria outperformed the 1990 ACR criteria.

There are some study limitations to consider. Acquisition of clinical and imaging data among patients with LVV and comparators was not standardised (eg, not all pulses were recorded by the investigators; patients with suspected diagnosis of TAK rarely underwent investigation of the cranial arteries; temporal artery biopsy was not performed in all patients with suspected GCA). However, this limitation reflects the existing differences in how these diseases are assessed in routine clinical practice. Most patients were recruited from Europe, Asia and North America, with fewer patients from Africa and Oceania. The performance characteristics of the criteria should be further tested in populations that were underrepresented in the DCVAS cohort and may have different clinical presentations of TAK. These criteria were developed using data collected from adult patients with vasculitis and should be tested in children with TAK.[15]

The 2022 ACR/EULAR classification criteria for TAK are the product of a rigorous methodologic process that used an extensive data set generated by the work of a remarkable international group of collaborators. These criteria have been endorsed by the ACR and EULAR and are now ready for use in research.

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CLINICAL SCIENCE

Olokizumab, a monoclonal antibody against interleukin-6, in combination with methotrexate in patients with rheumatoid arthritis inadequately controlled by tumour necrosis factor inhibitor therapy: efficacy and safety results of a randomised controlled phase III study

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ABSTRACT

Objectives To assess the efficacy and safety of olokizumab (OKZ), a monoclonal antibody against the interleukin-6 (IL-6) cytokine, versus placebo (PBO) in patients with prior inadequate response to tumour necrosis factor inhibitors (TNFi-IRs).

Methods In this 24-week multicentre, placebo-controlled, double-blind study, the patients were randomised in a 2:2:1 ratio to receive subcutaneously administered OKZ 64 mg once every 2 weeks (q2w), OKZ 64 mg once every 4 weeks (q4w) or PBO plus methotrexate. At week 16, the patients on PBO were randomised to receive either OKZ regime. The primary endpoint was the proportion of patients achieving an American College of Rheumatology 20% (ACR20) response at week 12. Disease Activity Score 28-joint count C-reactive protein (DAS28 (CRP))<3.2 at week 12 was the major secondary efficacy endpoint. Safety and immunogenicity were assessed.

Results In 368 patients randomised, ACR20 response rates were 60.9% in OKZ q2w, 59.6% in OKZ q4w and 40.6% in PBO ($p<0.01$ for both comparisons). Achievement of DAS28 (CRP) <3.2 was significantly different, favouring the OKZ arms. Improvements in efficacy and patient-reported outcomes were maintained throughout 24 weeks and were noted after week 16 in patients who switched from PBO.

Dose-related treatment-emergent serious adverse events were 7% in OKZ q2w, 3.2% in OKZ q4w and none in the PBO group.

Conclusions Direct inhibition of IL-6 with OKZ resulted in significant improvements in the signs and symptoms of rheumatoid arthritis compared with PBO in TNF-IR patients with a similar safety profile as observed for monoclonal antibodies to the IL-6 receptor.

Trial registration number NCT02760433.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disease that primarily affects the joints and is associated with significant morbidity, mortality

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Olokizumab (OKZ) is a new humanised monoclonal antibody targeting the interleukin-6 (IL-6) ligand in development for the treatment of rheumatoid arthritis (RA).
⇒ OKZ was previously shown to be safe and effective in two-dose ranging placebo controlled phase II studies conducted in patients with RA who had failed prior treatment with anti-tumour necrosis factor (TNF) biologics, and two phase III trials in those who were methotrexate inadequate responders.

WHAT THIS STUDY ADDS

⇒ This is a placebo-controlled randomised phase III trial conducted in patients with active RA despite prior treatment with anti-TNF agents.
⇒ In fact, an increasing medical need in patients with RA after failure of anti-TNF agents requires further adequately designed phase III trials to delineate their specific clinical outcomes.
⇒ The current CREDO 3 study met its predefined key efficacy endpoints and provided meaningful safety and efficacy data for two dose regimens of olokizumab.
⇒ It adds to accumulating knowledge about targeting the IL-6 axis in general, and IL-6 ligand specifically.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The CREDO programme includes three phase III randomised controlled trials (RCTs) each with its specific features to provide relevant clinical data for physicians in different clinical settings.
⇒ This study provides further evidence that OKZ, a direct inhibitor of IL-6, is safe and highly effective and thus represents a new treatment approach in the management of refractory RA.

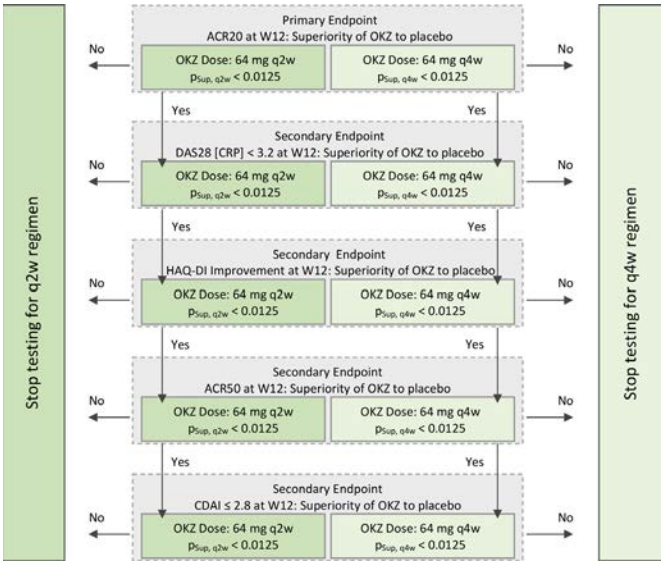

Figure 1 Gatekeeping strategy. $p_{Sup, q2w}$ and $p_{Sup, q4w}$ represent p values from a one-sided test of superiority vs placebo for OKZ dose regimens 64 mg q2w and q4w, respectively. ACR, American College of Rheumatology; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; DAS28 (CRP), Disease Activity Score 28 based on CRP; HAQ-DI, Health Assessment Questionnaire Disability Index; OKZ, olokizumab; q2w, every 2 weeks; q4w, every 4 weeks; Wk, week.

and reduced quality of life, when insufficiently treated.[1–3] Early treatment of RA with conventional synthetic disease modifying drugs (csDMARDs) such as methotrexate (MTX) in a treat-to-target setting is recommended. Although tumour necrosis factor inhibitors (TNFis) are frequently used in patients with active RA who fail to achieve their treatment goal with MTX,[4 5] both American College of Rheumatology (ACR) and European Alliance of Associations for Rheumatology (EULAR) suggest that after MTX, a biological DMARD (bDMARD) or targeted synthetic DMARD (tsDMARD) may be used especially in patients with poor prognosis.[3 6] There are several approved bDMARDs and tsDMARDs which target molecules beside TNF that have been shown to be effective in patients who fail to respond to TNFi. Interleukin-6 (IL-6) is a proinflammatory cytokine that has been shown to play a key role in the pathogenesis of RA.[7] Currently, there are two approved bDMARDs for RA that target IL-6 by blocking the IL-6 receptor.[8 9] While other agents have been studied that also target the IL-6 cytokine directly, none has been approved.[10] As a potential relevant difference with respect to the mode of action, these anti-IL-6 monoclonal antibodies all target site 1 of the cytokine, whereas olokizumab (OKZ) binds to site 3.[11] OKZ was previously shown to be generally safe and effective in reducing signs and symptoms of active RA in patients with an incomplete response to TNFi in two relatively small and short-term phase II randomised controlled trials (RCTs).[12 13] Two phase III study of OKZ in MTX-IR was previously reported with positive results.[14 15] In the present global phase III study, we evaluated the efficacy and safety of OKZ 64 mg every 2 weeks (q2w) and every 4 weeks (q4w) in patients with active RA and inadequate response to TNFi.

METHODS

Study design

This study was a 24-week phase III, randomised, double-blind, placebo-controlled, multicentre trial (ClinicalTrials.gov Identifier NCT02760433, CREDO 3), conducted at 123 centres in 11 countries across Asia, EU, Latin America, Russia and the USA from January 2017 to October 2019. After week 24, the patients were offered the opportunity to participate in an open-label extension study (OLE) or stop the drug and enter the safety follow-up period (SFU) of 20 weeks duration.

Patient inclusion and exclusion criteria

Adult patients with active RA (swollen joint count ≥6 (66-joint count), tender joint count ≥6 (68-joint count) and CRP >6 mg/L) meeting the ACR/EULAR 2010 revised classification criteria[8] for at least 24 weeks prior to screening, and who received treatment with MTX for at least 12 weeks prior to screening at a dose of 15 to 25 mg/week (or ≥10 mg/week if intolerant to higher doses) were enrolled. The patients had failed to achieve an adequate response to ≥1 anti-TNF agent after at least 12 weeks of treatment. Prior use of other bDMARDs, with the exception of other anti-IL-6 or anti-IL-6R products, and cell depleting agents other than rituximab, was allowed if the drugs were discontinued at least for a specified period of time before randomisation. Non-steroidal anti-inflammatory drugs and glucocorticoids in doses<10 mg/day prednisone or equivalent were allowed in stable doses. Patients with latent tuberculosis infection were allowed to participate if they had started appropriate anti-TB therapy at least 30 days prior to randomisation (online supplementary materials, online supplemental table S1; exclusion criteria in the online supplemental materials).

Randomisation and blinding

Patients were randomised 2:2:1 to receive subcutaneous injections of OKZ 64 mg q2w, OKZ 64 mg q4w or placebo (PBO) for 24 weeks using an automated randomisation system. At week 16, all subjects in the PBO group were randomised in a 1:1 ratio in a blinded fashion to receive either OKZ SC 64 mg q2w or 64 mg q4w. Subjects who discontinued the randomised treatment prior to week 24 were requested to continue the study without study treatment.

All patients, investigators, clinical site staff, contract research organisation's staff and the sponsor's staff involved in the study were blinded. Joint assessments were performed by independent assessors, blinded to study drug assignment and all other study assessments (online supplemental materials).

Rescue medication

At week 14, non-responders, defined as subjects who did not improve by at least 20% in both swollen and tender joint counts, in all study arms were prescribed rescue medication (sulfasalazine and/or hydroxychloroquine) in addition to the study treatment (online supplemental materials).

Endpoints

The primary endpoint was the proportion of patients achieving the American College of Rheumatology 20 (ACR20) response at week 12.

Ranked secondary endpoints were percentage of subjects achieving Disease Activity Score 28-joint count C-reactive protein (DAS28 (CRP))<3.2, improvement in the Health Assessment Questionnaire-Disability Index (HAQ-DI), ACR50 response and percentage of subjects with Clinical Disease Activity Index (CDAI) ≤2.8 (remission), all at week 12 (online supplemental materials).

Other patient-reported outcomes (PROs) were Short Form-36 Health Survey (SF-36), Functional Assessment of Chronic Illness

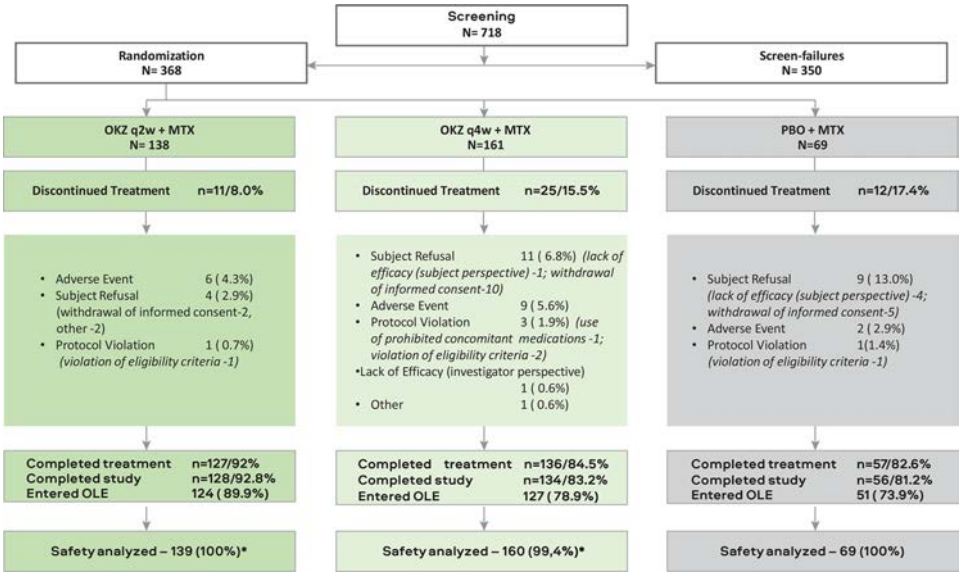

Figure 2 Patient disposition. *One patient was randomised to OKZ 64 mg q4w but actually received OKZ 64 mg q2w. Patients who discontinued treatment early and entered safety follow-up period were considered completers for the whole study if they performed all three follow-up visits. Therefore, the number of those who completed study can be higher than the number of treatment completers. AE, adverse event; IC, informed consent; ITT, intention-to-treat; MTX, methotrexate; N, number patient in the arm; N (%), number (%) patients; %, the percentage of subjects is calculated relative to the total number of subjects in the population; OKZ, olokizumab; OLE, open-label extension; PBO, placebo; q2w, every 2 weeks; q4w, every 4 weeks.

Therapy-Fatigue (FACIT-Fatigue) and European Quality of Life Questionnaire 5-Dimensions (EQ-5D).

Safety monitoring, including assessment of adverse events (AEs), serious adverse events (SAEs) and laboratory tests via central laboratory were performed at multiple time points.

Determination of anti-drug antibodies (ADAs) in plasma samples was accomplished using electrochemiluminescense assay (Covance Laboratories, Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK). For the detection of neutralising ADAs, a cell-based assay was used (Eurofins BioPharma Product Testing Munich GmbH, Robert-Koch-Str. 3a-Haus 2, 82152 Planegg/Munich, Germany).

An independent external Data and Safety Monitoring Board reviewed the safety data throughout the study. Major adverse cardiovascular events (MACEs) were adjudicated by a Cardiovascular Adjudication Committee. MACE included cardiovascular death or death from undetermined cause, non-fatal myocardial infarction, non-fatal stroke, transient ischaemic attack, hospitalisation for unstable angina requiring unplanned revascularisation and coronary revascularisation procedures.

Statistical analyses

To detect a difference between at least one OKZ dose regimen and placebo, a sample size of 320 patients randomised in a 2:2:1 ratio was estimated to ensure sufficient discriminatory power (99% for testing the primary hypothesis (ACR20 at week 12) and 82% for the primary secondary endpoint of DAS28 (CRP) <3.2 rate at week 12).

The ACR20 response at week 12 for each of the active treatment groups was compared with placebo using a 2×2 χ^2 test for equality of proportions. To control the overall type I error rate at a one-sided α=0.025, the Bonferroni adjustment was used for the tests related to each of the OKZ dose regimens versus placebo (ie, one-sided α=0.0125 for each dose group for primary and secondary endpoints). The secondary endpoints that were binary in nature were analysed as per primary endpoint. Efficacy endpoints that were continuous in nature were analysed using an analysis of covariance model adjusted for the baseline value of the corresponding parameter. A gatekeeping strategy with a fixed order of hypotheses was used for the primary and secondary endpoints within each OKZ dose regimen independently (figure 1).

For analyses of binary variables, inability to remain on randomised treatment through the time point of interest was defined as non-response with respect to the corresponding endpoint. In case of missing visits or assessments not performed for the reason other than treatment or study discontinuation, intermediate missing data were imputed using surrounding visits. For the analyses of continuous endpoints, subjects who discontinued randomised treatment prematurely but remained in the study through the time point of interest were included using all collected measurements, including those from assessments post-treatment discontinuation; in case of missing values, return to baseline values was assumed and was implemented using multiple imputation methodology allowing to account for the uncertainty of missing data according to the methodology of Rubin.[16]

The primary analysis was performed for the intent-to-treat (ITT) population defined as all randomised patients. The safety population included all subjects who received at least one dose of the study treatment (see online supplemental materials for additional details).

Protocol-specified statistical analyses were performed using Statistical Analysis System V.9.4 or higher (SAS Institute).

RESULTST

Disposition

A total of 368 patients were randomised to OKZ 64 mg q2w (n=138), OKZ 64 mg q4w (n=161) or placebo (n=69) (figure 2). The three treatment groups were well balanced for baseline demographic and disease characteristics (table 1, online

Table 1 Demographic and other baseline characteristics (ITT population)*

Characteristics, n (%) unless otherwise specified	OKZ q2w, n=138	OKZ q4w, n=161	PBO, n=69
Age, years; mean (SD)	53.4 (12.7)	53.9 (11.7)	53.0 (13.7)
Female	122 (88.4)	130 (80.7)	55 (79.7)
Race			
Asian	6 (4.3)	3 (1.9)	2 (2.9)
Black or African American	11 (8.0)	11 (6.8)	1 (1.4)
White	110 (79.7)	139 (86.3)	53 (76.8)
Other/mixed	11 (8.0)	8 (5.0)	13 (18.8)
Ethnicity			
Hispanic or Latino ethnicity	64 (46.4)	77 (47.8)	42 (60.9)
Not Hispanic or Latino	74 (53.6)	84 (52.2)	27 (39.1)
Duration of RA, years; mean (SD)	11.8 (9.2)	12.7 (8.8)	9.8 (7.0)
MTX dose, mg*; mean (SD)	16.3 (3.7)	16.7 (3.8)	16.5 (3.8)
Duration of prior MTX use, months; mean (SD)	74.7 (68.2)	71.3 (56.7)	66.3 (56.7)
Systemic corticosteroids use	78 (56.5)	94 (58.4)	46 (66.7)
Prednisone dose or equivalent, mg; mean (SD)	5.9 (2.3)	6.0 (2.3)	5.9 (2.1)
Prior exposure to ≥2 bDMARD	26 (18.8)	36 (22.4)	16 (23.2)
Prior exposure to ≥3 bDMARD	4 (2.9)	10 (6.2)	6 (8.7)
BMI, kg/m; mean (SD)	28.8 (7.0)	29.2 (6.0)	28.4 (5.6)
RF+ (≥20 IU/mL)	105 (76.1)	128 (79.5)	55 (79.7)
Anti-CCP+ (>10 U/mL)	96 (69.6)	124 (77.0)	58 (84.1)
CRP (mg/L)†; mean (SD)	20.7 (21.7)	21.4 (24.3)	19.4 (20.2)
TJC‡; mean (SD)	26.0 (13.7)	25.6 (12.8)	28.2 (13.7)
SJC‡; mean (SD)	16.8 (8.2)	17.0 (7.8)	19.3 (9.5)
DAS28 (CRP); mean (SD)	5.9 (0.9)	6.0 (0.8)	6.2 (0.9)
CDAI (0–76); mean (SD)	40.7 (12.5)	41.7 (10.6)	44.4 (11.7)
HAQ-DI; mean (SD)	1.8 (0.6)	1.8 (0.6)	1.8 (0.6)
HAQ-DI <0.5, n (%)	2 (1.4)	3 (1.9)	5 (7.2)
PtGA (VAS) (mm); mean (SD)	64.8 (20.5)	68.1 (19.1)	72.1 (18.5)
Pain (VAS) (mm); mean (SD)	67.2 (19.5)	69.3 (19.1)	69.6 (21.9)
PGA (VAS) (mm); mean (SD)	64.6 (17.8)	65.9 (17.5)	69.5 (14.9)

*100% patients were on MTX.
†Upper limit of normal=6 mg/L.
‡Joints were assessed based on 66–68 joint counts.
Anti-CCP, anti-cyclic citrullinated peptide positivity; BMI, body mass index; CDAI, Clinical Disease Activity Index; DAS28 (CRP), Disease Activity Score 28 based on C-reactive protein; HAQ-DI, Health Assessment Questionnaire Disability Index; IIT, intention-to-treat; MTX, methotrexate; N, number of subjects; OKZ, olokizumab; Pain, patient assessment of pain; PBO, placebo; PGA, Physician Global Assessment of Disease Activity; PtGA, Patient Global Assessment of Disease Activity; q2w, every 2 weeks; q4w, every 4 weeks; RA, rheumatoid arthritis; RF+, rheumatoid factor positivity; SJC, swollen joint count; TJS, tender joint count; VAS, visual analogue scale.

supplemental tables S2 and S3). The majority of patients had a previous exposure to TNF blockers of more than 6 months (online supplemental table S4).

A total of 326 patients completed week 16 of the study: 129 (93.5%) in OKZ q2w, 139 (86.3%) in OKZ q4w and 58 (84.1%) in the placebo treatment group. Of patients randomised to placebo, 32 and 26 were re-randomised to OKZ q2w and OKZ q4w groups, respectively.

A total of 87.0% (320) of randomised subjects completed the treatment period of 24 weeks: 127 (92.0%) in OKZ q2w, 136 (84.5%) in OKZ q4w, 31 (96.9%) in placebo to OKZ q2w and 26 (100%) in placebo to OKZ q4w group. Most of the patients in the study rolled over to OLE; a minority continued to SFU (9 (6.5%) in OKZ q2w, 14 (8.7%) in OKZ q4w, 2 (6.3%) in placebo to OKZ q2w and 3 (11.5%) in placebo to OKZ q4w group) (figure 2).

Efficacy

The primary efficacy endpoint, ACR20 response rate at week 12, was 60.9% in the OKZ q2w group and 59.6% in the OKZ q4w group compared with 40.6% in the placebo group ($p<0.01$ for both comparisons) (table 2, figure 3). Achievement of ACR20 response in the OKZ treatment groups separated from the placebo group as early as week 2 and persisted throughout the 24-week treatment period (figure 3, online supplemental figure S1). Statistically significant difference in the first secondary endpoint in the hierarchy (DAS28 (CRP) <3.2 at week 12) was observed in patients receiving either dose of OKZ compared with PBO ($p<0.0001$ for OKZ q2w and 0.0035 for OKZ q4w) (table 2).

While numerically higher improvements from baseline in HAQ-DI were observed at week 12 for subjects in OKZ q2w and OKZ q4w treatment groups in comparison to patients on PBO, the differences were not statistically significant at the prespecified level of $p<0.0125$ ($p=0.0227$ for OKZ q2w, $p=0.1814$ for OKZ q4w).

Due to the gatekeeping strategy of statistical testing, differences from placebo for the ranked outcomes of ACR50 and disease remission defined as CDAI <2.8 could not be assessed for statistical significance and should be considered nominal. Achievement of ACR70 was an exploratory endpoint and therefore not ranked in the hierarchy of statistical testing.

Subgroup analyses of the ACR20 response showed no influence of country, gender, age, weight, body mass index (BMI), baseline disease severity, time since diagnosis, duration of prior MTX use, or anti-CCP and RF status on the efficacy of OKZ (online supplemental figure S2 (region), other data available on request).

Re-randomisation from placebo to OKZ at week 16 resulted in prompt improvements in all assessed efficacy parameters (figure 3).

In parallel with the main efficacy endpoints, there were marked improvements in several PRO measurements such as SF-36 mental and physical component scores (table 3, online supplemental figure S1).

Safety

A total of 238 patients (64.7%) reported treatment emergent adverse events (TEAE) up to week 44: 110 (64.3%) in any OKZ q2w group (those on OKZ q2w from randomisation and those who were re-randomised to this group from placebo at week 16), 111 (59.7%) in any OKZ q4w group and 35 (50.7%) on placebo (up to week 16) (online supplemental table S7). Most TEAEs were mild to moderate in severity and non-serious and infections were the most common TEAEs. TEAEs leading to study treatment discontinuation were more commonly observed in OKZ q2w (7 (4.1%) and OKZ q4w (10 (5.4%)) than in the PBO-treated patients (1 (1.4%)) for 16 weeks prior to re-randomisation (online supplemental table S7).

In total, 197 patients reported TEAEs up to week 16 (table 4). TESAEs were reported in 9 (6.5%) subjects in OKZ q2w group and in 3 (1.9%) in OKZ q4w group, no serious events were reported in the placebo group (table 4). An anaphylaxis reaction with lip oedema and decreased blood pressure was reported in a patient from the OKZ q4w treatment group. This adverse drug reaction resolved with prednisone 10 mg orally two times per day and loratadine 10

Table 2 Main efficacy results at week 12 in the intent-to-treat population

Outcomes, n (%) **unless otherwise specified**	OKZ q2w, n=138	OKZ q4w, n=161	PBO, n=69
Primary endpoint			
ACR20 response (NRI)	84 (60.9)	96 (59.6)	28 (40.6)
Comparison vs PBO risk difference	0.203 (0.038 to 0.353)**	0.190 (0.030 to 0.337)**	
Secondary endpoints			
DAS28 (CRP) <3.2	55 (39.9)	45 (28.0)	8 (11.6)
Comparison vs PBO risk difference*	0.283 (0.139 to 0.396)***	0.164 (0.029 to 0.268)**	
HAQ-DI LSM (SE), mean difference from baseline	−0.49 (0.05)	−0.39 (0.04)	−0.32 (0.07)
Comparison vs PBO risk difference*	−0.17 (−0.35 to 0.02)*	−0.07 (−0.26 to 0.11)	
ACR50 response (NRI)	46 (33.3)	52 (32.3)	11 (15.9)
Comparison vs PBO risk difference*	0.174 (0.027 to 0.294)**	0.164 (0.020 to 0.278)**	
CDAI≤2.8 (NRI)	9 (6.5)	5 (3.1)	0
Comparison vs PBO risk difference*	0.065 (−0.023 to 0.134)*	0.031 (−0.052 to 0.083)	
Other endpoints			
DAS28 (CRP) <2.6†	30 (21.7)	25 (15.5)	3 (4.3)
Comparison vs PBO risk difference*	0.174 (0.059 to 0.267)**	0.112 (0.005 to 0.192)*	
CDAI <10†	43 (31.2)	41 (25.5)	9 (13.0)
Comparison vs PBO risk difference*	0.181 (0.040 to 0.296)**	0.124 (−0.011 to 0.231)*	
ACR70 response (NRI)	27 (19.6)	21 (13.0)	4 (5.8)
Comparison vs PBO risk difference*	0.138 (0.021 to 0.232)**	0.072 (−0.037 to 0.153)	
HAQ-DI improvement of ≥0.22 (NRI)	75 (54.3)	89 (55.3)	33 (47.8)
Comparison vs PBO risk difference*	0.08 (−0.086 to 0.236)	0.074 (−0.084 to 0.229)	

*p≤0.025; **p<0.01; ***p<0.001 compared with placebo.
*97.5% CI was calculated for comparison of OKZ vs PBO
†Not predefined by protocol (post hoc).
ACR, American College of Rheumatology response; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; DAS28 (CRP), Disease activity Score 28 based on CRP; HAQ-DI, Health Assessment qQuestionnaire Disability Index; LSM, least squares mean; n (%), number and percentage of responders; N, number of subjects; NRI, non-responder imputation; OKZ, olokizumab; PBO, placebo; q2w, every 2 weeks; q4w, every 4 weeks.

mg orally two times per day for 2 days. No TEAEs leading to death, MACE, active TB, or gastrointestinal perforations were reported during the study. TESAEs up to week 44 were numerically higher for the any OKZ 64 mg q2w group (online supplemental table S6). One opportunistic infection (non-serious Herpes zoster infection) was reported in the study in any OKZ q2w group (online supplemental table S5).

Elevations in serum ALT value from 1x ULN to ≤3x ULN at any time during the study were seen in 17 (12.2%) patients in any OKZ q2w, in 12 (7.5%) subjects in any OKZ q4w and in 6 (8.7%) in the PBO group; and elevations above 3x ULN ALT were seen in OKZ arms only: 12 subjects (8.7%) and 16 subjects (10%), respectively, none with concomitant elevation of bilirubin >2x ULN (online supplemental table S9). Other selected abnormal results of haematology and chemistry assessments are presented (online supplemental tables S8 and S9), as well as mean changes in laboratory values dynamic are shown (figure 4).

Overall, 23 subjects (6.9%) had positive confirmatory ADA results at any time post-baseline among patients who received OKZ with no neutralising antibodies detected. Although the clinical significance of this is not clear for the general RA population, there was no difference in clinical responses or safety outcomes in the patients who developed ADA compared with those who did not in this study.

DISCUSSION

This phase III study was conducted to assess efficacy and safety of OKZ in TNFi-IR patients with active RA, a population of patients in high need of additional therapies. The study met the primary endpoint and the first secondary endpoint of DAS28 (CRP) <3.2: it was shown that both dose regimens of OKZ were statistically superior to placebo for these two key endpoints. Moreover, there were numerically higher clinical responses observed in most clinical and some PRO domains with OKZ every 2 week compared with the OKZ every 4 week, but the study was neither designed nor powered to detect differences between doses.

Several clinical outcomes did not show significant improvement by week 12 including HAQ-DI and evidence of deep response determined by CDAI remission. However, more stringent endpoints generally do not plateau by 12 weeks (which was chosen as the time for assessment of the primary endpoint for ethical reasons); they usually plateau by week 20 to week 24 and achieve significance compared with placebo.[17 18] Indeed, increased levels of improvement were also observed in this study between week 12 and week 24, as seen in figure 3. Regarding the HAQ-DI, it is well established that with increasing disease duration the difference between active treatment and placebo decreases until it disappears, presumably due to an increasing irreversibility of functional impairment with increasing damage, related to RA duration.[19 20]

Because of the failure of statistical significance for HAQ-DI, subsequent secondary endpoints could only be statistically evaluated with nominal p values. Using nominal p values, the ranked secondary endpoints of ACR50 and CDAI <2.8 were supportive of the primary endpoint. Clinical efficacy of OKZ was sustained throughout the entire 24-week treatment period. Importantly, re-randomisation from placebo to OKZ at week 16 resulted in prompt improvements in all disease activity parameters to the degree that these patients approached the same level of disease control by week 24 as those who received OKZ for the entire 24-week period.

Reductions in disease activity were paralleled by improvements in most PROs including SF-36 (both physical and mental), pain, EQ-5D and fatigue.

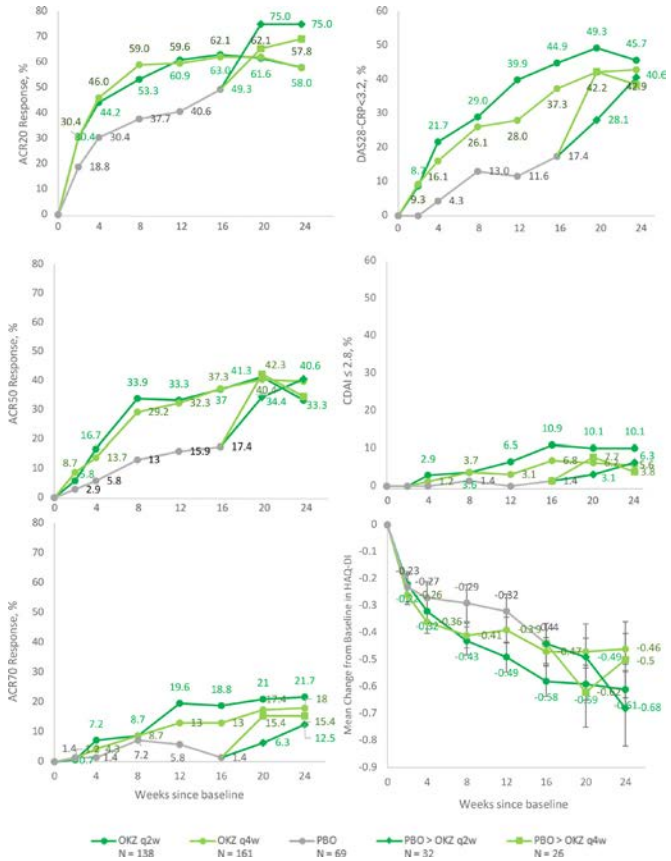

Figure 3 Efficacy results during the double-blind treatment period (ITT population). ACR, American College of Rheumatology; CDAI, Clinical Disease Activity Index; DAS28 (CRP), Disease Activity Score 28 based on C-reactive protein; HAQ-DI, Health Assessment Questionnaire Disability Index; ITT, intention-to-treat; OKZ, olokizumab; PBO, placebo; q2w, every 2 weeks; q4w, every 4 weeks.

Table 4 Incidence of treatment-emergent adverse events by system organ class in >than 3% of patients and serious adverse events up to week 16 (safety population)

System organ class, n (%)	OKZ q2w n=139	OKZ q4w n=160	PBO n=69
Subjects with ≥1 TEAE	**74 (53.2)**	**88 (55.0)**	**35 (50.7)**
Blood and lymphatic system disorders	7 (5.0)	8 (5.0)	5 (7.2)
Gastrointestinal disorders	12 (8.6)	10 (6.2)	6 (8.7)
General disorders and administration site conditions	7 (5.0)	12 (7.5)	3 (4.3)
Hepatobiliary disorders	6 (4.3)	5 (3.1)	1 (1.4)
Infections and infestations	28 (20.1)	36 (22.5)	18 (26.1)
Injury, poisoning and procedural complications	3 (2.2)	10 (6.2)	1 (1.4)
Investigations	21 (15.1)	21 (13.1)	4 (5.8)
Metabolism and nutrition disorders	9 (6.5)	11 (6.9)	1 (1.4)
Musculoskeletal and connective tissue disorders	9 (6.5)	8 (5.0)	5 (7.2)
Nervous system disorders	3 (2.2)	5 (3.1)	2 (2.9)
Skin and subcutaneous tissue disorders	9 (6.5)	12 (7.5)	1 (1.4)
Vascular disorders	4 (2.9)	3 (1.9)	3 (4.3)
TEAE, leading to death	**0**	**0**	**0**
Subjects with ≥1 TESAE*	**9** (6.5)	**3** (1.9)	**0**

n, number of subjects; %, percentage of subjects calculated relative to the total number of subjects in the treatment arm. MedDRA (Medical Dictionary for Regulatory Activities) V.21.1 was used to code AEs. A TEAE is defined as an AE that first occurred or worsened in severity after the first dose of the study treatment.
*TEASE by organ class/*preferred term* were: 1 pt with hepatobiliary disorders/*cholecystitis*; 1 pt with immune system disorders/*anaphylactic reaction*; 3 pts with infections and infestations/*cellulitis* (1pt), *pilonidal cyst* (1pt), *sepsis* (1pt); 3 pts with investigations/*alanine aminotransferase increased* (1pt), *aspartate aminotransferase increased* (1pt), *transaminases increased* (1pt); 2 pts with musculoskeletal and connective tissue disorders/*intervertebral disc protrusion* (1pt), *musculoskeletal chest pain* (1pt); 1pt with psychiatric disorders/*anxiety* and 1 pt with vascular disorders/*hypertensive crisis*.
pt, patient; TEAE, treatment-emergent adverse event; TESAE, treatment-emergent serious adverse event.

OKZ was generally safe and well tolerated with few subjects discontinuing treatment. However, a dose-dependent increase of SAEs was observed with more SAE in the q2w regimen; this had not been observed in other studies with OKZ in RA.[14 21]

There were no deaths, few serious infections and no unexpected safety findings. The safety profile of OKZ, including its effect on serum lipids and hepatic transaminases, was consistent with that seen in other studies of OKZ as well as the approved anti-IL-6 drugs tocilizumab and sarilumab.[8 9] The findings suggest that there may be a numerical advantage with respect to some clinical outcomes with the q2w regimen versus the q4w regimen counterbalanced by better safety with the q4w regimen; however, this trial may be too small to draw any definitive conclusions with respect to the optimal dose

Table 3 Mean baseline values and LSM changes from baseline to week 12 for PROs

	Baseline, mean (SD)			12 weeks LSM changes (SE)		
	OKZ q2w, n=138	OKZ q4w, n=161	PBO, n=69	OKZ q2w, n=138	OKZ q4w, n=161	PBO, n=69
PtGA-VAS (mm)	64.8 (20.5)	68.1 (19.1)	72.1 (18.5)	−24.9 (2.1)	−25.0 (1.9)	−16.9 (2.9)
Pain-VAS (mm)	67.2 (19.5)	69.3 (19. 1)	69.6 (21.9)	−28.2 (2.2)**	−27.5 (2.0)**	−15.0 (3.0)
HAQ-DI	1.79 (0.53)	1.78 (0.56)	1.78 (0.64)	−0.49 (0.05)*	−0.39 (0.04)	−0.32 (0.07)
SF-36 PCS score	31.4 (6.8)	30.6 (7.2)	30.6 (5.9)	6.9 (0.7)**	5.7 (0.6)	3.9 (0.9)
SF-36 MCS score	44.3 (12.6)	44.5 (11.1)	45.1 (10.2)	4.1 (0.8)*	3.4 (0.8)	0.5 (1.1)
FACIT-Fatigue	27.0 (10.2)	26.6 (10.6)	27.3 (9.9)	7.8 (0.9)	6.8 (0.8)	4.6 (1.2)
EQ-5D Health Today Score	45.0 (23.35)	43.7 (22.42)	50.4 (28.31)	17.8 (2.06)	18.0 (1.92)	12.6 (2.92)

Missing data resulted from study withdrawal imputed based on the return to baseline assumption.
*p≤0.025; **p<0.01; ***p<0.001 compared with placebo.
*Secondary endpoint: OKZ q2w p=0.0227 and OKZ q4w p=0.1814 compared with placebo.
EQ-5D, EuroQol 5-Dimensions; FACIT, Functional Assessment of Chronic Illness Therapy-Fatigue; HAQ-DI, Health Assessment Questionnaire Disability Index; LSM, least squares mean; MCS, Mental Component Summary; OKZ, olokizumab; PBO, placebo; PCS, Physical Component Summary; PRO, patient-reported outcome; PtGA, Patient Global Assessment of Disease Activity; q2w, every 2 weeks; q4w, every 4 weeks; SF-36, Short Form-36 Health Survey; VAS, visual analogue scale.

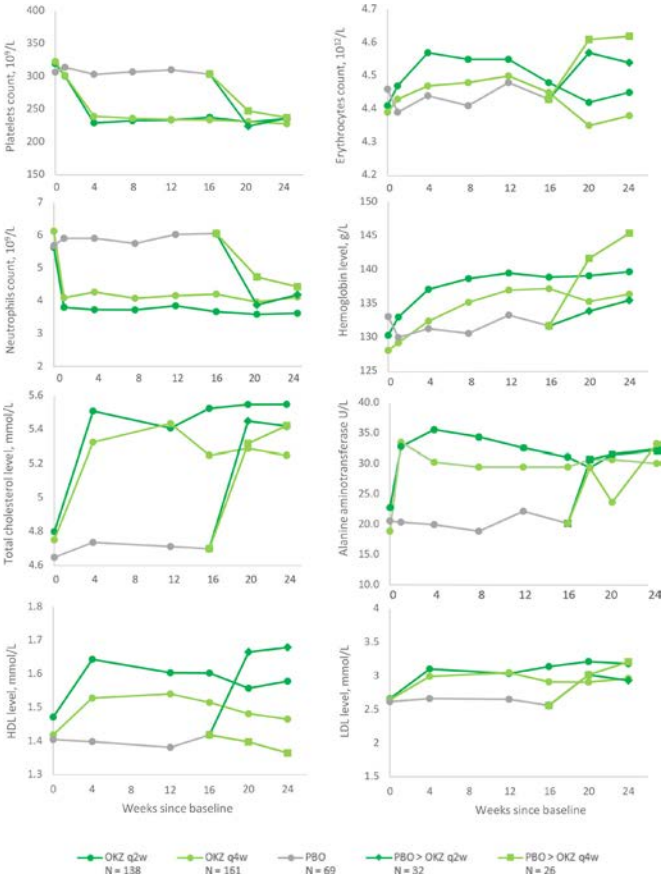

Figure 4 Mean changes in laboratory values during the double-blind treatment period (Safety population). HDL, high-density lipoproteins; LDL, low-density lipoproteins; OKZ, olokizumab; PBO, placebo; q2w, every 2 weeks; q4w, every 4 weeks. Elements of these data were presented at the annual meeting of the American College of Rheumatology 2021[29] and the British Society of Rheumatology Conference 2021.[30]

of OKZ in an individual patient. Post-marketing surveillance and registry data are required to capture further information on rare safety issues, as has been done with other agents.

It has been shown that proinflammatory cytokines such as IL-6 play an essential role in the pathogenesis of RA and the inhibition of the signal cascade at the IL-6 receptor is an established and highly effective approach in the treatment of RA. The IL-6 ligand itself has the potential to be a particularly attractive therapeutic target due to the presumable different levels of the circulating pluripotent cytokine and expression of its soluble as well as cell-associated receptors. It is thus important to fully explore this mode of action, especially in patients who have failed an anti-TNF agent.

With respect to the potential antigenic sites of IL-6,[22] sirukumab and clazakizumab target site 1; interfering with the binding of IL-6 to the cognate IL-6R (IL-6Rα) in the trimolecular IL-6–IL-6R–gp130 complex. Of note, olokizumab binds to site 3 and inhibits the interaction of IL-6 and the IL-6–IL6-R dimer with the signal-transducing β-receptor subunit gp130 of the receptor complex.[12–14 21] As a result, OKZ blocks the final hexamer formation on the molecular level, while the other anti-IL-6 inhibitors prevent dimer formation. This has the advantage that dimers of IL-6 and the soluble IL-6R cannot continue to bind to the signalling moiety of the receptor on the cell membrane with continued cell activation.

The mode of action is also different from the two approved IL-6 pathway inhibitors, which are monoclonal antibodies to the IL-6 receptor. In theory, sIL-6R levels far exceeds those of the IL-6 cytokine in patients with RA and therefore neutralisation of the ligand requires less monoclonal antibody than targeting the IL-6R. This could represent a significant pharmacokinetic and pharmacodynamic difference compared with the IL-6R blockers.[23 24]

The advantages of OKZ are, that as a direct inhibitor of IL-6, less protein needs to be injected to obtain a therapeutic response, and every 4-week dosing may be advantageous to the patient rather than the weekly or every 2-week dosing required with the two approved anti-IL-6R antibodies.

Two other IL-6 ligand blockers, sirukumab and clazakizumab, have been evaluated in RA. Although both drugs have demonstrated clinical efficacy, sirukumab was not approved by the United States Food and Drug Administration for RA due to an observed increased mortality with prolonged treatment. (NCT01604343).[10] Although clazakizumab showed efficacy in phase 2 (NCT02015520), the company stopped further development in RA in favour of an ongoing investigation in chronic kidney transplant rejection (NCT03744910).

Major limitations of this study are its relatively small size, although comparable to studies of other molecules in this patient group, which limits the generalisability of our findings, and the short placebo-controlled portion (for ethical reasons).

The high placebo response rate is another limitation. This phenomenon has been observed in the more recent trials in RA.[25 26] Proposed reasons for this are better adherence to MTX due to the scrutiny of the investigators in current clinical trials.[27 28] Similar to other studies in patients with RA who are TNF-IR, an active comparator arm was not used.

In summary, this study confirms and extends the results of the two previous phase III trials demonstrating significant efficacy with acceptable toxicity for this novel IL-6 inhibitor.

CONCLUSION

In this phase III trial in patients with active RA inadequately controlled by TNF-α inhibitor therapy, treatment with OKZ 64 mg q2w and 64 mg q4w plus MTX was associated with significant improvements in the signs and symptoms of RA compared with PBO plus MTX over a 24-week period with a safety profile similar to approved IL-6 inhibitors.

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

Patient consent for publication Not applicable.

Ethics approval The study protocol was approved by all individual centres' ethics committees and regulatory authorities and written informed consent was obtained from each patient. The study was conducted in accordance with the ICH GCP and the Declaration of Helsinki requirements.

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TRANSLATIONAL SCIENCE

Stage-specific roles of microbial dysbiosis and metabolic disorders in rheumatoid arthritis

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ABSTRACT

Objective Rheumatoid arthritis (RA) is a progressive disease including four stages, where gut microbiome is associated with pathogenesis. We aimed to investigate stage-specific roles of microbial dysbiosis and metabolic disorders in RA.

Methods We investigated stage-based profiles of faecal metagenome and plasma metabolome of 76 individuals with RA grouped into four stages (stages I–IV) according to 2010 RA classification criteria, 19 individuals with osteoarthritis and 27 healthy individuals. To verify bacterial invasion of joint synovial fluid, 16S rRNA gene sequencing, bacterial isolation and scanning electron microscopy were conducted on another validation cohort of 271 patients from four RA stages.

Results First, depletion of *Bacteroides uniformis* and *Bacteroides plebeius* weakened glycosaminoglycan metabolism ($p<0.001$), continuously hurting articular cartilage across four stages. Second, elevation of *Escherichia coli* enhanced arginine succinyltransferase pathway in the stage II and stage III ($p<0.001$), which was correlated with the increase of the rheumatoid factor ($p=1.35\times10^{-3}$) and could induce bone loss. Third, abnormally high levels of methoxyacetic acid ($p=1.28\times10^{-8}$) and cysteine-S-sulfate ($p=4.66\times10^{-12}$) inhibited osteoblasts in the stage II and enhanced osteoclasts in the stage III, respectively, promoting bone erosion. Fourth, continuous increase of gut permeability may induce gut microbial invasion of the joint synovial fluid in the stage IV.

Conclusions Clinical microbial intervention should consider the RA stage, where microbial dysbiosis and metabolic disorders present distinct patterns and played stage-specific roles. Our work provides a new insight in understanding gut–joint axis from a perspective of stages, which opens up new avenues for RA prognosis and therapy.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Rheumatoid arthritis (RA) is a progressive disease, clinically including four stages.
⇒ Intestinal microbiome is associated with the pathogenesis of RA.
⇒ Joint synovial fluid is generally considered as sterile.

WHAT THIS STUDY ADDS

⇒ This is the first study focusing on RA stages to report microbial and metabolic profiles and roles, particularly their enhancement of inflammation, bone loss and bone erosion in the stage II and stage III.
⇒ Joint synovial fluid is not sterile, where bacterial invasion happened in the stage IV.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study provides microbial and metabolic targets for each stage of RA.
⇒ Further experiments and intervention on microbiota of joint synovial fluid are warranted for patients in the stage IV.

INTRODUCTION

Rheumatoid arthritis (RA) affects over tens of millions of people worldwide.[1] RA is recognised clinically as a progressive, inflammatory and autoimmune disease that primarily affects the joints and typically has four stages[2–5]: (1) In the first stage, the synovium of the joints is inflamed and most people have minor symptoms such as stiffness on awakening; (2) In the second stage, the inflamed synovium has caused damage to the joint cartilage and people begin to feel swelling, and have a restricted range of motion; (3) In the third stage, RA has proceeded to a severe state when bone erosion begins and the cartilage on the surface of the bones has deteriorated, resulting in the bones rubbing against one another and (4) In the fourth stage, certain joints are severely deformed and lose function. To inhibit RA progression, specific therapeutic strategies are necessary for people across different RA stages.

Gut microbial dysbiosis has been implicated in the pathogenesis of RA via a range of mechanisms such as metabolic perturbation and immune response regulation, which is known as the gut–joint axis,[6 7] for instance, increased abundance of *Prevotella* and *Collinsella* in patients with RA are correlated with the production of T_H17 cell cytokines.[8 9] Moreover, Gut microbes and their products were likely to be transited to the joint due to the increased gut permeability.[6] Metabolites have also been correlated to immunity regulation in RA: administration of Short-chain fatty acids to mice with collagen-induced arthritis (CIA) can reduce the severity of arthritis by modulation of IL-10.[6 10] Comprehensive metagenomic and metabolomic analyses could

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Table 1 General characteristics at stool collection of multiomics cohort (122 participants)

	Patients with RA from four stages					
	RAS1 n=15	RAS2 n=21	RAS3 n=18	RAS4 n=22	OA n=19	HC n=27
Age (years), median (IQR)	52 (50–60)	64 (59–67)	59 (50–66)	60 (54–66)	66 (64–71)	56 (50–60)
Female sex, n (%)	12 (80)	16 (76)	12 (67)	21 (95)	15 (79)	19 (70)
Classification score						
A (IQR)	3 (3–3)	3 (3–3)	5 (5–5)	5 (5–5)	1 (0–1)	0
B (IQR)	2 (2–2)	3 (3–3)	3 (3–3)	3 (3–3)	0	0
C (IQR)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	0
D (IQR)	1 (1–1)	1 (1–1)	0 (0–0)	1 (1–1)	1 (1–1)	0
Sum score (IQR)	7 (7–7)	8 (8–8)	9 (9–9)	10 (10–10)	3 (2–3)	0
ACPA positivity, n (%)	5 (33)	18 (86)	13 (72)	17 (77)	0	
ESR (IQR)	60.00 (30.50–87.00)	72.00 (45.00–116.00)	48.00 (29.50–60.00)	60.00 (40.50–80.00)	23.00 (15.75–41.50)	
CRP (IQR)	29.10 (9.20–53.40)	43.00 (15.95–56.55)	38.60 (12.50–44.40)	27.15 (20.32–64.45)	17.45 (4.62–65.75)	
RF (IQR)	52.00 (39.50–60.50)	421.00 (186.00–590.00)	34.00 (19.50–80.00)	279.50 (106.00–359.00)	26.00 (22.00–29.00)	

ACPA positivity was defined as a concentration of greater than 5 µL/mL.
Classification scores were summarised according to 2010 RA classification criteria: A, joint involvement; B, serology; C, acute-phase reactants; D, duration of symptoms.
ACPA, anticitrullinated protein antibody; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; HC, healthy controls; OA, osteoarthritis; RA, rheumatoid arthritis; RAS1–4, the first to fourth stage of RA; RF, rheumatoid factor.

therefore enhance our understanding about the gut–joint axis. However, the role of the gut–joint axis across successive stages of RA is understudied,[6 11] where more examinations may provide an alternative approach to ameliorate RA progression.

Here, we aimed to investigate the stage-based profiles and roles of the gut–joint axis in RA pathogenesis, and whether or in which stage gut microbial invasion of the joint synovial fluid happened.

MATERIALS AND METHODS

Study design and sample collection

Data collection for this multiomics study was conducted in The First Affiliated Hospital of Shandong First Medical University (Jinan, Shandong, China), which was a provincial-level large-scale comprehensive tertiary first-class hospital and had tens of thousands of outpatients with arthritis per year. A total of 122 faecal and 122 plasma samples were collected from 122 outpatients of the The First Affiliated Hospital of Shandong First Medical University from 2017 to 2020.[7] These outpatients included 76 patients with RA, 19 patients with OA and 27 healthy individuals (table 1). Patients with RA were grouped into four RA stages including RAS1 (n=15), RAS2 (n=21), RAS3 (n=18) and RAS4 (n=22) according to the rheumatoid diagnostic score,[3] where RAS1, RAS2, RAS3 and RAS4 has a score of 6–7, 8, 9 and 10, respectively. The score was evaluated by the sum of four categories as summarised in the 2010 RA classification criteria.[3] Faecal samples were collected and sequenced and plasma samples were used to test the plasma metabolites, anticitrullinated protein antibody, erythrocyte sedimentation rate, C reactive protein, rheumatoid factor, cytokines and plasma metabolites.

To confirm the bacterial invasion of the joint synovial fluid, another cohort of 271 with RA of four distinct stages were recruited, including 52 patients in RAS1, 66 in RAS2, 67 in RAS3 and 86 in RAS4. Synovial fluid samples were collected aseptically from knee joints during therapeutic aspiration. The entire experiment was conducted in a completely sterile atmosphere. For each patient, a total of 7 mL synovial fluid was collected, of which 5 mL was used for 16S rRNA gene sequencing, 1 mL was used for bacteria isolation and 1 mL synovial fluid was prepared for scanning electron microscopy.

All of the participants were at fasting status during the sample collection in the morning. Only participants who met the standard were recruited in this study: Recruited individuals had not received treatment in the recent month and were in the active period, and had no malignant tumour, no other rheumatic diseases such as ankylosing spondylitis, psoriasis, gout, no gastrointestinal diseases such as diarrhoea, constipation and haematochezia in the recent month, no infections, no other comorbidity such as diabetes and hepatitis B.

Metagenomic sequencing and processing to analyse the faecal microbiome

Whole-genome shotgun sequencing and processing of faecal samples, non-redundant gene catalogue construction, identification of metagenomic species (MGS), functional annotation to Kyoto Encyclopaedia of Genes and Genomes (KEGG) were performed (details in online supplemental text). Two parallel processes were used for gut metagenomic data analysis: One was based on 4 million non-redundant genes and investigated the functional composition across RA stages and OA, as well as the MGS that most drove the correlation of these microbial functions with RA or OA and (2) The other reported the 232 classified microbial species composition across RA stages and OA, profiled by MetaPhlAn2[12] (V.2.7.8).

UHPLC-QTOF-mass spectrometry analysis of plasma metabolites

Untargeted plasma metabolome was examined by ultra-performance liquid chromatography-quadrupole time-of-flight (UHPLC-QTOF) mass spectrometry: liquid chromatography with tandem mass spectrometry on an UHPLC system (1290, Agilent Technologies) with a UPLC BEH Amide column (1.7 µm 2.1×100 mm, Waters) coupled to TripleTOF 6600 (Q-TOF, AB Sciex) and QTOF 6550 (Agilent) (details in online supplemental text).

16S rRNA gene sequencing and processing to analyse the synovial fluid microbiota

Bacterial DNA was extracted from 271 5 mL synovial fluid samples. The tube containing PBS serves as environmental

control. Only a total of 86 synovial fluid samples from patients in RAS4 had enough bacteria DNA content (≥10 ng) (Bacterial DNA Kit, TIANGEN) for bacteria 16S rRNA gene high-throughput sequencing. The V1/V2 hypervariable regions of the 16S ribosomal RNA gene were sequenced using the Illumina HiSeq platform. The 16S sequence paired-end data set was joined and quality filtered using the FLASH as previously described.[13] Taxonomic annotation was then performed (details in online supplemental text).

Bacterial isolation and scanning electron microscopy

Six synovial fluid samples (1 mL) per RA stage were used for bacteria isolation, and the obtained isolated colonies were identified using 16S rRNA gene sequencing (details in online supplemental text). For the samples from which bacterial can be isolated, synovial fluid samples (1 mL) of the same individuals were then filtered and imaged with scanning electron microscope (ZEISS Sigma 300, details in online supplemental text).

Statistical analysis

Samples were divided into three groups including the healthy group, the OA group and the RA group. Samples of the RA group were further divided into four subgroups including RAS1, RAS2, RAS3, RAS4. For comparisons of vectors across groups or subgroups, such as microbial species abundance, KO abundance, metabolite intensity. Mann-Whitney-Wilcoxon test (p values) with Benjamini and Hochberg correction (q values) was used to test the significance. A threshold for statistical significance was $p<0.05$, and for multiple testing the threshold was $p<0.05$ and $q<0.1$.

For correlations between KEGG modules and clinical phenotypes including arthritis (healthy=0, OA=1, RA=2), cytokine levels and rheumatic factor level, owing to that a KEGG module contained multiple KOs, Spearman correlation coefficients (SCC) between abundances of KOs and clinical phenotypes were first calculated. Subsequently, Mann-Whitney-Wilcoxon test (p values) with Benjamini and Hochberg correction (q values) was used to test if SCC between the KOs in a given KEGG module and phenotypes were different from that between all the other KOs out of the KEGG module and phenotypes. In this process, the KEGG module with statistical significance was viewed as significantly correlated with the clinical phenotypes. A threshold for statistical significance was $p<0.05$ and $q<0.1$. Considering that sex and age might have potential effects on gut microbiome,[14] partial SCCs with age and gender adjusted were also calculated and compared, and a threshold for statistical significance was $p_{partial}<0.05$ and $q_{partial}<0.1$.

Leave-one-out analysis was used to test which MGS was driving the observed correlations between KEGG modules and arthritis. Owing to that one MGS contained multiple genes that were mapped to KOs, if one MGS was excluded in the dataset, the overall profiles of the KO abundance would change, resulting in the change of the correlations between KEGG modules and arthritis. Therefore, to determine the driving effects of each of MGS, the calculation of the KO abundance was iterated excluding the genes from a different MGS in each iteration, and the correlations between each KEGG module and arthritis were recalculated. Finally, the driving effects of a given MGS on a specified correlation was defined as the change in median SCC between KOs and arthritis when genes from the respective MGS were left out.

To determine the diagnostic potential of RA stages using multiomics features, random forest algorithm was performed on 6,224 KOs, 232 microbial species and 277 plasma metabolites, using the R package 'randomForest'. Function 'trainControl' in R package 'caret' was used to perform 10 repeats of 10-fold cross-validation for each data set. Function 'train' in R package 'caret' was used to fit models over different tuning parameters to determine the 'mtry' for random forest algorithm. Gini coefficients were used to measure how each variable contributed to the homogeneity of the nodes and leaves in the resulting random forest.

RESULTS

Stage-specific microbial taxonomic profiles

We obtained a total of 231 classified microbial species from metagenomic data, and tested their alterations in each stage of RA, as compared with healthy controls (see online supplemental figure S1, table S1–S5). The elevated species in RA progression were mostly from the phyla Firmicutes and Actinobacteria, while the depleted species were predominantly from the phylum Bacteroides ($q<0.1$). We found certain microbes did not remain altered across RA stages, as compared with healthy controls. *Bifidobacterium dentium*, for instance, was reported to be associated with the development of dental caries and periodontal disease, both of which were particularly prevalent in patients with RA.[15 16] Compared with healthy controls, it remained elevated across RA stages except for RAS1 (RAS2: $p=7.16\times10^{-3}$, RAS3: $p=3.70\times10^{-3}$, RAS4: $p=9.15\times10^{-4}$). Moreover, we noticed that 29 species that were altered exclusively in a specific stage (see online supplemental table S1–S5). We found that *Collinsella aerofaciens* was elevated exclusively in RAS1 ($p=0.043$). *C. aerofaciens* was previously reported to generate severe arthritis when inoculated into CIA-susceptible mice, and an in vitro experiment showed that *C. aerofaciens* could increase gut permeability and induce IL-17A expression, a key cytokine involved in RA pathogenesis.[9] The elevation of *C. aerofaciens* in RAS1 might contribute to the early breach in gut barrier integrity, through which the translocation of microbial products would then trigger the subsequent clinical arthritis.[6] Moreover, *Veillonella parvula*, whose infection could cause osteomyelitis,[17] was found elevated exclusively in RAS3 ($p=0.027$). *Eggerthella lenta* ($p=0.018$) and *Bifidobacterium longum* ($p=0.022$) were found elevated exclusively in RAS4. The gavage of *E. lenta* were reported to increase gut permeability and produce proinflammatory cytokines.[18] We also recognised species altered exclusively in OA, such as elevated *Dialister invisus* ($p=0.041$) that was positively correlated with spondyloarthritis severity.[19] These stage-specific altered species had the potential to serve as the targets for intervention in a given RA stage.

Stage-specific microbial functional profiles

Next, we sought to detect the microbial dysfunction across stages of RA. We grouped 4 047 645 metagenomic genes into 6,224 KOs and 404 KEGG modules. We identified 12 KEGG modules that were significantly correlated with RA or OA ($q<0.1$ or $q_{partial}<0.1$, see online supplemental figure S2) and presented their variation across stages (figure 1A). We then used leave-one-out analysis to identify the MGS that most drove the correlations of these KEGG modules with RA or OA (figure 1B, online supplemental figure S3).

We found an evident decrease in glycosaminoglycan (CAG) metabolism across four RA stages and OA. It was mainly reflected by the significant decrease in K01197 (hyaluronoglucosaminidase) of dermatan sulfate (DS) degradation and the significant decrease in K10532 (heparan-alpha-glucosaminide

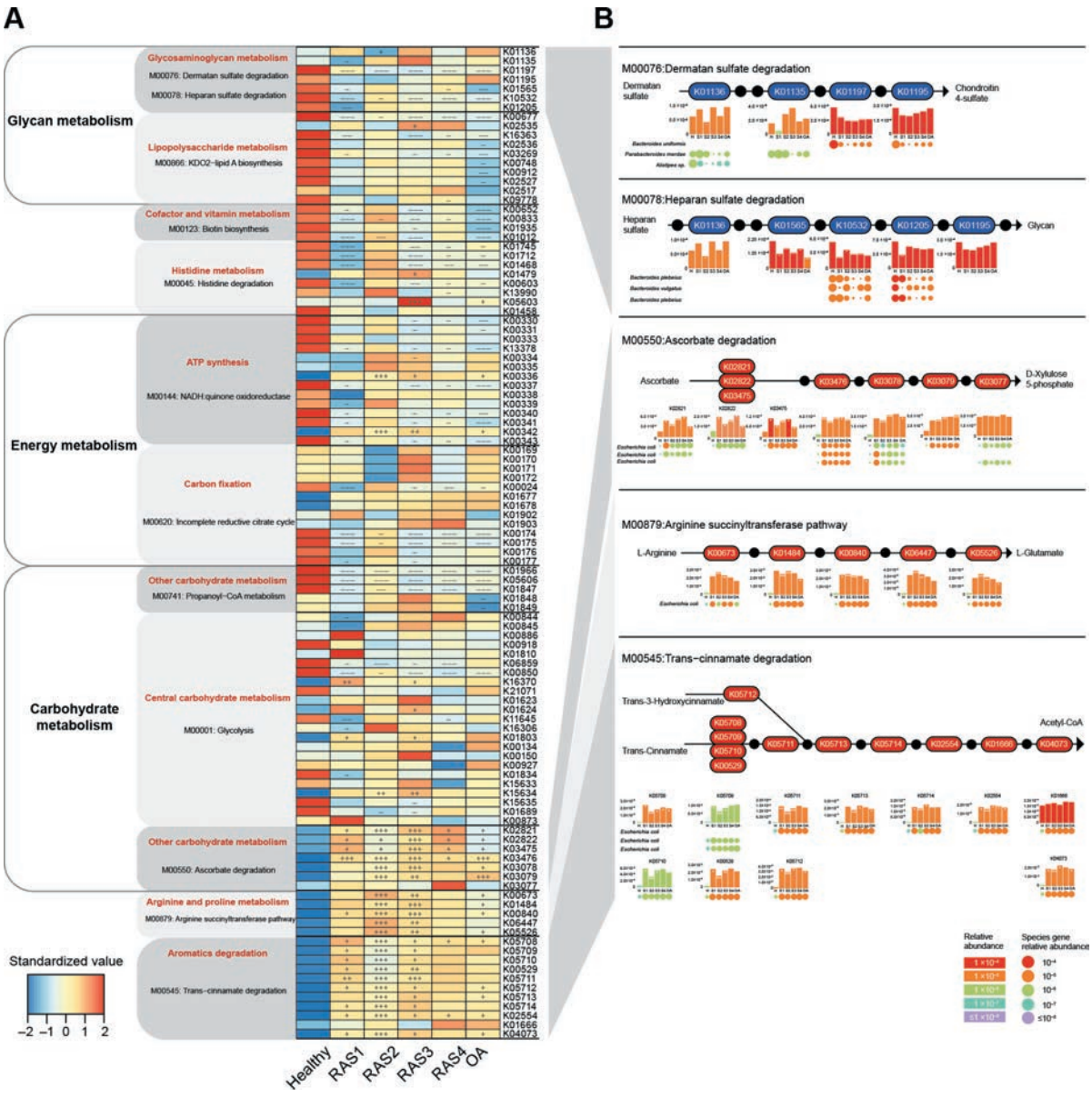

Figure 1 Stage-specific microbial functional profiles. Gene abundances were assessed for elevation or depletion in each of the arthritis stages, RAS1 (n=15), RAS2 (n=21), RAS3 (n=18), RAS4 (n=22) and OA (n=19) compared with the healthy individuals (n=27). (A) Relative abundance of KO genes in the KEGG modules that were significantly correlated with arthritis ($q<0.1$ or $q_{partial}<0.1$, see online supplemental figure S1). KO genes with a prevalence of 5% or higher are shown. (B) KO genes involved in specific KEGG pathway modules in (A) are shown in the KEGG pathway maps. Each box in a pathway represents a KO gene and is marked in red for elevation or in blue for depletion at any of the stages compared with healthy individuals. Bar plots show relative gene abundances averaged over samples within each of the five groups (healthy (H), RAS1 (S1), RAS2 (S2), RAS3 (S3), RAS4 (S4) and OA) and are coloured according to the values. Each KO gene is composed of MGS genes represented by circles. The sizes and colours of the circles are proportional to the relative abundances of the MGS genes. MGS genes are grouped into one row and indicated by the taxonomic name. The three MGS that most drove the correlation of the KEGG modules with arthritis types are shown. In all panels, significant changes are denoted as follows: +++, elevation with $p<0.005$; ++, elevation with $p<0.01$; +, elevation with $p<0.05$; −−−, depletion with $p<0.005$; −−, depletion with $p<0.01$; −, depletion at $p<0.05$; Mann-Whitney-Wilcoxon test. KEGG, Kyoto Encyclopaedia of Genes and Genomes; KO, KEGG ortholog; MGS, metagenomic species; OA, osteoarthritis.

N-acetyltransferase) of heparan sulfate (HS) degradation ($p<0.05$, figure 1A). Chondroitin 4-sulfate is a major component of the extracellular matrix of many connective tissues, such as cartilage, bone and skin.[12] We found that the significant depletion of DS degradation would inhibit the production of chondroitin 4-sulfate (figure 1B), which might hurt the mechanical properties of the articular cartilage.[12] Moreover, the significant depletion of HS degradation might be a potential cause of the higher plasma level of HS observed in RA and OA patients,[20 21] which could promote arthritis progression by regulating protease activity.[22] The most driving species of DS degradation and HS degradation were MGS *Bacteroides uniformis* and MGS *Bacteroides plebeius*, respectively. The genes of MGS *B. uniformis* related to K01197 were found most depleted in RAS2, while the genes of MGS *B. plebeius* related to K10532 were found most depleted in RAS3 and RAS4 (figure 1B). These results indicated that the depleted microbial function in DS degradation and HS degradation driven by *B. uniformis* and *B.*

plebeius, respectively, could promote RA and OA in a way of hurting articular cartilage.

We also identified elevated microbial functions that were related to inflammation such as the previously reported ascorbate degradation.[7] Here, we found most of the KOs related to ascorbate degradation retained a higher level across RA stages and OA, especially in RAS2 and RAS3 ($p<0.05$, figure 1A). Genes of K02821 (phosphotransferase system) in RAS1, K03475 (phosphotransferase system), K03476 (L-ascorbate 6-phosphate lactonase), and K03479 (L-ribulose-5-phosphate 3-epimerase) were mostly driven by MGS *Escherichia coli* (figure 1B). The enhanced ascorbate degradation might contribute to the deficiency of the ascorbate reported in patients with RA[23] and were found positively correlated with multiple plasma cytokines ($q<0.1$ or $q_{partial}<0.1$, see online supplemental table S6), such as IL-1β ($p=5.44\times10^{-4}$), TNF-α ($p=6.59\times10^{-4}$) and IL-6 ($p=1.12\times10^{-3}$). Moreover, to confirm the effects of ascorbate on RA progression, we examined the plasma TNF-α level and IL-6 level, bone CT scans, and bone density of (1) normal DBA/1 mice, (2) DBA/1 mice with CIA and (3) DBA/1 mice with CIA and gavage of ascorbate. We found that the 3-month gavage of ascorbate to CIA mice can prevent the increase of TNF-α and IL-6 levels by half, inhibit bone destruction, and maintain bone density ($1.58\pm0.0034\,g/cm^3$), as compared with the CIA mice without ascorbate ($1.53\pm0.013\,g/cm^3$), and the normal group ($1.61\pm0.021\,g/cm^3$, see online supplemental figure S4).

For other elevated microbial functions, the trans-cinnamate degradation driven by MGS *E. coli*, where most KOs were notably elevated in RAS2, was also correlated with multiple cytokines ($q<0.1$ or $q_{partial}<0.1$, see online supplemental table S6), such as IL-13 ($p=1.63\times10^{-5}$), IL-1β ($p=2.87\times10^{-5}$) and IL10 ($p=4.10\times10^{-5}$). Moreover, the arginine succinyltransferase pathway driven by MGS *E. coli* was found significantly elevated mainly in RAS2 and RAS3 (figure 1). L-arginine is able to prevent bone loss induced by zinc oxide nanoparticles or by ciclosporin A, through anti-inflammatory mechanism[24] or nitric oxide production, respectively.[25] Both arginine succinyltransferase pathway and trans-cinnamate degradation was positively correlated with the elevation of rheumatoid factor ($p=1.35\times10^{-3}$). Taken together, these results suggested that microbial dysfunction could promote RA progression mainly by hurting bone tissue and strengthening inflammation. The inflammation-related microbial dysfunction was extremely active in RAS2 and RAS3 and largely driven by *E. coli*.

Microbial invasion of the joint synovial fluid

Next, we investigated whether or in which stage microbial invasion of the joint synovial fluid happened. Enhanced gut permeability may render it possible for microbes and their products to translocate, triggering an immune response.[6 26] We thus speculated that gut microbes might invade the joint synovial fluid of patients with RA through the gut–joint axis. To test this, we performed 16S rRNA gene sequencing on the synovial fluid samples from another cohort of 271 patients in four RA stages, including RAS1 (n=52), RAS2 (n=66), RAS3 (n=67) and RAS4 (n=86). Notably, we were not able to obtain enough bacterial DNA for sequencing in samples of RAS1, RAS2 or RAS3, however, we could identify many microbes in samples of RAS4 (see online supplemental figure S5). We found that most of the microbes in joint synovial fluid were from phyla Proteobacteria and Firmicutes, and a total of 98 genera could also be detected in faecal metagenomic data (see online supplemental table S7). Moreover, we could recognise *E. lenta* and *B. longum* in most of the synovial fluid samples, both of which were observed to be exclusively elevated in faecal metagenome of patients in RAS4 from the multiomics cohort (see online supplemental table S4). In addition, *Prevotella copri* that has been reported highly correlated with RA[8 27] was also found abundant in most synovial fluid samples of patients in RAS4. We then randomly selected six synovial fluid samples per RA stage for bacteria isolation. Only from three synovial fluid samples of RAS4 can we separate bacteria. We then picked and sequenced three single colonies per synovial fluid sample. Five of the nine colonies were identified as *Clostridium sporogenes* strain, and three were identified as *Enterococcus gallinarum* strain, and one was identified as *Citrobacter freundii* strain (see online supplemental table S8). Interestingly, *Enterococcus gallinarum* and *Citrobacter freundii* could also be detected in faecal metagenomic data of 18% of patients with RA. We subsequently observed the corresponding synovial fluid samples using scanning electron microscopy, and found substances shaped like bacteria in rod-like or spherical forms (figure 2). Taken together, this multifaceted investigation has provided unprecedented evidence to support the existence of microbial invasion of the joints in the fourth stage of RA.

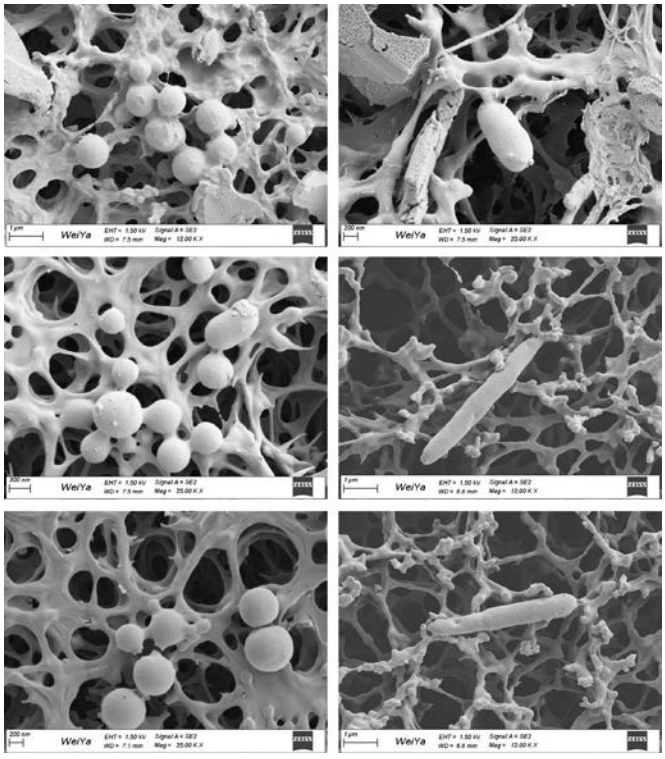

Figure 2 Scanning electron microscopy of the joint synovial fluid.

Stage-specific metabolomic profiles

We then introduced metabolomic data, and performed a random forest algorithm on 232 microbiome species, 6224 KOs and 277 metabolites to test their diagnostic potential for each stage of RA and OA (figure 3A–E). Metabolites exhibited the best area under the receiver operating characteristic curve (AUROC) in discriminating samples of four RA stages or OA from healthy samples, with AUROC ranging from 0.974 to 0.998. Other characteristics at the species and KO levels exhibited weaker discriminant ability, with AUROC ranging from 0.760 to 0.838 and from 0.799 to 0.852, respectively. The most prominent changes in metabolites were the significant increase of DL-lactate and gly-glu in RAS1 ($p=2.15\times10^{-6}$, $p=4.70\times10^{-4}$), the decrease of

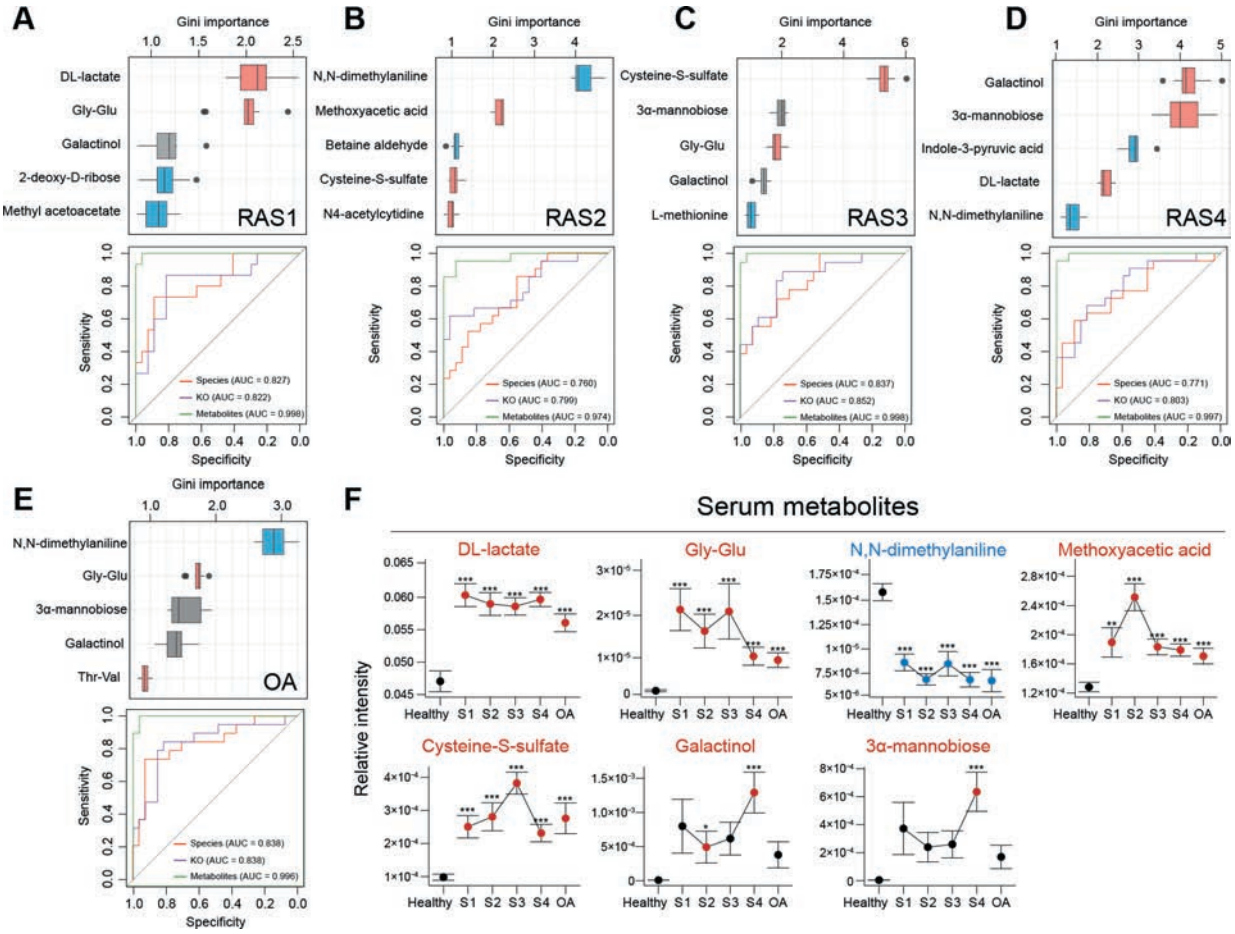

Figure 3 Multiomics diagnostic potential for the RA stage. A random forest algorithm was performed on 6224 KOs, 232 microbial species and 277 plasma metabolites in RAS1 (A), RAS2 (B), RAS3 (C), RAS4 (D) and OA (E). The Gini importance of the top five most discriminant metabolites are displayed. Boxes represent the IQR between the first and third quartiles and the line inside represents the median. Whiskers denote the lowest and highest values within the 1.5×IQR from the first and third quartiles, respectively. Boxes are marked in a specific colour to show the significant elevation (p<0.05, red, Mann-Whitney-Wilcoxon test) or depletion (p<0.05, blue, Mann-Whitney-Wilcoxon test) of the features in each of the arthritis stages compared with the healthy group. The ROC curves of the random forest model using microbial species, KOs, or metabolites were plotted, with AUC calculated by 10 randomised 10-fold cross-validation. The colour of the curve represents the category of the used features. (F) The dot plots show stage-specific abundance or concentration (mean±SE) of plasma metabolites, which are specified in (A–E). Four RA stages are connected to display the variance. Dots are coloured differently if the features are significantly elevated (red) or significantly depleted (blue), as compared with those of the healthy group. *p<0.05, **p<0.01, ***p<0.005; Mann-Whitney-Wilcoxon test. AUC, area under curve; KEGG, Kyoto Encyclopaedia of Genes and Genomes; KO, KEGG ortholog; OA, osteoarthritis; RA, rheumatoid arthritis; ROC, receiver operating characteristic.

N,N-dimethylaniline and the increase of methoxyacetic acid in RAS2 ($p=4.60\times10^{-8}$, $p=1.28\times10^{-8}$), the increase of cysteine-S-sulfate ($p=4.66\times10^{-12}$) in RAS3, the increase of galactinol and 3α-mannobiose in RAS4 ($p=5.71\times10^{-5}$, $p=5.68\times10^{-4}$), and the decrease of N,N-dimethylaniline and increase of gly-glu ($p=2.00\times10^{-7}$, $p=1.74\times10^{-3}$) in OA, as compared with a healthy state. The predominant metabolic disorders implicated a critical involvement in pathogenesis and a great diagnostic potential for RA stages.

Moreover, metabolic disorders could distinguish a given RA stage from not just healthy controls but also other RA stages or OA (figure 3F): Methoxyacetic acid in RAS2 ($p=1.68\times10^{-4}$) or cysteine-S-sulfate in RAS3 ($p=2.42\times10^{-4}$) or Galactinol and 3α-mannobiose in RAS4 ($p=9.37\times10^{-3}$, $p=4.89\times10^{-3}$), respectively, was higher than that in all the other RA stages and OA. Methoxyacetic acid was reported to have inhibitory effects on osteoblasts and could cause reductions in bone marrow cellularity.[28–30] Additionally, cysteine-S-sulfate was a structural analogue of glutamate, acting as an agonist of N-methyl-D-aspartate receptor (NMDA-R) whose expression and function in osteoclasts engaged in bone resorption.[31] Therefore, notable elevations of methoxyacetic acid in RAS2 might hinder osteoblasts, whereas notable elevations of cysteine-S-sulfate in RAS3 might encourage osteoclasts. The imbalance between osteoblasts and osteoclasts would promote the bone erosion that occurred clinically in the third stage of RA. Moreover, DL-lactate in OA was less than that in all RA stages ($p=0.037$), which might improve clinical differentiation of early RA from OA.

DISCUSSION

Our findings reveal dynamic shifts in gut microbiome and plasma metabolome, and their continuous roles in pathogenesis of RA across four successive stages (figure 4). Moreover, we demonstrate that microbial invasion of the joint synovial fluid happens in the fourth stage of RA.

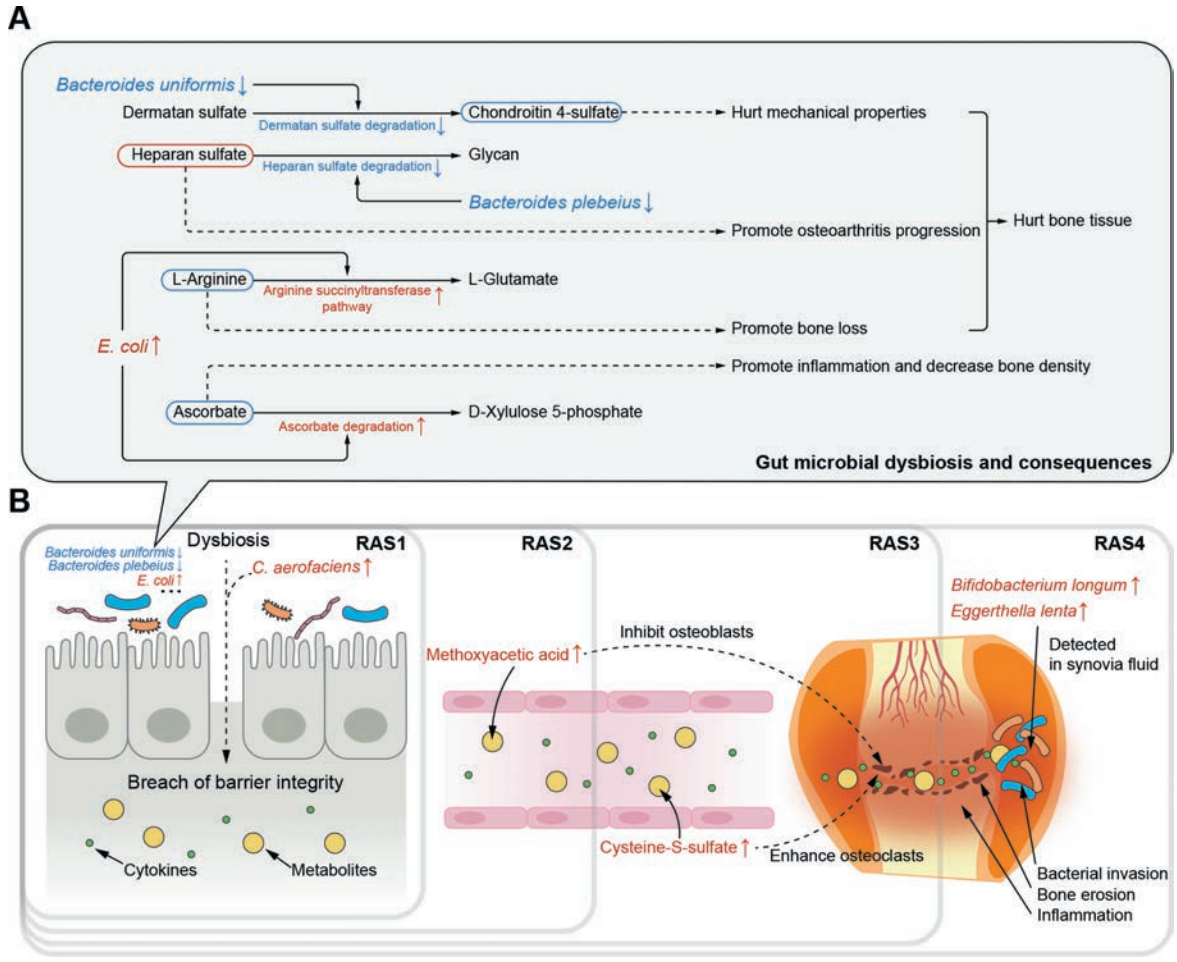

Figure 4 Potential pathogenesis across successive RA stages from multiomics perspective. (A) Potential mechanisms by which gut microbial dysbiosis play roles in RA pathogenesis through hurting hone tissue and increasing inflammation. The driving species, microbial dysfunction and related metabolites were extracted from figure 1B. The red or blue box of metabolites represents their speculated elevation or depletion according to the KEGG map. The dotted line represents the speculated effects of microbial and metabolic variation on arthritis pathogenesis. (B) The most representative effects of microbial dysbiosis and metabolic disorders on RA progression across successive stages. KEGG, Kyoto Encyclopaedia of Genes and Genomes; RA, rheumatoid arthritis.

The samples used for this study can fairly represent gut microbiome of each RA stage. Our hospital had tens of thousands of outpatients with arthritis per year and we have kept collecting samples from patients diagnosed with each stage of RA from 2017 to 2020. Considering the potential effects of clinical intervention on gut microbiome,[32] in this study, we only recruited samples of those patients who had not received treatment within 1 month and were in the active period. Therefore, the microbial dysbiosis and metabolic disorders depicted here could serve as a profound reference for future studies in each stage of RA.

Clinical microbial intervention should take into account the stage of RA. We found each RA stage had its special elevated or depleted microbes that played a role in RA pathogenesis (figure 4A). Hence, it may not be adequate for clinical guidance to generally report microbial alterations in RA without information of the stage, as many studies have done.[6 11] For instance, early inhibition of *C. aerofaciens* that was elevated exclusively in the first stage could help prevent increasing of gut permeability.[9] Additionally, inhibition of *E. coli* in the second and third stage could help maintain the content of L-arginine that acted as an inhibitor of bone lose,[24 25] as well as the content of anti-inflammatory ascorbate.[33] Moreover, certain species may need intervention across stages owing to its depletion during the whole RA progression. A cross-stages restoration of *B. uniformis* could help maintain the content of chondroitin 4-sulfate to keep mechanical properties of the articular cartilage.[12]

Moreover, metabolic alterations kept considerable throughout RA progression, in spite of which we found that certain of these metabolites need a higher priority of intervention in a specific stage. In the second stage of RA, the aberrant elevation of methoxyacetic acid might have inhibitory effects on osteoblasts and cause reductions in bone marrow cellularity[28–30] (figure 4B). The inhibited osteoblasts then drew the foreshadowing for the bone erosion that happened in the next stage. In the third stage, the considerable elevation of cysteine-S-sulfate might enhance the osteoclasts by NMDA-R interaction.[31] The imbalance between osteoblasts and osteoclasts would then promote bone erosion that happened in the third stage and persisted in the late RA stages. Thus, methoxyacetic acid may be a targeted metabolite for treatment to patients in the second stage of RA and serve as a precaution against the upcoming third stage.

Our findings suggested that bacterial invasion of joint synovial fluid happened in the fourth stage of RA (figure 4B). Joint synovial fluid was generally considered sterile, and indeed, we failed to either extract enough DNA or isolate bacteria from the synovial fluid in the first three stages. However, in the

fourth stage, we succeeded to obtain bacterial 16S reads, isolate bacteria and observe substances shaped like bacteria in rod-like or spherical forms under scanning electron microscopy. Moreover, in the multiomics cohort, we found two faecal microbes elevated exclusively in the fourth stage of RA, *E. lenta* and *B. longum*, and their existence in the joint synovial fluid was validated by the other cohort. It might due to the buildup of the continuous damages in gut barrier and microbes and microbial metabolites would then be transferred to the joints via blood.[6] Hence, for patients in the fourth stage of RA, in addition to routine medical therapies, specific treatments to the microbes in the joint synovial fluid may ameliorate the joint microenvironment to decrease synovial inflammation and inhibit potential bacterial effects.

This study also has limitations and prospects. First, a long-term follow-up investigation on a single individual throughout his/her RA development may reinforce the conclusions of this study. Second, it remains unclear how bacterial genetic materials are transferred from intestine to joint. It might be realised by bacteria transmission through blood or by means of extracellular vesicles or both. Third, the proposed links between microbial dysbiosis/metabolic disorders and RA can serve as a guidance for future experiments on RA pathogenesis. Lastly, additional researches into the synovial fluid microbiome and metabolome have the potential to reveal more sophisticated mechanisms underlying RA pathogenesis.

In conclusion, this study demonstrates microbial and metabolic roles in RA pathogenesis across four successive stages. A stage-specific intervention of microbial dysbiosis and metabolic disorders is warranted for prognosis and prevention of RA.

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Ethics approval The study was approved by the Ethics Committee of The First Affiliated Hospital of Shandong First Medical University (NO.2017-02 and NO.2020-011). Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available in a public, open access repository. Whole-genome shotgun sequencing data are available in the Genome Sequence Archive (GSA) section of the National Genomics Data Center (project accession number CRA004348). 16S rRNA gene sequencing data are available in the Genome Sequence Archive (GSA) section of the National Genomics Data Center (project accession number CRA005811). Plasma metabolomic data are available in the MetaboLights (project accession number MTBLS5297).

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EPIDEMIOLOGICAL SCIENCE

Isolated axial disease in psoriatic arthritis and ankylosing spondylitis with psoriasis

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ABSTRACT

Objectives To compare isolated axial psoriatic arthritis (PsA), axial PsA with peripheral involvement and isolated axial ankylosing spondylitis (AS) with psoriasis. To evaluate predictors for developing peripheral disease from isolated axial PsA over time.

Methods Two PsA and AS cohorts identified patients with PsA with axial disease and isolated axial patients with AS with psoriasis. Logistic regression compared isolated axial PsA to axial PsA with peripheral involvement and isolated axial AS with psoriasis. Cox proportional hazards model evaluated predictors for developing peripheral disease from isolated axial PsA.

Results Of 1576 patients with PsA, 2.03% had isolated axial disease and 29.38% had axial and peripheral disease. human leucocyte antigen HLA-B*27 positivity (OR 25.00, 95% CI 3.03 to 206.11) and lower Health Assessment Questionnaire scores (OR 0.004, 95% CI 0.00 to 0.28) were associated with isolated axial disease. HLA-B*27 also predicted peripheral disease development over time (HR 7.54, 95% CI 1.79 to 31.77). Of 1688 patients with AS, 4.86% had isolated axial disease with psoriasis. Isolated axial patients with PsA were older at diagnosis (OR 1.06, 95% CI 1.01 to 1.13), more likely to have nail lesions (OR 12.37, 95% CI 2.22 to 69.07) and less likely to have inflammatory back pain (OR 0.12, 95% CI 0.02 to 0.61) compared with patients with isolated axial AS with psoriasis.

Conclusions Isolated axial PsA and AS with psoriasis are uncommon. HLA-B*27 positivity is associated with isolated axial PsA and may identify those who develop peripheral disease over time. Isolated axial PsA is associated with better functional status. Isolated axial PsA appears clinically distinct from isolated axial AS with psoriasis.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

⇒ Pure axial disease is uncommon in psoriatic arthritis (PsA), comprising <5% of patients, while the remainder have concomitant peripheral involvement.

WHAT DOES THIS STUDY ADD?

⇒ Isolated axial patients with PsA may have better functional status when compared with those with concomitant peripheral disease. human leucocyte antigen-B*27 predicted the development of peripheral involvement from isolated axial PsA over clinic follow-up.

⇒ Isolated axial PsA appears distinct from isolated axial AS with psoriasis.

HOW MIGHT THIS IMPACT ON CLINICAL PRACTICE OR FUTURE DEVELOPMENTS?

⇒ Isolated axial PsA and isolated axial ankylosing spondylitis with psoriasis may be two distinct clinical phenotypes and may warrant different treatment approaches.

INTRODUCTION

Psoriatic arthritis (PsA) is a multisystem disease characterised by psoriasis and musculoskeletal manifestations.[1] The presentation of PsA can involve five distinct disease domains, including peripheral disease, axial disease, enthesitis, dactylitis, skin and nail disease.[2] Given the considerable clinical overlap between ankylosing spondylitis (AS) and PsA within the spondyloarthritis (SpA) family, cross-sectional studies in the past have sought to better delineate their associated disease features and clinical outcomes. Epidemiological studies have aimed to study their genetics, clinical features, imaging findings, prognosis and optimal treatment modalities.[3] Within the PsA disease entity, patients with axial only disease pose an area of research interest, given its similarities to AS. Pure axial involvement exists in less than 5% of all patients with PsA, while the majority of patients have concomitant peripheral involvement.[4] A recent longitudinal study concluded that axial PsA appears to be distinct clinically from AS and is associated with worse peripheral arthritis and less back pain.[5]

A cross-sectional study performed in 2017 found that patients with AS without psoriasis, those with axial PsA and those with peripheral PsA all had similar disease activity as measured by composite clinical indices, namely, the Ankylosing Spondylitis Disease Activity Score, metrology and disability scores as measured by the Health Assessment Questionnaire Disability Index.[6] A subsequent registry-based study has demonstrated a higher proportion of moderate/severe psoriasis, higher disease activity and lower quality of life among patients with PsA with axial disease.[7] However, the PsA population with isolated axial disease without peripheral involvement has not been exclusively studied. Furthermore, it is unknown at this time which clinical variables increase the chance of developing peripheral disease among patients with PsA with isolated axial disease at initial presentation. This is also clinically significant, as patients with axial disease have distinct disease characteristics that

Table 1 Clinical parameters between patients with PsA isolated axial disease versus those with concomitant peripheral disease at first presentation of axial disease

Variable	Isolated axial group (N=32)	Axial and peripheral group (N=463)	P value
Demographics			
Age	43.09 (14.06)	45.54 (13.22)	0.346
Male (%)	71.88	63.28	0.431
Caucasian (%)	87.50	85.53	0.963
Age at diagnosis of PsA in years	37.44 (12.36)	35.06 (13.36)	0.302
Age at diagnosis of psoriasis in years	25.78 (17.31)	27.32 (14.31)	0.627
Smoker (%)	59.38	46.65	0.226
Clinical features			
Sacroiliitis grade*	2.75 (0.67)	2.59 (0.69)	0.189
Enthesitis† (%)	3.13	14.90	0.068
Elevated ESR (%)	39.29	46.71	0.560
PASI	6.06 (8.00)	7.29 (9.61)	0.517
BSA	10.13 (18.30)	9.79 (18.01)	0.946
Nail lesion (%)	53.13	74.95	0.013
Uveitis (%)	18.75	9.72	0.185
Inflammatory bowel disease† (%)	6.25	7.78	1.000
Inflammatory back pain‡ (%)	50.00	33.96	0.172
Back metrology§			
Neck rotation (degrees)	67.81 (22.80)	71.84 (20.81)	0.506
Lateral flexion, Domjan method (degrees)	15.03 (5.78)	15.58 (4.54)	0.709
Schober test (cm)	4.07 (1.65)	4.52 (1.31)	0.263
Chest expansion (cm)	5.67 (2.76)	5.35 (2.65)	0.551
Comorbidities			
BMI	26.61 (6.08)	29.26 (6.61)	0.101
Cardiovascular disease† (%)	12.50	18.36	0.485
Diabetes† (%)	7.41	7.19	1.000
Patient-reported outcomes			
BASDAI	1.97 (1.73)	4.65 (2.58)	<0.001
Fatigue	2.90 (2.51)	5.32 (2.87)	0.015
Spinal pain	1.50 (1.90)	4.45 (3.20)	<0.001
Joint pain/swelling	1.30 (1.49)	4.61 (2.84)	<0.001
Areas of localised tenderness	1.40 (1.84)	4.41 (2.99)	<0.001
Morning stiffness severity	2.70 (2.58)	4.35 (3.11)	0.084
Morning stiffness duration	2.80 (3.16)	3.60 (3.02)	0.462
HAQ	0.16 (0.29)	0.68 (0.61)	<0.001
SF-36 physical	46.75 (10.36)	36.61 (12.07)	0.009
SF-36 mental	52.68 (8.66)	46.50 (12.11)	0.042
Human leucocyte antigen (HLA) types			
HLA-B†27 (%)	34.62	21.46	0.188
HLA-B†38† (%)	15.38	15.66	1
HLA-B†39† (%)	0	8.84	0.152
HLA-B†8† (%)	11.54	19.95	0.442
HLA-B†13 (%)	11.54	7.32	0.435
HLA-B†40† (%)	0	1.52	1
HLA-C†6 (%)	23.08	25.38	0.977
Medications			
NSAIDs (%)	50.00	69.98	0.031
DMARDs (%)	28.13	47.30	0.055
Biologics (%)	18.75	13.39	0.558

Continued

Table 1 Continued

Variable	Isolated axial group (N=32)	Axial and peripheral group (N=463)	P value

Where applicable, figures reported as mean (SD); % denotes percentage of patients in the respective groups;
*The sacroiliac joint with the highest grade was used preferentially for analysis.
†Fisher's exact test used due to low sample size in each sub-group.
‡Low back pain or neck pain and stiffness for more than 3 months that improves with exercise but is not relieved by rest.
§The side with the lowest numeric value was used preferentially for analysis, where applicable.
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BMI, body mass index; BSA, body surface area of psoriasis; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; NSAID, nonsteroidal anti-inflammatory drug; PASI, psoriasis area and severity index; PsA, psoriatic arthritis; SF-36, Short Form Health Survey.

may warrant and subsequently respond to different treatment approaches.[8–13]

Our previous study looked at all patients with PsA with axial disease.[5] The primary objective of this study was to compare patients with PsA with isolated axial disease (ie, do not have peripheral arthritis at presentation to the PsA clinic) to those with axial and peripheral disease. We also aimed to delineate predictors for developing peripheral disease in patients with PsA who present with isolated axial disease. Finally, we described the subset of patients with AS with axial disease with psoriasis who do not have peripheral disease (isolated axial disease) and compared their clinical features to patients with isolated axial PsA.

METHODS

Setting

This longitudinal study was conducted at the University of Toronto Psoriatic Arthritis Clinic, which is an observational cohort of patients with PsA and the University of Toronto Ankylosing Spondylitis Clinic, which is an observational cohort of patients with AS. At both clinics, patients are followed prospectively at 6–12 month intervals by a rheumatologist according to a standard protocol. All patients with PsA included in this study fulfilled the 2006 CASPAR criteria and have axial and/or peripheral inflammatory arthritis in the presence of psoriasis.[14] Patients in the AS cohort fulfilled the modified New York AS criteria.[15] The protocols recorded information on clinical, laboratory and radiographic variables at initial consultation and at each follow-up visit. At the time of analysis, 1576 patients were followed in the PsA cohort, while 1688 patients were followed in the AS cohort.

Patient selection and case definitions

The longitudinal single-centre PsA and AS cohorts were analysed to identify patients from cohort inception in January 1978 to October 2020 inclusive for the PsA cohort and July 2003 to November 2019 inclusive for the AS cohort. In general, patients with psoriasis and predominant peripheral symptoms are referred to the PsA clinic while those with predominant lower back symptoms are referred to the AS clinic for assessment. Patients with axial disease were identified from the database according to the presence of sacroiliitis on prior imaging. In this study, axial disease was defined as having features of ≥grade 2 sacroiliitis bilaterally or ≥grade 3 sacroiliitis unilaterally on radiographs of the sacroiliac joint, according to the modified New York criteria, and as interpreted by a radiologist with additional expertise in

Table 2 Clinical parameters between patients with PsA isolated axial disease versus those with isolated axial AS with psoriasis at first presentation of axial disease

Variable	Isolated axial PsA (N=32)	Isolated axial AS with psoriasis (N=82)	P value
Demographics			
Age	43.09 (14.06)	36.92 (12.57)	0.035
Male (%)	71.88	73.17	1
Caucasian (%)	87.50	76.83	0.362
Age at diagnosis of PsA/AS in years	37.44 (12.36)	29.65 (11.25)	0.003
Smoker (%)	59.38	39.02	0.079
Clinical features			
Enthesitis* (%)	3.13	7.32	0.671
Elevated ESR (%)	39.29	25.00	0.256
PASI	6.06 (8.00)	1.72 (2.08)	0.030
BSA	10.13 (18.30)	4.61 (9.03)	0.328
Nail lesion (%)	53.13	6.06	<0.001
Uveitis (%)	18.75	34.15	0.166
Inflammatory bowel disease* (%)	6.25	18.29	0.146
Inflammatory back pain† (%)	50.00	77.33	0.021
Back metrology‡			
Neck rotation (degrees)	67.81 (22.80)	66.57 (22.96)	0.849
Lateral flexion, Domjan method (degrees)	15.03 (5.78)	13.49 (5.95)	0.344
Schober test (cm)	4.07 (1.65)	4.00 (1.61)	0.870
Chest expansion (cm)	5.67 (2.76)	4.99 (2.04)	0.230
Comorbidities			
BMI	26.61 (6.08)	25.96 (4.50)	0.696
Cardiovascular disease* (%)	12.50	6.10	0.265
Diabetes* (%)	7.41	3.70	0.597
Patient reported outcomes			
BASDAI	1.97 (1.73)	4.23 (2.59)	0.002
Fatigue	2.90 (2.51)	5.05 (2.82)	0.027
Spinal pain	1.50 (1.90)	4.92 (3.04)	<0.001
Joint pain/swelling	1.30 (1.49)	2.93 (2.86)	0.011
Areas of localised tenderness	1.40 (1.84)	3.86 (3.29)	0.002
Morning stiffness severity	2.70 (2.58)	4.67 (3.15)	0.047
Morning stiffness duration	2.80 (3.16)	4.08 (3.45)	0.256
HAQ	0.16 (0.29)	0.59 (0.58)	<0.001
SF-36 physical	46.75 (10.36)	39.14 (10.47)	0.041
SF-36 mental	52.68 (8.66)	45.33 (12.53)	0.027
Human leucocyte antigen (HLA) types			
HLA-B*27 (%)	34.62	75.95	<0.001
Medications			
NSAIDs (%)	50.00	60.98	0.392
DMARDs (%)	28.13	10.98	0.049
Biologics (%)	18.75	59.76	<0.001

Continued

Table 2 Continued

Where applicable, figures reported as mean (SD); % denotes percentage of patients in the respective groups.
*Fisher's exact test used due to low sample size in each sub-group.
†Low back pain or neck pain and stiffness for more than 3 months that improves with exercise but is not relieved by rest.
‡The side with the lowest numeric value was used preferentially for analysis, where applicable.
§The sacroiliac joint with the highest grade was used preferentially for analysis.
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BMI, body mass index; BSA, body surface area of psoriasis; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; NSAID, nonsteroidal anti-inflammatory drug; PASI, psoriasis area and severity index; PsA, psoriatic arthritis; SF-36, Short Form Health Survey.

musculoskeletal imaging.[15 16] Furthermore, those with isolated axial disease were characterised by the absence of inflammatory peripheral arthritis, damaged joints and/or dactylitis documented on clinical examination or imaging at any point in the patient's clinical course, leading up to the initial presentation of axial disease, whereas those with axial PsA and peripheral disease were characterised as having inflammatory peripheral joint involvement, damaged joints and/or dactylitis along with axial disease, at any point prior to first presentation of axial disease. For patients in the AS cohort, the presence of psoriasis was defined as at least one documented occurrence of psoriasis from initial consultation to most recent follow-up, or any previous history of psoriasis as diagnosed by a rheumatologist or dermatologist. Hence, the presence of isolated axial AS with psoriasis is defined as patients with AS and psoriasis but without peripheral involvement, which is likewise defined as the absence of inflammatory peripheral arthritis, damaged joints and/or dactylitis.

Data collection

For the PsA cohort, variables on demographics, clinical features, comorbidities, patient-reported outcomes, human leucocyte antigen (HLA) types and treatments at first presentation of axial disease in addition to each subsequent follow-up visit for those with isolated axial disease and for those with concomitant peripheral disease were retrieved for analysis, if collected and available in the database. For the AS cohort, clinical features were collected at the baseline clinic visit only.

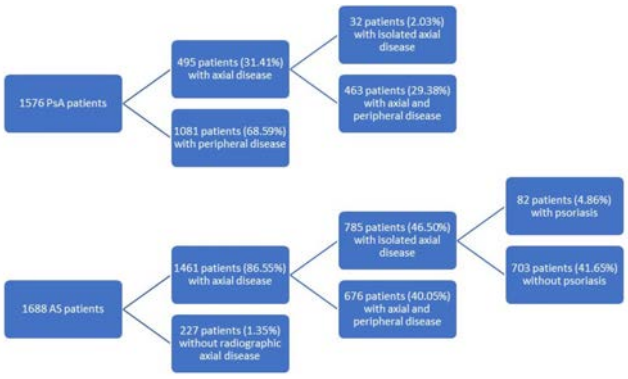

Figure 1 Flow diagram of the patients among the PsA and AS cohorts at baseline visit. AS, ankylosing spondylitis; PsA, psoriatic arthritis.

Table 3 Logistic regression analysis for factors associated with isolated axial PsA at first presentation of axial disease adjusted for sex and age at PsA diagnosis (N=495)

Sex (male)				0.588	(0.097, 3.570)	0.564
Age at diagnosis of PsA in years				1.061	(0.990, 1.138)	0.095
Sacroiliitis grade*	1.332	(0.811, 2.188)	0.257	2.021	(0.698, 5.852)	0.195
Enthesitis	0.176	(0.024, 1.312)	0.090	0.000	(0.000, ∞)	0.995
Elevated ESR	0.689	(0.312, 1.521)	0.357	3.298	(0.599, 18.173)	0.170
Nail lesion	0.357	(0.171, 0.745)	0.006	0.445	(0.075, 2.633)	0.372
HLA-B*27	2.013	(0.848, 4.779)	0.113	25.000	(3.033, 206.114)	0.003
Uveitis	2.195	(0.854, 5.644)	0.103	0.859	(0.040, 18.311)	0.922
HAQ	0.063	(0.008, 0.502)	0.009	0.004	(0.000, 0.284)	0.010
SF-36 PCS	1.080	(1.014, 1.151)	0.017	0.919	(0.817, 1.034)	0.161

*The sacroiliac joint with the highest grade was used preferentially for analysis.
ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; PsA, psoriatic arthritis; SF-36 PCS, Short Form Health Survey Physical Health.

Statistical analyses

Descriptive statistics determined the percentages of patients with isolated axial and axial with peripheral disease among the PsA cohort, in addition to the percentage of patients with isolated axial disease with psoriasis in the AS cohort. Student's two sample t tests determined differences between baseline continuous variables and χ^2 tests or Fisher's exact tests for baseline categorical variables, all at first presentation of axial disease between the two groups. Univariate and multivariate logistic regression models, adjusted for sex and age at PsA diagnosis, was performed to calculate ORs for presenting with isolated axial disease, when compared with patients with concomitant axial and peripheral disease at initial presentation of axial disease. Subsequently, survival analysis was performed to determine covariates that predicted the development of concomitant peripheral disease over time, in patients with PsA with isolated axial disease at presentation. This was done using Cox proportional hazards models using baseline and time-dependent covariates. Patients with axial disease who did not develop peripheral disease during the entire follow-up period were right censored at their last clinic visit. Survival models were adjusted for sex and age at PsA diagnosis. Time to the event of developing peripheral disease was measured from time of first radiographic evidence of axial disease. Finally, univariate and multivariate logistic regression models, adjusted for sex and age at PsA/AS diagnosis, were performed to compare clinical features associated with patients with isolated axial patients with PsA versus AS with isolated axial disease with psoriasis.

For all models, missing data were imputed from the closest previous clinic visit data point, if available. All statistically significant thresholds were set at $p<0.05$. Statistical analysis was performed using R V.4.0.5. Informed consent was obtained for the patients who participated in the cohort and the study was approved by the University Health Network Research Ethics Board (REB 18–5538).

RESULTS

Patients in the PsA cohort were compared with those in the AS cohort (tables 1 and 2). Of the 1576 patients with PsA in the cohort, 495 (31.41%) had axial disease at presentation to the clinic. Of those, 32 patients (2.03%) had isolated axial disease and 463 patients (29.38%) had axial with peripheral disease (figure 1). At first presentation of axial disease, significantly fewer patients with isolated axial disease had nail lesions or used non-steroidal anti-inflammatory drugs. Furthermore, those with

Table 4 Cox proportional regression for the development of peripheral disease among PsA patients who presented with isolated axial disease at first presentation of axial disease adjusted by sex and age at diagnosis of PsA (N=32)

	Univariate model		
Variable	**HR**	**95% CI**	**P value**
Sex (male)			
Age at diagnosis of PsA in years			
Sacroiliitis grade*	0.899	(0.466 to 1.733)	0.751
Enthesitis	0.940	(0.121 to 7.342)	0.953
Elevated ESR	0.626	(0.230 to 1.708)	0.360
Nail lesion	0.826	(0.319 to 2.141)	0.694
PASI	1.030	(0.960 to 1.106)	0.408
Uveitis	2.130	(0.786 to 5.769)	0.137
HLA-B*27	1.069	(0.394 to 2.901)	0.896
NSAIDS	1.009	(0.434 to 2.346)	0.983
DMARDS	0.825	(0.341 to 1.993)	0.669
Biologics	1.137	(0.381 to 3.392)	0.818

*The sacroiliac joint with the highest grade was used preferentially for analysis.
DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; NSAID, non-steroidal anti-inflammatory drug; PASI, psoriasis area and severity index; PsA, psoriatic arthritis.

Table 5 Time-dependent univariate Cox proportional regression for the development of peripheral disease among PsA patients who presented with isolated axial disease, adjusted by sex and age at diagnosis of PsA (N=32)

Variable	**HR**	**95% CI**	**P value**
Sex (male)			
Age at diagnosis of PsA in years			
Sacroiliitis grade*	1.302	(0.497 to 3.406)	0.591
Enthesitis	0.000	(0.000 to ∞)	0.998
Elevated ESR	1.292	(0.454 to 3.672)	0.631
Nail lesion	0.984	(0.388 to 2.500)	0.974
PASI	1.039	(0.984 to 1.098)	0.167
Uveitis	2.752	(0.883 to 8.572)	0.081
HLA-B*27	7.544	(1.792 to 31.769)	0.006
NSAIDS	0.924	(0.322 to 2.655)	0.884
DMARDS	1.454	(0.570 to 3.710)	0.434
Biologics	1.086	(0.335 to 3.515)	0.891

*The sacroiliac joint with the highest grade was used preferentially for analysis.
DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; NSAID, nonsteroidal anti-inflammatory drug; PASI, psoriasis area and severity index; PsA, psoriatic arthritis.

Table 6 Logistic regression analysis for factors associated with isolated axial PsA at first presentation of axial disease adjusted for sex and age at psa/as diagnosis among the isolated axial PsA and isolated axial AS with psoriasis cohorts (N=114)

	Univariate model			Multivariate model		
Variable	OR	95% CI	P value	OR	95% CI	P value
Sex (male)				0.638	(0.131 to 3.106)	0.578
Age at diagnosis of PsA or AS in years				1.063	(1.002 to 1.129)	0.043
Nail lesion	17.295	(4.923 to 60.760)	<0.001	12.370	(2.215 to 69.073)	0.004
Inflammatory back pain	0.170	(0.054 to 0.537)	0.003	0.116	(0.074 to 1.320)	0.011
HLA-B*27	0.200	(0.074 to 0.540)	0.001	0.312	(0.074 to 1.320)	0.113
Biologics	0.125	(0.043 to 0.368)	<0.001	0.319	(0.074 to 1.380)	0.126

AS, ankylosing spondylitis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; HAQ, Health Assessment Questionnaire; PsA, psoriatic arthritis.

isolated axial disease also had significantly lower Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Health Assessment Questionnaire (HAQ) scores but higher short form health survey (SF-36) physical and mental scores, when compared with patients with axial and peripheral disease (table 1). Of note, the lower BASDAI scores associated with patients with isolated axial disease were across all individual components with fatigue, spinal pain, joint pain/swelling and areas of localised tenderness reaching statistical significance. In the AS cohort, 82 (4.86%) of the 1688 patients had isolated axial disease with psoriasis (figure 1). When compared with patients with AS with isolated axial disease with psoriasis at first presentation of axial disease, those with isolated axial PsA were older, had a later age of diagnosis, had higher Psoriasis Area Severity Index scores, a higher chance of having psoriatic nail lesions but a lower chance of having inflammatory back pain, a lower chance of HLA-B*27 positivity as well as were less likely to be on biologics but more likely to be on a disease-modifying antirheumatic drug. From a functional perspective, they had lower BASDAI, HAQ scores and higher SF-36 physical and mental scores (table 2). The lower BASDAI scores associated with patients with isolated axial PsA were across all individual components with fatigue, spinal pain, joint pain/swelling, areas of localised tenderness and morning stiffness severity reaching statistical significance.

In univariate logistic regression analysis for the 495 patients with PsA with axial disease irrespective of peripheral involvement, isolated axial disease was significantly associated with a lower probability of having nail lesions (OR 0.357, 95% CI 0.171 to 0.745, p<0.006), lower HAQ scores (OR 0.063, 95% CI 0.008 to 0.502, p<0.009) and higher SF-36 PCS scores (OR 1.080, 95% CI 1.014 to 1.151, p<0.017) at first presentation of axial disease, when adjusted for sex and age at PsA diagnosis, when compared with patients with concomitant axial and peripheral disease. Full protocol data were available for 237 patients to perform multivariate logistic regression analysis. In the multivariate model, HLA-B*27 positivity (OR, 25.000, 95% CI 3.033 to 206.114, p<0.003) and lower HAQ scores (OR 0.004, 95% CI 0.000 to 0.284, p<0.010) were significantly associated with isolated axial disease when compared with patients with concomitant axial and peripheral disease (table 3).

For the 32 patients with isolated axial disease, 25 ultimately developed peripheral disease by most recent clinic follow-up. Survival analysis using univariate Cox proportional-hazards models adjusted for sex and age of PsA diagnosis did not reveal any significant predictors for the development of peripheral disease, at first presentation of axial disease (table 4). However, with time-dependent variables over clinic follow-up, HLA-B*27 positivity was associated with the development of peripheral disease (HR 7.544, 95% CI 1.792 to 31.769, p<0.006) (table 5). The development of multivariate models was not possible due to the small sample size of the survival models (n=32).

In univariate logistic regression analysis for the 114 patients with isolated axial disease within both PsA and AS cohorts, those in the PsA cohort were more likely to have nail lesions (OR 17.295, 95% CI 4.923 to 60.760, p<0.001), but less likely to have inflammatory back pain (OR 0.170, 95% CI 0.054 to 0.537, p<0.003), to have HLA-B*27 positivity (OR 0.200, 95% CI 0.074 to 0540, p<0.001) and to be on biologics (OR 0.125, 95% CI 0.043 to 0.368, p<0.001) compared with the subset of AS patients with psoriasis. In the multivariate model, those in the PsA cohort were older (OR 1.063, 95% CI 1.002 to 1.129, p<0.043), more likely to have nail lesions (OR 12.370, 95% CI 2.215 to 69.073, p<0.004) and less likely to have inflammatory back pain (OR 0.116, 95% CI 0.022 to 0.606, p<0.011) (table 6).

DISCUSSION

In our PsA cohort, 2.03% patients had isolated axial disease at first presentation of axial disease, which is congruent with previously reported studies, citing a range between 2% and 5% of all patients with PsA.[16–18] While severe peripheral arthritis and HLA-B*27 positivity have been previously found to be risk factors for axial PsA, whether patients with PsA with isolated axial disease represent a distinct clinical phenotype has not been well studied thus far.[18] Our data suggest that those with isolated axial disease appear to have a milder form of PsA than those with axial and concurrent peripheral disease at baseline. The signal towards better patient-reported outcomes in the isolated axial group was reinforced in logistic regression analysis, with lower HAQ scores both in univariate and multivariate models compared with those patients with axial and peripheral disease.

Furthermore, in multivariate logistic regression, genetic factors appeared to influence the chances of presenting with isolated axial disease, as HLA-B*27 positivity was found to increase the odds by 25 times when compared with patients with PsA with concomitant axial and peripheral involvement, suggesting that HLA-B*27 may be used to identify patients with isolated axial disease at baseline. Patients with HLA-B*27 positivity were also found to have a higher likelihood of developing peripheral disease from isolated axial disease. In general, the impact of HLA risk alleles has been studied among patients with PsA of various clinical phenotypes. The association between HLA-B*27 and psoriatic spondylarthritis has been well established in prior cohort studies, but not within the subset of patients with PsA with isolated axial disease.[6] A 2012 study demonstrated an increase in HLA-B*39 prevalence positivity in patients with axial PsA compared with patients with psoriasis, while other studies

have previously linked HLA-B*27, HLA-B*08, HLA-Cw* 07:02 and HLA-B*38 to axial PsA.[19–21]

Additionally, we aimed to specifically study and compare the subset of patients with AS with isolated axial disease and psoriasis to patients with isolated axial PsA. While axial disease and psoriasis in AS have been evaluated before in the literature individually, isolated axial disease without peripheral involvement accompanied by concomitant psoriasis has not.[5 22–25] Compared with a recent study which found that 12% of patients with AS have concurrent psoriasis, our data indicate that 4.86% of patients with AS have isolated axial disease with psoriasis, which is higher than the 2.03% of patients with isolated axial disease in PsA.[5] As per our logistic regression analysis, isolated axial PsA appears to be a different clinical entity than isolated axial AS with psoriasis, with older age at diagnosis, a higher chance of nail lesions and lower odds of inflammatory back pain. This is congruent with a 2020 study, which compared the whole group of axial PsA, irrespective of peripheral involvement, to both AS as an umbrella group in addition to AS with psoriasis.[5] Hence, this study further solidifies the concept that axial PsA is indeed different from AS.

To our knowledge, this is the first study in the literature to exclusively evaluate the prevalence of and factors associated with isolated axial disease within the general umbrella of axial PsA, differentiating patients with concomitant peripheral involvement. It is also the first study to exclusively study isolated axial AS with psoriasis. The advantages of our study include long-term follow-up data as well as the comprehensiveness of clinical parameters collected in our research protocol, facilitating analysis of two cohorts within the SpA family. Moreover, the weakness of small sample size in our PsA patient group with axial only disease (n=32) within our study can be attributed to the rarity of isolated axial PsA within the PsA clinical phenotypes, comprising only 2.03% of our cohort. As a result, despite our PsA cohort having 1576 patients at the time of data collection, only 32 patients had isolated axial disease. This low patient number limited our model development, precluding multivariate analysis. Moreover, a potentially non-modifiable limitation lies in the classification of patients with PsA as having isolated axial disease versus AS with psoriasis, as this is not based on existing classification criteria, leading to natural overlap. By using objective disease activity and characteristic markers present in our protocolised data within our two cohorts, referral bias to each individual clinic (PsA vs AS) can hopefully be minimised. Another limitation inherent to the retrospective cohort design of this study is that we are relying on radiographs in defining axial involvement. The lack of MRI data in our study population results in a potential underreporting of axial disease in our cohorts.

Recognising the importance of evaluating this important subset of patients with PsA, international efforts are being made to recruit patients for a multinational, multicentre study to better evaluate the impact of axial involvement in PsA via the Axial Involvement in Psoriatic Arthritis Cohort (AXIS).[26] The AXIS cohort will hopefully be able to address other risk factors for the development of peripheral disease over time, including further exploring the role of HLA-B*27 positivity, as our findings were unfortunately limited by the small sample size of patients presenting with isolated axial PsA.[27] From a methodological perspective, with the development of larger cohorts of patients with PsA with isolated axial disease, hopefully future analysis using multistate models can help determine predictors for transitions between axial and peripheral disease in this seldomly studied population.

Patients with isolated axial disease represent a small subset of total patients, and in PsA, accounts for 2.03% of patients in our cohort. Whether patients with PsA present with axial disease by coincidence versus real inflammatory back pain on presentation is an important area of focus for future study. Based on the clinical parameters in table 1, whereby only 50% of patients in the isolated axial group had inflammatory back pain symptoms, we would favour a large contributor being incidental axial disease picked up on imaging. As our data suggest, those with isolated axial PsA have a significantly higher chance of HLA-B*27 positivity, and better functional status as evidenced by improved HAQ scores at first presentation of axial disease, compared with those with concomitant peripheral involvement. Moreover, HLA-B*27 positivity appears to be a predictor for the development of peripheral disease among patients who present with isolated axial disease, though analysis was limited by small sample size. When isolated axial AS with psoriasis was evaluated in our study, this subset of patients comprised 4.86% of patients with AS in our cohort. Furthermore, isolated asial PsA patients were differentiated from isolated axial AS with psoriasis patients by an older age at diagnosis, a higher chance of having psoriatic nail lesions and lower chance of having inflammatory back pain.

While there may be different opinions regarding nomenclature of axial disease, and whether axial PsA is just axSpA our patients all fulfilled CASPAR criteria for PsA, and the majority accrued peripheral disease over time. If the group with isolated axPsA at presentation was the same as AS without peripheral arthritis, one would expect there to be no differences in clinical parameters between these groups. Our data indicate that the group with axial PsA was indeed different from the group with AS, supporting the validity of our findings and conclusions in the manuscript.

Given the paucity of studies focused on the uncommon clinical phenotype of isolated axial disease within the SpA family, more research is needed to further evaluate longitudinal clinical outcomes among those with isolated axial disease, including the possible use of multistate models to evaluate the impact of clinical changes such as peripheral involvement over time. Hopefully, with the upcoming recruitment for the AXIS cohort, and continued international collaboration, we may better understand the subgroup of patients with PsA with isolated axial disease. By extension, more longitudinal studies are required to study the subset of patients with AS with isolated axial disease with psoriasis, including associations for the development of peripheral disease over time as well as prognosis compared with those with peripheral disease.

Contributors TSHK, MS, RJC and D-DG were involved in study design. D-DG, VC, DP, NH and RDI were involved in patient recruitment. TSHK and MS were involved in data collection and analysis. TSHK prepared the initial draft and all authors were involved in reviewing the manuscript and providing critical comments.

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

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by University Health Network Research Ethics Board. Participants gave informed consent to participate in the study before taking part.

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CLINICAL SCIENCE

Low-dose interleukin-2 therapy in active systemic lupus erythematosus (LUPIL-2): a multicentre, double-blind, randomised and placebo-controlled phase II trial

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ABSTRACT

Objectives A regulatory T cell (Treg) insufficiency due to shortage of interleukin-2 (IL-2) is central to the pathophysiology of systemic lupus erythematosus (SLE). We performed a multicentre, double-blinded, randomised, placebo-controlled phase II proof-of-concept trial to evaluate the efficacy of low-dose IL-2 therapy in patients with SLE having moderate-to-severe disease activity while receiving standard treatment.

Methods We randomly assigned 100 patients in a 1:1 ratio to receive either 1.5 million IU/day of subcutaneous IL-2 (ILT-101) or placebo for 5 days followed by weekly injections for 12 weeks. Clinical efficacy was assessed at week 12 in a predefined hierarchical analysis of (1) the SLE responder index-4 (SRI-4) response as a primary end point, and of (2) relative and (3) absolute changes in the Safety of Estrogens in Lupus Erythematosus National Assessment-Systemic Lupus Erythematosus Disease Activity Index scores as key secondary end points.

Results The primary end point was not met in the intention-to-treat population (ILT-101: 68%, placebo: 58%; p=0.3439), due to a 100% SRI-4 response rate in the placebo group from the two sites from Bulgaria. A post hoc per-protocol analysis on a prespecified population that excluded patients from these two sites (n=53) showed a statistically significant difference for the SRI-4 response rate (ILT-101: 83.3%; placebo: 51.7%; p=0.0168), and for the two key secondary end points, accompanied by differences in several secondary exploratory end points. ILT-101 was well tolerated and there was no generation of antidrug antibodies.

Conclusions The post hoc hierarchical analysis of the primary and key secondary end points in a per-protocol population, complemented by the exploratory analyses of multiple other secondary end points, support that low-dose IL-2 is beneficial in active SLE.

Trial registration number NCT02955615.

WHAT IS ALREADY KNOWN ON THIS TOPIC?

⇒ Preclinical studies provided evidence that an acquired deficiency of the cytokine interleukin-2 (IL-2) and associated disturbances in regulatory T cell (Treg) homeostasis play a crucial role in the pathogenesis of systemic lupus erythematosus (SLE).

⇒ Up to now, there are no approved treatments available that address the modulation of Treg biology by low-dose IL-2 therapy in SLE.

⇒ Several early phase uncontrolled phase I/II pilot trials suggested that expansion of the Treg population by low-dose IL-2 therapy is safe and could be effective in reducing disease activity in patients with active and refractory SLE.

⇒ More recently, when data analysis of our trial was still ongoing, the results from one single-centre randomised placebo-controlled trial were reported showing that the primary end point was close to statistical significance and several secondary efficacy end points were achieved.

WHAT THIS STUDY ADDS

⇒ LUPIL-2 is the first international, multicentre, randomised and placebo-controlled phase II clinical trial evaluating the safety and efficacy of low-dose IL-2 therapy with ILT-101 (aldesleukin) as add on to standard therapies in patients with moderate-to-severe SLE.

⇒ The results of this trial confirm that low-dose IL-2 therapy can safely and selectively expand the Treg population and is capable to reduce disease activity in patients with SLE.

INTRODUCTION

In health, there is a homeostatic balance between regulatory T cells (Treg) and effector T cells (Teff) that prevents the development of inflammation and autoimmunity.[1] In many autoimmune diseases, this homeostasis is breached because of a Treg cell insufficiency. In systemic lupus erythematosus (SLE), an autoimmune disease characterised by generalised loss of immune tolerance leading to autoantibody production and inflammation of multiple organs, the role of Treg cells has been extensively documented.[2 3] In brief, while interleukin-2 (IL-2) is the key cytokine promoting Treg homeostasis, survival and fitness, IL-2 production and signalling are impaired in patients with SLE and mouse models of SLE.[4–8] As a result of low IL-2 availability, Treg from patients with SLE are unfit, expressing low amounts

⇒ Although the primary efficacy end point in the intention-to-treat population was not met, hierarchical post hoc analyses in a per -protocol population revealed statistically significant differences between the ILT-101 and the placebo group in the primary efficacy end point (systemic lupus erythematosus responder index-4 response) and in several important secondary efficacy end points including the two key secondary end points.
⇒ We further provide evidence that the clinical outcome is associated with the magnitude of the Treg response supporting the concept that clinical efficacy of low-dose IL-2 is driven by Treg activation and expansion.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The results of this trial in conjunction with data from previous studies justify the further development of low-dose IL-2 therapy in SLE and other autoimmune diseases and provide a valuable scientific basis for the design of confirmatory phase III clinical trials in SLE.
⇒ Low-dose IL-2 therapy can be considered a promising targeted treatment strategy in SLE with a unique mode of action which may have potential to change the current therapeutic concept in autoimmune diseases from immunosuppression to the boosting of physiological mechanisms of immune regulation.

of IL-2Rα, the key component of the high-affinity IL-2 receptor. This low IL-2Rα expression correlates with disease activity and anti-double-stranded DNA antibody (anti-dsDNA-Ab) levels, underlining its pathophysiological relevance and can be relieved by in vitro and in vivo stimulation with IL-2.[9] Therefore, stimulation of Treg cells with IL-2 should have therapeutic value in SLE.

Besides its ability to expand Treg, IL-2 also blocks the differentiation of $CD4^+$naïve T cells into proinflammatory T helper 17 (Th17) cells or T follicular helper (Tfh) cells. This should lead to reduced inflammation and autoantibody production, respectively,[10–12] and should thus be beneficial in SLE. In accordance with these properties, IL-2 has been reported to suppress disease in >30 experimental inflammatory and autoimmune diseases in mice.[10 13] In three different mouse models of SLE, treatment with low-dose IL-2 ($IL\text{-}2_{LD}$) expanded the Treg population and improved the course of disease.[14–19]

Following the initial demonstration that, in humans, $IL\text{-}2_{LD}$ can stimulate and expand Treg without activating Teffs in systemic vasculitis[20] and Graft versus Host Disease (GvHD),[21] several subsequent studies have suggested safety and clinical efficacy of $IL\text{-}2_{LD}$ therapy in numerous autoimmune diseases.[13 22] Recently, we reported a robust expansion of Treg cells and clinical benefit following the administration of $IL2_{LD}$ in patients with 11 different autoimmune-autoinflammatory diseases, suggesting a causal relationship.[23] However, while Treg are widely seen as targets for novel therapies of autoimmune diseases and IL-2 as a first-in-class Treg activator, it is noteworthy that the clinical efficacy of $IL2_{LD}$ has not yet been documented in multicentre, double-blind, randomised and placebo-controlled trials. In patients with SLE, $IL\text{-}2_{LD}$ was first shown to be remarkably efficient in the treatment of a single patient with severe refractory SLE.[24] Subsequently, three open-label studies[9 23 25 26] and one single-centre randomised controlled trial (RCT)[27] reported clinical improvement. We report here the results of an international multicentre RCT done at 36 clinical sites from 10 different countries that investigated the clinical efficacy, safety and Treg responses of $IL\text{-}2_{LD}$ therapy in patients with SLE having a moderate-to-severe disease activity.

METHODS

Study design and participants

LUPIL-2 was a multicentre, randomised, double-blind, placebo-controlled phase II study evaluating the safety, clinical efficacy and biological responses of ILT-101 (aldesleukin) subcutaneously administered for 12–24 weeks in patients with moderately to severely active SLE. The trial was set up at 36 sites in Austria, Bulgaria, France, Germany, Italy, Mauritius, Mexico, Portugal, Romania and Spain. Patients were included in 22 sites in all countries but Austria and Italy.

Eligible patients were aged 18 years or older with a confirmed diagnosis of SLE according to the revised classification criteria of the American College of Rheumatology from 1997 (presence of at least four criteria) or of the Systemic Lupus International Collaborating Clinics and having a moderate-to-severe disease activity characterised by a Safety of Estrogens in Lupus Erythematosus National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) score of at least 6 at baseline and the presence of antinuclear antibodies with a titre of ≥1:160 including a positivity for at least one of the following SLE-associated autoantibodies: anti-dsDNA-Abs, anti-Smith antibodies, anti-Sjögren's syndrome-related antigen A antibodies (anti-Ro-Abs) or antiphospholipid antibodies (anti-cardiolipin-Abs, anti-beta-2-glycoprotein-Abs or lupus anticoagulant). Patients had to be under stable background therapy (dose and type): glucocorticoids (GC) at doses <30 mg/day or 0.5 mg/kg/day (whichever was the lowest) and antimalarial drugs must have been stable for at least 1 month before first dosing, and allowed immunosuppressive therapies (mycophenolate mofetil, leflunomide, thalidomide, methotrexate and azathioprine) for at least 2 months. Adjustments in dose or class of background therapy were not permitted during the study, with the exception of GC with the following rules: for patients with a daily GC dose of >20 mg at inclusion, any improvement of the SELENA-SLEDAI to a score ≤6 together with a systemic lupus erythematosus responder index-4 (SRI-4) response from week 4 triggered a reduction of the daily GC dose by 2.5 mg of prednisone (or equivalent) every week to the limit of 15 mg/day up to week 12. After week 12, any improvement of the SELENA-SLEDAI down to a score ≤6 together with an SRI-4 response triggered a reduction of the daily GC dose by 2.5 mg every week up to 7.5 mg/day (for additional information on patients and eligibility criteria, see online supplemental methods, appendix pp 12–13; 17–18).

Randomisation and masking

After confirmation of their eligibility at baseline, patients were randomised in a 1:1 ratio to receive either ILT-101 (n=50) or placebo (n=50). Treatment randomisation was performed through an interactive web response system (IWRS), and the randomisation list prepared by the independent unblinded statistician was uploaded into the IWRS. Patients, investigators, nurses, people involved in the evaluation of patients and data managers were kept blinded to the treatment allocation for the whole duration of the study and up to the final database lock. The study sponsor remained masked to the individual treatment arm allocation up to the freezing of the database at the end of the study.

Treatment

ILT-101 at a dose of 1.5 million IU/day or placebo was administered by subcutaneous injection every day for consecutive 5 days (induction period) and then once every week from day 8 to week 12 (maintenance period). Patients who met the SRI-4 criteria without treatment failure at week 12 (responders) continued to receive ILT-101 or placebo once every week until week 24 (extended maintenance period). These patients were followed up for another 12 weeks after the last treatment administration until week 36. Patients who did not have an SRI-4 response at week 12 (non-responders) discontinued treatment after week 12 and were followed up for another 12 weeks after the last treatment administration until week 24.

End points

The primary efficacy end point was the proportion of patients who achieved a SRI-4 response at week 12, defined as a reduction by at least 4 points in the SELENA-SLEDAI score as compared with baseline, no new British Isles Lupus Assessment Group (BILAG) A or ≤1 new BILAG B score and no deterioration from baseline in the PGA score by ≥0.3 points, without any treatment failure during the first 12 weeks in the ILT-101 group compared with the placebo group. Treatment failure was defined as any worsening of the patient's condition requiring an increased dose of GC that was above the baseline dose or any change in dose or class of background therapies other than GC. Key secondary end points were the absolute and relative change in the SELENA-SLEDAI score from baseline to week 12. Additional secondary efficacy end points included the SRI-4 response at week 8 without treatment failure, and the SRI-6 and SRI-8 response at week 12, defined as a reduction by at least 6 or 8 points, respectively, in the SELENA-SLEDAI score as compared with baseline, no new BILAG A or ≤1 new BILAG B score, and no deterioration from baseline in the PGA score by ≥0.3 points, without treatment failure. The SRI-4, SRI-6 and SRI-8 response was also evaluated at all other visits, and the time to first SRI-4 response was calculated, as well as the proportion of patients in remission (defined as patients with a SELENA-SLEDAI score ≤2) at all visits. The absolute and relative change from baseline in SELENA-SLEDAI score, defined as the two key secondary end points, was determined at each visit. The number of patients with mild/moderate or severe flares was evaluated at each visit using the SELENA-SLEDAI Flare Index, and the time to flare was calculated. The daily dose of GC was recorded at each visit. Absolute changes from baseline in levels of anti-dsDNA-Abs and of the complement factors C3 and C4 were analysed at different time points during the study. The numbers and percentages of $CD3^+CD4^+FoxP3^+CD127^{lo}CD25^{hi}$ Treg and of other immune cells were measured by flow cytometry at different time points during the study and the change from baseline was calculated for each patient and treatment arm. Within each treatment arm, patients were subclassified into responders and non-responders and Treg responses were compared in between. Additional information on efficacy, safety and biological assessments are mentioned in online supplemental methods, appendix pp 12-16.

Statistical analysis

A hierarchical statistical analysis was planned to test in a sequential order the primary end point (step 1), the relative change from baseline in the SELENA-SLEDAI score at week 12 (first key secondary end point, step 2) and the absolute change from baseline in the SELENA-SLEDAI score at week 12 (second key secondary end points, step 3). This step-down testing procedure, which obeys the closed testing principle, strongly controls the overall type I error at the 0.05 two-sided level. For exploratory end points, no adjustments for multiplicity were planned.

The primary efficacy end point was analysed using a logistic regression model (ILT-101 vs placebo). The relative and absolute changes from baseline in the SELENA-SLEDAI score at week 12 were analysed in a mixed model for repeated measures assuming an unstructured covariance matrix and including treatment, baseline SELENA-SLEDAI score, intake of GC at baseline (none or >0 and ≤7.5 mg or >7.5 mg), country, visit (week 4, week 8 and week 12) and treatment-by-visit interaction.

Additional statistical analyses are described in the online supplemental methods, appendix p 14.

RESULTS

Between 20 February 2017 and 30 May 2018, 189 patients were screened for eligibility and 100 patients were randomly assigned in a 1:1 ratio to receive either ILT-101 (n=50) or placebo (n=50) (figure 1). Baseline disease characteristics and background therapy were balanced between the treatment groups and indicated moderate-to-severe disease activity (table 1).

In the intention-to-treat (ITT) population, the proportion of patients who achieved a SRI-4 response without treatment failure at week 12 was 68.0% (n=34) in the ILT-101 group and 58.0% (n=29) in the placebo group (difference 10.0%, OR 1.52, 95% CI 0.64 to 3.60, p=0.3439) (figure 2A, table 2). Thus, in this population, the primary end point was not met.

The response rate in the placebo group of the ITT population (58.0%) was unexpectedly high.[27–33] Covariate analyses revealed a relevant country effect that was attributed to two sites from the same country where the SRI-4 response rate in the placebo group was 100% each (online supplemental table S1, appendix p 7). This 100% response rate falls far outside the 95% CI (27.75% to 36.64%) of the weighted mean percentage of the SRI-4 placebo response calculated from 7 recent studies in SLE (32.12%) involving 449 placebo-treated patients.[27–33] This excludes that the placebo response in these centres just reflects a placebo effect, and signs a yet unexplained major deviation to the protocol (online supplemental table S2, appendix p 8). This justified a post hoc analysis in a per-protocol (PP) population prespecified in the statistical analysis plan, which excluded patients with major deviation from the protocol: (i) patients who were identified to have had a SELENA-SLEDAI score <6 at baseline (ILT-101: n=3, placebo: n=2) in a retrospective data review performed before unblinding by an independent adjudication committee, as these patients should have been screening failures; (ii) patients with a clinical SELENA-SLEDAI score <4 at baseline (ILT-101: n=2, placebo: n=1) because only the clinical items composing the SELENA-SLEDAI score can improve at week 12, thus requiring a minimum clinical SELENA-SLEDAI score of 4 to reach an SRI-4 at this time point; (iii) patients that had not received at least 80% of their scheduled injections of ILT-101 or placebo (ILT-101: n=8, placebo: n=4) and (iv) all patients from the two sites with a placebo response rate of 100% (ILT-101: n=13; placebo: n=14) (online supplemental table S3, appendix p9). Ultimately, this post hoc PP analysis included 24 and 29 patients treated with ILT-101 or placebo, respectively, having comparable baseline characteristics and disease activity between the treatment groups (mean SELENA-SLEDAI score ILT-101 vs placebo: 11.3 vs 10.4), except for the mean disease duration, which was higher in the ILT-101 group (13.7 vs 8.4 years), and the proportion of patients with ≥2 BILAG B scores, which was lower in the ILT-101 group (29.2% vs 44.8%)

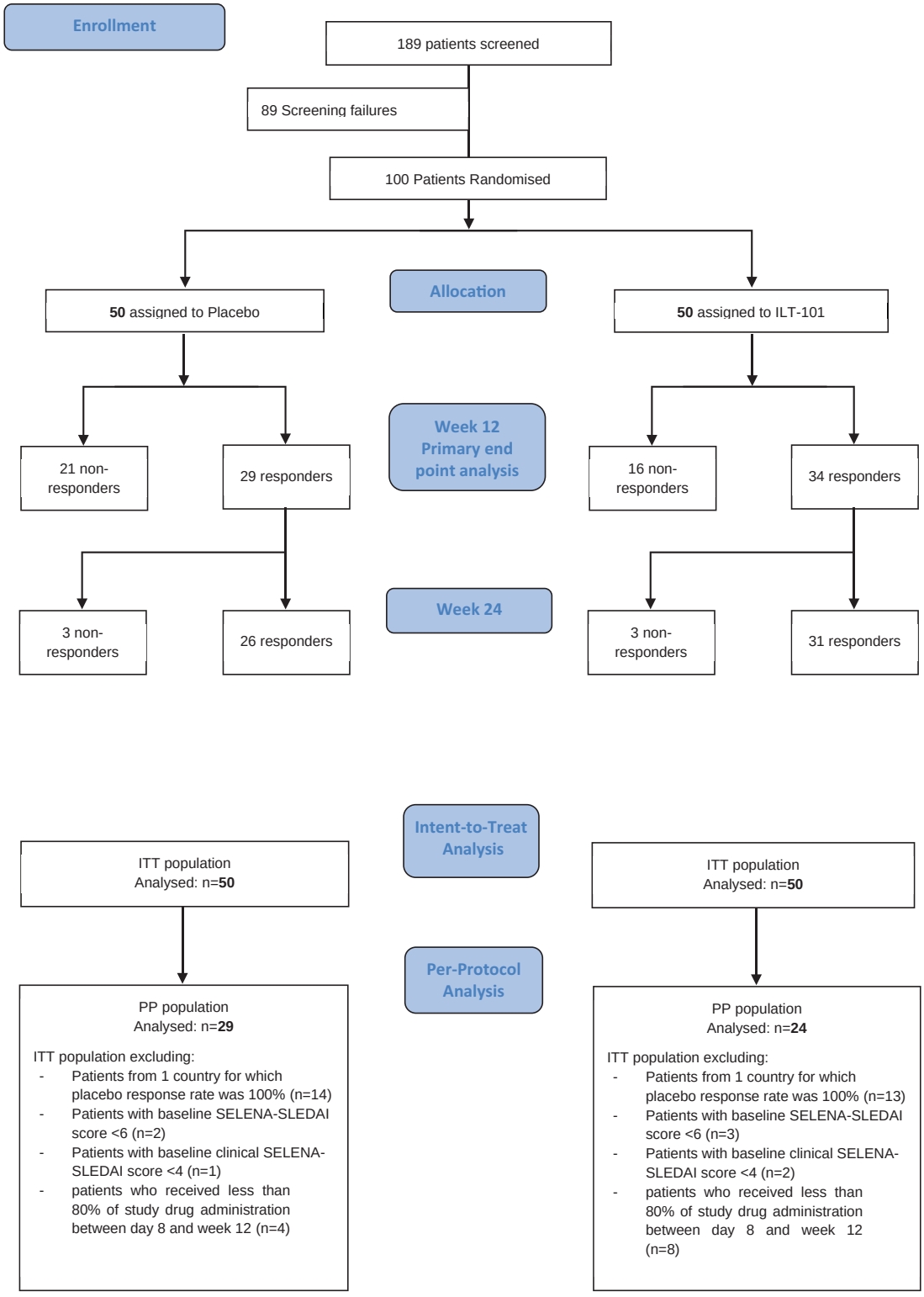

Figure 1 Randomisation, follow-up and definition of analysed patients' populations. ITT, intention-to-treat; PP, per protocol; SELENA-SLEDAI, Safety of Estrogens in Lupus National Assessment Systemic Lupus Erythematosus Disease Activity Index.

(table 1). The most notable difference between patients of the PP population and those of the ITT population was a lower proportion of patients with ≥1 BILAG A score in both groups of the PP population (ITT: 48% vs 38%; PP: 25% vs 17.2%; ILT-101 vs placebo, respectively), because a high number of patients with ≥1 BILAG A were recruited from the two excluded sites with a 100% placebo response rate (n=24). The post hoc PP analysis followed the predefined hierarchical statistical analyses of the primary and the two key secondary end points.

Despite the smaller number of patients composing this PP population, the primary evaluation criterion was met in the ILT-101 group compared with the placebo group (figure 2D) (difference 31.6%, 95% CI 1.35 to 20.42, p=0.0168; table 2). This was accompanied by significant differences between the

Table 1 Baseline demographics and disease characteristics of the intention-to-treat (ITT) and the per-protocol (PP) population

	ITT population (n=100)		PP population (n=53)	
	ILT-101 (n=50)	Placebo (n=50)	ILT-101 (n=24)	Placebo (n=29)
Age (years)	41.7	40.4	41.3	38.2
Sex				
Female	49 (98%)	42 (84%)	24 (100%)	27 (93.1%)
Male	1 (2%)	8 (16%)	0 (0%)	2 (6.9%)
Race				
Asian	1 (2%)	1 (2%)	0 (0%)	1 (3.4%)
Black or African-American	2 (4%)	0 (0%)	2 (8.3%)	0 (0%)
Caucasian/White	35 (70%)	39 (78%)	14 (58.3%)	21 (72.4%)
Caucasian/Mix	9 (18%)	8 (16%)	7 (29.2%)	7 (24.2%)
Unknown	3 (6%)	2 (4%)	1 (4.2%)	0 (0%)
BMI	25.1 (4.88)	25.0 (5.43)	25.4 (5.4)	26.3 (6.6)
Disease duration (years)	10.7 (8.2)	8.4 (7.1)	13.7 (9.59)	8.4 (7.99)
SELENA-SLEDAI	10.8 (3.9)	10.3 (3.2)	11.3 (3.37)	10.4 (3.26)
SELENA-SLEDAI >10	18 (36%)	21 (42%)	11 (45.8%)	12 (41.4%)
≥1 BILAG A	24 (48%)	19 (38%)	6 (25%)	5 (17.2%)
≥2 BILAG B	11 (22%)	16 (32%)	7 (29.2%)	13 (44.8%)
PGA score (scale 0–3)	1.9 (0.4)	1.9 (0.5)	1.85 (0.42)	1.73 (0.45)
History of lupus nephritis	3 (6%)	0 (0%)	2 (8.3%)	0 (0%)
Positive for ANA	50 (100%)	50 (100%)	24 (100%)	29 (100%)
Positive for anti-dsDNA-Abs	29 (58%)	29 (58%)	16 (66.7%)	20 (69%)
Low C3 and/or C4 concentrations	15 (30%)	20 (40%)	9 (37.5%)	10 (34.5%)
GC dose, mg/day	10.9 (4.2)	11.2 (6.7)	11.4 (5.60)	12.3 (8.04)
GC dose >7.5 mg/day	41 (82%)	33 (66%)	18 (75.0%)	18 (62.1%)
Use of antimalarials	38 (76%)	39 (78%)	20 (83.3%)	25 (86.2%)
Use of immunosuppressants	25 (50%)	20 (40%)	16 (66.8%)	15 (51.7%)
Mycophenolate mofetil	8 (16%)	6 (12%)	7 (29.2%)	4 (13.8%)
Azathioprine	15 (30%)	14 (28%)	7 (29.2%)	11 (37.9%)
Methotrexate	1 (2%)	0 (0%)	1 (4.2%)	0 (0%)
Leflunomide	1 (2%)	0 (0%)	1 (4.2%)	0 (0%)

Data are n (%) or mean (SD) unless otherwise stated.
Abs, antibodies; ANA, antinuclear antibodies; BILAG, British Isles Lupus Assessment Group; BMI, body mass index; ds, double-stranded; GC, glucocorticoids (prednisolone or equivalent); PGA, physician's global assessment; SELENA-SLEDAI, Safety of Estrogens in Lupus National Assessment Systemic Lupus Erythematosus Disease Activity Index.

ILT-101 and the placebo group in the two key secondary efficacy end points: (i) a greater relative decrease from baseline in the SELENA-SLEDAI score at week 12 in the ILT-101 group (figure 2E) (LS mean difference: −16.11%, 95% CI −30.79 to 1.43, p=0.0322; table 2) and (ii) a greater absolute decrease from baseline in the SELENA-SLEDAI score at week 12 in the ILT-101 group (figure 2F) (LS mean difference: −1.92, 95% CI −3.49 to −0.34, p=0.0181; table 2), which was not noted in the ITT population (figure 2B,C).

We then performed exploratory analyses of other secondary end points. At week 12, in the ILT-101 group compared with the placebo group of the PP population, we observed (i) higher proportions of patients achieving a SRI-6 (figure 3A) and a SRI-8 response (figure 3B) without treatment failure (difference 32.9%, 95% CI 1.37 to 15.28, p=0.0133 and difference 32.0%, 95% CI 1.28 to 20.73, p=0.0212, respectively; table 2); (ii) a shorter time to first SRI-4 response (figure 3C) (8.0 vs 12.0 weeks, HR 2.28, 95% CI 1.14 to 4.55, p=0.0117; table 2); (iii) a reduction of the daily GC dose from baseline to week 12 (figure 3D) (LS mean difference: −0.89, 95% CI −1.70 to −0.08, p=0.0327; table 2); (iv) a higher proportion of patients in clinical remission (SELENA-SLEDAI ≤2) (figure 3E) (difference 24.5%, 95% CI 1.18 to 21.22, p=0.0291; table 2); and (v) higher proportions of patients resolved arthritis (82.6% vs 60.0%), rash (68.7% vs 33.3%), mucosal ulcers (90.9% vs 61.5%) and alopecia (76.5% vs 55.5%) (figure 3F).

No significant differences between the ILT-101 and the placebo group in the proportion of patients achieving a BICLA response or in changes of the BILAG or PGA score were observed at week 12 in both the ITT and PP populations (table 2). There were also no major differences in absolute changes of Lupus Quality of Life, Fatigue Severity Scale (FSS) and Visual Analogue Scale to Evaluate Fatigue Severity (VAS-F) from baseline to week 12 (online supplemental table S4, appendix p 11).

Patients with an SRI-4 response without treatment failure at week 12 (ILT-101: n=20; placebo: n=15) continued to receive weekly injections of either ILT-101 or placebo until week 24. In the PP population, 85% and 80% of the responders of the ILT-101 or placebo group, respectively, maintained an SRI-4 response without treatment failure until week 24 (online supplemental table S4, appendix p 10). This corresponded to an overall SRI-4 response rate of 70.8% in the ILT-101 group and of 41.3% in the placebo group at week 24 (p=0.0619).

In patients from both the ITT and PP populations who had low serum levels of complement factors C3 and/or C4 at baseline, there were small increases in concentrations of C3 and C4

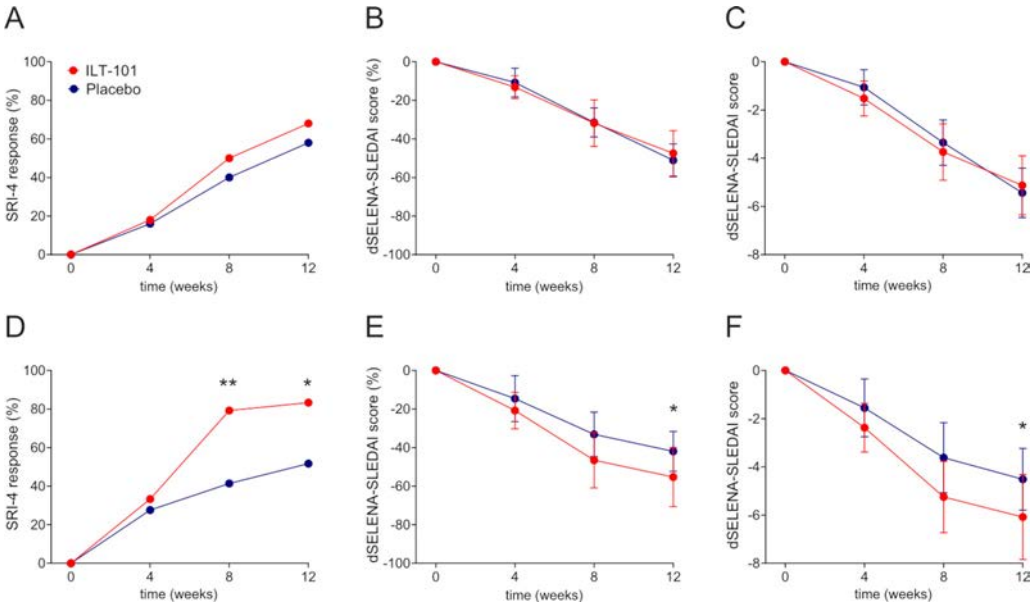

Figure 2 Sequential analysis of the primary and two key secondary efficacy outcomes in the intention-to-treat (ITT) population (A–C) and in the per-protocol (PP) population (D–F). Proportions of patients with SRI-4 response without treatment failure† (A, D), relative change from baseline (d=delta) in SELENA-SLEDAI score (B, E) and absolute change from baseline (d=delta) in SELENA-SLEDAI score (C, F) (data are means; error bars indicate 95% CI). SRI-4, systemic lupus erythematosus responder index-4; SELENA-SLEDAI, Safety of Estrogens in Lupus National Assessment Systemic Lupus Erythematosus Disease Activity Index. †Treatment failure was defined as any worsening of the patient's condition requiring an increased daily dose of glucocorticoids that was above the baseline daily dose or any change in dose or class of background therapies other than glucocorticoids (*p<0.05, **p<0.01).

during the treatment phase in the ILT-101 group (online supplemental figure S1 A-B and D-E; appendix p 2). There were no changes in serum levels of anti-dsDNA-Abs in either of the two groups of both populations (online supplemental figure S1 C and F, appendix p 2).

In both the ITT and PP populations, the numbers and percentages of total $CD3^+CD4^+FoxP3^+CD127^{lo}$ Treg cells were significantly and persistently increased following treatment with ILT-101 (online supplemental figure S2, appendix p 3). Importantly, highly significant increases from baseline in percentages of the $CD3^+CD4^+FoxP3^+CD127^{lo}CD25^{hi}$ Treg subset ($CD25^{hi}$ Treg), which is enriched for activated Tregs with a high suppressive capacity and which are known to be reduced in active SLE,[9] were observed in patients receiving ILT-101, but not in those receiving placebo (figure 4A and D). Increased levels of $CD25^{hi}$ Treg were detectable from day 5 after the start of the treatment, peaked at week 4 and remained significantly elevated until week 12. Concomitantly, there were highly significant increases in the ratio between absolute numbers of $CD25^{hi}$ Treg and absolute numbers of $CD3^+CD4^+FoxP3^-$ conventional T cells in the ILT-101 group (figure 4B and E), indicating that $IL\text{-}2_{LD}$ therapy promotes the selective expansion of the $CD25^{hi}$ Treg subset. This is further substantiated by the observation that the percentage of $CD25^+$ cells among $CD3^+CD4^+FoxP3^-$ conventional T cells did not increase, whereas the percentage of $CD25^{hi}$-expressing cells among $FoxP3^+CD127^{lo}$ Treg cells increased significantly (online supplemental figure S3, appendix p 4).

Comparison of Treg responses between clinical responders and non-responders of the ILT-101 group revealed that a significant increase in Treg numbers was only detectable in the responding patients (figure 4C and F), which implies that the clinical outcome is associated with the magnitude of the Treg response.

Analysis of other cell subsets which can respond to stimulation with IL-2 revealed changes reflecting a global recirculation of immune cells. In the ILT-101 group of the ITT and PP populations, absolute numbers of conventional $CD3^+CD4^+FoxP3^-$ T cells and of $CD3^+CD8^+$ cytotoxic T cells were significantly decreased from baseline at day 5 after the start of the treatment. This early decrease was followed by an increase at week 4 and numbers declined again to baseline levels at week 12 (online supplemental figure S4, appendix p 5). There was also a moderate increase in the numbers of $CD3^-CD56^+$ natural killer (NK) cells in the ILT-101 group of the PP population at week 4 and week 12 (online supplemental figure S4F, appendix p 5). Apart from a slight increase in NK cells in the ITT population at week 12, no relevant changes in the numbers of these lymphocyte subsets were observable in placebo-treated patients (online supplemental figure S4C, appendix p 5).

Treatment with ILT-101 was generally well tolerated and treatment-emergent adverse events (TEAEs) were mostly transient and mild to moderate in severity. In total, 235 TEAEs were experienced by 39 patients (78%) in the ILT-101 group compared with 104 TEAEs that were experienced by 30 patients (60%) in the placebo group (table 3). Of those TEAEs, 139 were considered treatment-related in the ILT-101 group (26 patients, 52%) and 32 in the placebo group (8 patients, 16%). The most frequent TEAE in the ILT-101 group was injection site reactions (n=66 in 21 patients). Three serious AEs (SAEs) in the ILT-101 group, one of which was considered treatment-related (deep vein thrombosis), and two SAEs in the placebo group. occurred during the entire study time. There were no fatal AEs. In agreement with our previous studies, we could not detect the induction of anti-IL-2 antibodies in ILT-101-treated patients (online supplemental figure S5, appendix p 6).

DISCUSSION

Early phase open-label trials and a single-centre RCT indicated that expansion and activation of Treg by low-dose IL-2 therapy

Table 2 Primary and main secondary and exploratory end points in the ITT and PP populations

	ITT population (n=100)				PP population (n=53)			
	ILT-101 (n=50)	Placebo (n=50)	OR* difference† HR†* (95% CI)	P value	ILT-101 (n=24)	Placebo (n=29)	OR* difference† HR†* (95% CI)	P value
Primary end point								
SRI-4 response at week 12 w/o treatment failure*	34 (68.0%)	29 (58.0%)	1.52* (0.64 to 3.60) 10%†	0.3439	20 (83.3%)	15 (51.7%)	5.25* (1.35 to 20.42) 31.6%†	0.0168
Secondary end points								
Changes in SELENA-SLEDAI score from baseline to week 12								
Absolute change	−5.13 (4.13)	−5.44 (3.54)	−0.38† (−1.42 to 0.66)	0.4696	−6.08 (4.18)	−4.52 (3.37)	−1.92† (−3.49 to 0.34)	0.0181
Relative change	−47.46% (41.28)	−51.07% (30.00)	−0.85%† (−11.05 to 9.34)	0.8683	−55.27% (36.41)	−41.94% (27.25)	−16.11† (−30.79 to 1.43)	0.0322
SRI-4 response at week 8 w/o treatment failure*	25 (50.0%)	20 (40.0%)	1.76* (0.74 to 4.14)	0.1983	19 (79.2%)	12 (41.4%)	6.85* (1.78 to 26.33) 37.8%†	0.0051
SRI-6 response at week 12 w/o treatment failure*	28 (56.0%)	24 (48.0%)	1.33* (0.59 to 3.01) 8.0 %†	0.4881	17 (70.8%)	11 (37.9%)	4.58* (1.37 to 15.28) 32.9%†	0.0133
SRI-8 response at week 12 w/o treatment failure*	15 (30.0%)	16 (32.0%)	0.81* (0.33 to 1.98) 2.0%†	0.6419	11 (45.8%)	4 (13.8%)	5.15* (1.28 to 20.73) 32.0%†	0.0212
BICLA response at week 12	32 (64%)	34 (68.0%)	1.56* (0.31 to 7.72) 4.0%†	0.4843	17 (70.8%)	16 (55.2%)	2.08* (0.65 to 6.69) 15.6%†	0.2175
Absolute change in BILAG score from baseline to week 12	−10.22 (7.28)	−11.08 (8.22)	0.07† (−2.32 to 2.46)	0.9553	−8.42 (4.92)	−8.76 (8.34)	−1.69† (−5.82 to 2.44)	0.4144
Absolute change in PGA score from baseline to week 12	−0.84 (0.54)	−0.92 (0.52)	−0.02† (−0.20 to 0.16)	0.8113	−0.79 (0.55)	−0.77 (0.52)	−0.15† (−0.45 to 0.15)	0.3258
Patients in remission at week 12†	16 (32.0%)	13 (26.0%)	1.24* (0.49 to 3.17) 6.0%†	0.6484	10 (41.7%)	5 (17.2%)	5.00* (1.18 to 21.22) 24.5%†	0.0291
Time to first SRI-4 response (weeks)	8.1 (95% CI: 8.0, 12.0)	12.0 (95% CI: 8.1, 12.1)	1.61†* (0.97 to 2.68)	0.1397	8.0 (95% CI: 4.0, 8.0)	12.0 (95% CI: 8.0, NC)	2.28†* (1.14 to 4.55)	0.0117
Time to first flare to week 12 (weeks)	NC (95% CI: 12.4, NC)	NC (95% CI 12.1, NC)	0.59†* (0.23 to 1.52)	0.2600	NC (95% CI: 12.4, NC)	12.1 (95% CI: 12.0, NC)	0.10†* (0.01 to 0.83)	0.0150
Flares until week 12: mild/moderate (SFI)	4 (9.1%)	6 (13.6%)	1.06†* (0.38 to 2.95) −4.5%†	0.9086	2 (8.7%)	5 (20%)	4.24†* (0.91 to 19.76) −12.3%	0.0660
Mean GC dose								
At baseline	10.93 mg/day	11.20 mg/day	−2.09†	0.0248	11.02 mg/day	12.33 mg/day	−4.08†	0.0216
At week 12	10.10 mg/day	11.61 mg/day	(−3.90 to 0.27)		9.35 mg/day	12.33 mg/day	(−7.52 to 0.63)	
Change in GC dose (mg/day) from baseline to week 12	−0.89 (3.35)	0.05 (0.81)	−0.52 (−1.00 to 0.04)	0.0358	−1.67 (3.51)	0.00 (0.00)	−0.89 (−1.70 to 0.08)	0.0327

Data are n (%) or mean (SD) unless otherwise stated.
*Treatment failure was defined as any worsening of the patient's condition requiring an increased daily dose of GC that was above the baseline daily dose or any change in dose or class of background therapies other than GC.
†Patients in remission were defined as having a SELENA-SLEDAI score ≤2.
BICLA, BILAG-based composite lupus assessment; BILAG, British Isles Lupus Assessment Group; GC, glucocorticoids; ITT, intention-to-treat; NC, not calculated; PGA, physician's global assessment; PP, per protocol; SELENA-SLEDAI, Safety of Estrogens in Lupus National Assessment Systemic Lupus Erythematosus Disease Activity Index; SFI, SLEDAI Flare Index; SRI-4, systemic lupus erythematosus responder index-4.

is safe and could be effective in reducing disease activity in patients with active SLE.[9 23 25–27] We report here the results from the first international multi-centre RCT addressing the clinical efficacy, safety and Treg responses of low-dose IL-2 therapy in patients with active SLE. As all previous trials reported an early improvement of patients treated with IL-2_{LD}, we elected to evaluate clinical improvement at week 12 as our primary end point. From previous clinical trials in SLE, we estimated that the placebo response would be between 25% and 45% and calculated a sample size of 100 patients. Despite a high SRI-4

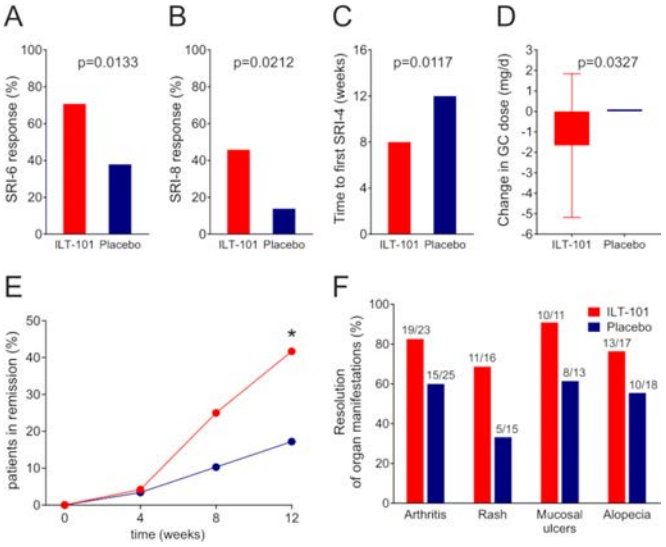

Figure 3 Exploratory analyses of other secondary efficacy outcomes in the per-protocol (PP) population. Proportions of patients with SRI-6 response without treatment failure† at week 12 (A), proportions of patients with SRI-8 response without treatment failure† at week 12 (B), time to first SRI-4 response (C), absolute change in the daily dose of glucocorticoids (GC) from baseline to week 12 (data are means; error bars indicate 95% CI) (D), proportions of patients in remission‡ (E) and proportions of patients who resolved the indicated organ manifestations at week 12 (F). SRI, systemic lupus erythematosus responder index. †Treatment failure was defined as any worsening of the patient's condition requiring an increased daily dose of GC that was above the baseline daily dose or any change in dose or class of background therapies other than GC. ‡Patients in remission were defined as having a Safety of Estrogens in Lupus National Assessment Systemic Lupus Erythematosus Disease Activity Index score ≤2. (*p<0.05).

response rate of 68% in the IL-2_{LD}-treated group, we failed to demonstrate clinical efficacy as the response rate in the placebo group appeared unusually and excessively high at 58%. This high placebo response rate was mainly driven by the two centres from Bulgaria, which had an unprecedented SRI-4 placebo response rate of 100%. Actually, the response rate of 449 placebo-treated patients similar to ours and treated in 7 recent trials[27–33] ranged between 17% and 44%, with a weighted mean of 32%. Thus, this excludes that the placebo response in these centres just reflects a placebo effect, and this signs a major deviation to the protocol. We speculate that this placebo response was driven by better adherence to concomitant background medications, including GC, during the trial period compared with their prior care. A similar assumption has been made for trials in patients with rheumatoid arthritis in which high placebo rates had been observed.[34]

We thus turned to a prespecified post hoc analysis excluding patients with major deviations and retaining 24 and 29 patients treated by IL-2_{LD} or placebo, respectively. Despite this substantially reduced size of the trial population, we observed significantly better clinical responses in this post hoc analysis of the primary and two key secondary efficacy end points in IL-2_{LD} compared with placebo-treated patients. Noteworthily, these observations at week 12 indicate that the clinical improvement driven by Treg stimulation by IL-2_{LD} occurs rapidly, faster than with other treatments in development which often do not show clinical benefit before month 12. Actually, a clear-cut difference in the SRI-4 response between the ILT-101 and the placebo group could be observed as early as week 8 (figure 2D), which is in line with findings from previous trials.[9 25–27] In addition, exploratory analyses also showed that SRI-6 and SRI-8 responses and the proportion of patients in remission were also all higher in the ILT-101 group, highlighting the strength of the response. In agreement

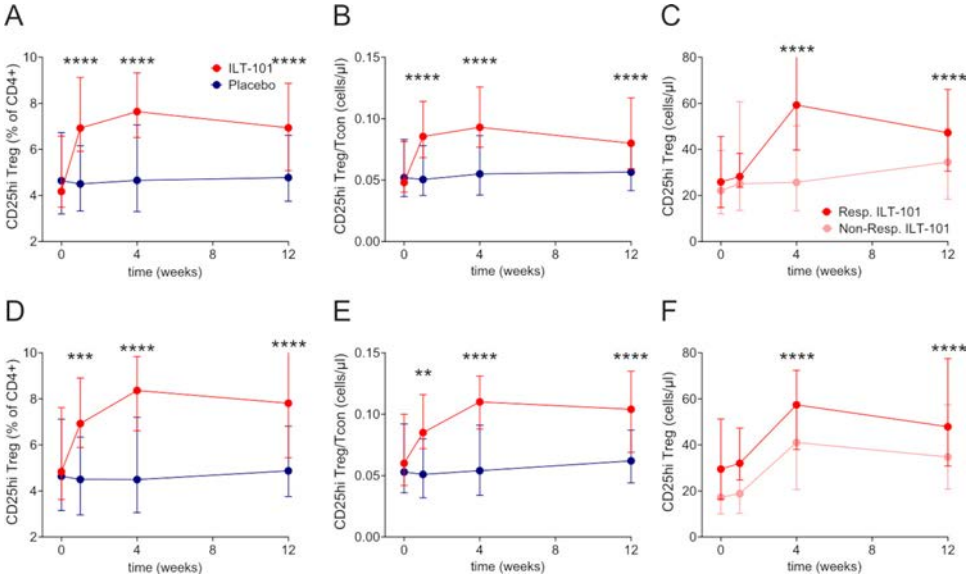

Figure 4 Assessment of regulatory T cell responses in the intention-to-treat (ITT) population (A–C) and in the per-protocol (PP) population (D–F). Changes between baseline and week 12 in the percentages of FoxP3$^+$CD127^{lo}CD25^{hi} regulatory T cells (CD25hi Treg) among CD3$^+$CD4$^+$ T cells (A, D) and changes in the calculated ratio between absolute numbers of CD25hi Treg and absolute numbers of CD3$^+$CD4$^+$FoxP3$^-$ conventional T cells (Tcon) (B, E) in patients receiving ILT-101 (red line) or placebo (blue line). Changes between baseline and week 12 in absolute numbers of CD25hi Treg in patients of the ILT-101 group who achieved a systemic lupus erythematosus responder index-4 (SRI-4) response without treatment failure at week 12 (responders (Resp.) ILT-101, red line) compared with those who failed to achieve this response (non-responders (Non-Resp.) ILT-101, pink line) (C, F). Data are medians (ITT: ILT-101 n=44, placebo n=41; PP: ILT-101 n=23, placebo n=23); error bars indicate IQR. The Wilcoxon signed-rank test was used to compare changes between baseline and the indicated time points (**p<0.01, ***p<0.001, ****p<0.0001).

Table 3 Summary of treatment-emergent adverse events (TEAEs)

	ILT-101 (n=50)		Placebo (n=50)		Total (n=100)	
	E	N (%)	E	N (%)	E	N (%)
Any adverse event	235	39 (78%)	104	30 (60%)	339	69 (69%)
Adverse events of severe intensity	6	4 (8%)	2	2 (4%)	8	6 (6%)
Treatment-related adverse events	139	26 (52%)	32	8 (16%)	171	34 (34%)
Most frequently reported treatment-related adverse events*						
Injection site reaction	66	21 (42%)	4	3 (6%)	70	24 (24%)
Diarrhoea	4	4 (8%)	0	0 (0%)	4	4 (4%)
Asthenia	5	3 (6%)	7	1 (2%)	12	4 (4%)
Fever	6	3 (6%)	0	0 (0%)	6	3 (3%)
Influenza-like symptoms	26	2 (4%)	0	0 (0%)	26	2 (2%)
Cough	2	2 (4%)	1	1 (2%)	3	3 (3%)
Nasopharyngitis	2	2 (4%)	0	0 (0%)	2	2 (2%)
Arthralgia	2	2 (4%)	0	0 (0%)	2	2 (2%)
Serious adverse events (SAEs)	3	3 (6%)	2	2 (4%)	5	5 (5%)
Treatment-related SAEs	1	1 (2%)	0	0 (0%)	1	1 (1%)
Deep vein thrombosis	1	1 (2%)	0	0 (0%)	1	1 (1%)
Unrelated SAEs	2	2 (4%)	2	2 (4%)	4	4 (4%)
Pleuropericarditis	1	1 (2%)		0 (0%)	1	1 (1%)
Fatigue	0	0 (0%)	1	1 (2%)	1	1 (1%)
Lower respiratory tract infection	1	1 (2%)	0	0 (0%)	1	1 (1%)
Vasculitis cerebral	0	0 (0%)	1	1 (2%)	1	1 (1%)

Results are reported in total number of adverse events (E) and in n (%) of patients having experienced at least one adverse event.
*Adverse events reported in >2% of patients in the ILT-101 arm.

with these evaluations, the mean daily GC dose from baseline to week 12 was slightly, but significantly reduced in the IL-2_{LD}-treated patients, while it was slightly increased in the placebo group. This decrease is remarkable as there were strict rules (online supplemental methods, appendix pp 12–13) that much limited the possible decrease at week 12. This suggests that IL-2_{LD} could be a substitution treatment for GC sparing for patients with SLE in remission under minimal GC treatment; 80% and 85% of the placebo or ILT-101 responders at week 12 maintained their SRI-4 response at week 24, respectively. This corresponds to an approximate 30% effect size for IL-2_{LD} at both weeks 12 and 24.

As in all previous studies, Treg from patients with diverse autoimmune diseases expanded and were activated by IL-2_{LD}. Interestingly, SLE is characterised by the loss of CD25, the IL-2 receptor α chain, on a large proportion of Treg. This low IL-2Rα expression appears as the consequence of an IL-2 insufficiency in these patients as in vitro and in vivo exposure to IL-2 rapidly re-induces IL-2Rα expression on Tregs.[9 26] This 'low' IL-2 state of the Treg population correlates with disease activity and circulating anti-dsDNA antibody levels, in line with its presumed pathophysiological relevance. In our study, treatment with IL-2_{LD} also led to a marked and rapid increase of IL-2Rα expression on Treg and a robust expansion of Treg that express high levels of the IL-2Rα ($CD25^{hi}$ Treg). In line with these findings, clinical responses in our trial appeared to be associated with the magnitude of such a Treg expansion, which was greater in responders than in non-responders. These observations are important as they support that IL-2-induced Treg fitness is the driver of the clinical response to IL-2_{LD} in patients with SLE, building confidence in the robustness of the clinical benefit observed.

Our study has several limitations. First, the clinical efficacy of ILT-101 in SLE could not be appreciated in the ITT population, mainly because of the 100% placebo response in two centres; however, we believe that given the significant improvements in the primary and two key secondary end points, tested in a predefined hierarchical manner, seen in the PP population, a ≈30% difference of SRI-4 response can serve as a realistic target for the design of phase III studies. Second, we did not include patients with severe renal or central nervous system involvement in this study. The biological and clinical response to IL-2 in these populations remains to be investigated.

In conclusion, the present study warrants further clinical assessment of IL-2_{LD} in SLE. The observation that the clinical improvement of patients with SLE treated with IL-2_{LD} is driven by Treg cell fitness adds confidence in the overall concept of a Treg insufficiency in SLE and in its treatment by IL-2_{LD}.

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Competing interests PC, MR and DK are inventors for patents related to the therapeutic use of IL-2, which belongs to their academic institutions and have been licensed to ILTOO Pharma in which they hold interests. HPP, JG, DL and TV are employees of ILTOO Pharma.

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TRANSLATIONAL SCIENCE

Lower disease activity but higher risk of severe COVID-19 and herpes zoster in patients with systemic lupus erythematosus with pre-existing autoantibodies neutralising IFN-α

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ABSTRACT

Objectives Type-I interferons (IFNs-I) have potent antiviral effects. IFNs-I are also overproduced in patients with systemic lupus erythematosus (SLE). Autoantibodies (AAbs) neutralising IFN-α, IFN-β and/or IFN-ω subtypes are strong determinants of hypoxemic COVID-19 pneumonia, but their impact on inflammation remains unknown.

Methods We retrospectively analysed a monocentric longitudinal cohort of 609 patients with SLE. Serum AAbs against IFN-α were quantified by ELISA and functionally assessed by abolishment of Madin-Darby bovine kidney cell protection by IFN-α2 against vesicular stomatitis virus challenge. Serum-neutralising activity against IFN-α2, IFN-β and IFN-ω was also determined with a reporter luciferase activity assay. SARS-CoV-2 antibody responses were measured against wild-type spike antigen, while serum-neutralising activity was assessed against the SARS-CoV-2 historical strain and variants of concerns.

Results Neutralising and non-neutralising anti-IFN-α antibodies are present at a frequency of 3.3% and 8.4%, respectively, in individuals with SLE. AAbs neutralising IFN-α, unlike non-neutralising AAbs, are associated with reduced IFN-α serum levels and a reduced likelihood to develop active disease. However, they predispose patients to an increased risk of herpes zoster and severe COVID-19 pneumonia. Severe COVID-19 pneumonia in patients with SLE is mostly associated with combined neutralisation of different IFNs-I. Finally, anti-IFN-α AAbs do not interfere with COVID-19 vaccine humoral immunogenicity.

Conclusion The production of non-neutralising and neutralising anti-IFN-I antibodies in SLE is likely to be a consequence of SLE-associated high IFN-I serum levels, with a beneficial effect on disease activity, yet a greater viral risk. This finding reinforces the recommendations for vaccination against SARS-CoV-2 in SLE.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Anti-interferon (IFN)-α autoantibodies (AAbs) have been reported in 5%–27% of patients with systemic lupus erythematosus (SLE), it is, however, as yet unclear whether their occurrence is pathogenic, protective or a reflection of a general tendency towards autoreactivity.

WHAT THIS STUDY ADDS

⇒ Neutralising and non-neutralising anti-IFN-α AAbs are present at a frequency of 3.3% and 8.4%, respectively, in patients with SLE.

⇒ AAbs neutralising IFN-α are associated with reduced IFN-α serum levels and a reduced likelihood to develop active disease.

⇒ AAbs neutralising IFN-α are associated with a history of severe COVID-19 pneumonia and episodes of cutaneous herpes zoster.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Monitoring anti-IFN-α antibodies in patients with SLE can help identify patients at risk of developing serious viral infections.

INTRODUCTION

Type-I interferons (IFNs-I) play a central role in the early control of viral infections. Inborn errors of IFN-I immunity were recently found in patients with life-threatening COVID-19.[1 2] Autoantibodies (AAbs) neutralising IFNs-I were also found in 7% and 15% of patients with severe and critical COVID-19 pneumonia, respectively.[3–6] They were also found in about a third of a cohort of patients with yellow fever vaccine-associated disease.[7] However, little is known about the circumstances in which neutralising AAbs directed at IFNs-I appear and whether they might also have anti-inflammatory effects. The IFN family of cytokines is indeed involved in systemic lupus erythematosus (SLE) pathogenesis, an autoimmune disease affecting mostly young women and where persistent overexpression of

IFNs-I, notably IFN-α, is observed.[8] While anti-IFN-α AAbs have been reported in 5% to 27% of patients with SLE,[9–12] it is, however, as yet unclear whether the occurrence of these AAbs in the context of SLE is pathogenic, protective or a reflection of a general tendency towards autoreactivity. It has been suggested that endogenous anti-IFN-α AAbs may have a regulatory, protective, role against disease activity.[10 11] However, it is difficult to draw firm conclusions from these studies involving only small numbers of patients. Indeed, if the presence of anti-IFN-α AAbs has reportedly been associated with reduced downstream IFN pathway activity in patients with SLE, it was either not[11] or only weakly[10] associated with a decrease in disease activity. Anti-IFN-α antibodies were previously described in two patients with SLE with severe COVID-19,[13] but their clinical impact on SLE activity was not explored. Furthermore, although targeting IFN-I signalling pathways represents a promising therapeutic approach for SLE, as evidenced by the recent approval of the IFN-I receptor antagonist anifrolumab by the US Food and Drug Administration[14] and the European Medicines Agency,[15] the potential long-term viral risk caused by this type of treatment is of concern.

In the present study, we retrospectively analysed immunological and clinical data in a monocentric longitudinal cohort of 609 patients with SLE and focused on the association between the presence and the neutralisation capacity of serum anti-IFN-α AAbs, infectious complications and disease evolution. We hypothesised that neutralising anti-IFN-α AAbs might confer an additional viral risk to patients with SLE but could also have a disease-ameliorating effect.

PATIENTS, MATERIALS AND METHODS

Study design and patients

The retrospective longitudinal study reported here was conducted between June 2006 and June 2021 at the French National Referral Center for SLE and Antiphospholipid Antibody Syndrome and Other Autoimmune Disorders, Paris, France, regrouping out or inpatients with active or quiescent, untreated or treated disease. Serum samples were randomly obtained from patients diagnosed with SLE according to the 1997 American College of Rheumatology criteria for SLE classification or the 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for SLE.[16 17] Patients seen in outpatient clinic or during hospital care were randomly included in the study, regardless of disease activity and treatment. Serum samples were kept frozen until anti-interferon-α AAbs were assessed. See online supplemental file for the designs of the clinical studies. The study was approved by the ethical committee of Sorbonne Université (CER2020-012, CER2021-011 and CER2021-099) and informed consent was obtained from all participants.

Measurement of anti-IFN-α AAbs

Auto-Abs against IFN-α were quantified using the anti-IFN-α Antibody Human ELISA Kit (Thermo Fisher, Invitrogen), according to the manufacturer's instructions. The positivity threshold of the assay was 15 ng/mL.

Determination of biological activity of IFN-α by IFN-α bioassay

Serum IFN-α biological activity was determined by assessing the protection conferred by each patient's serum to cultured Madin-Darby bovine kidney (MDBK) cells challenged with vesicular stomatitis virus (VSV), as previously described.[18–21] Serum IFN-α levels are expressed in IU/mL after comparison with IFN-α2b reference (Introna, Shering Plough), standardised against the National Institutes of Health reference Ga 023-902-530 titrated under the same conditions as the SLE patients' serum samples. The lower limit of detection was 2 IU/mL. Serum IFN-α activity in healthy individuals is undetectable (ie, <2 IU/mL).[22 23]

Functional evaluation of anti-IFN-α AAbs by VSV assay

The blocking activity of anti-IFN-α AAb-containing serum was assessed as previously described.[24] Neutralisation experiments were performed by the titration of serial dilutions of serum positive for anti-IFN-α AAbs against 10 IU/mL (50 pg/mL) of IFN-α2b (Introna, Shering Plough), following the previously described antiviral assay. Serum and IFN-α were incubated together for 30 min at room temperature before being added to MDBK cells. End points were scored at 50% cytopathic effect (CPE). Sera to be tested for their anti-IFN-α neutralisation capacity were previously inactivated at 56°C for 60 min to remove endogenous IFN-α activity. Neutralising titres correspond to the serum dilution at 50% CPE × 10. For clinical studies, only sera with neutralisation titres >30 were considered significant.

Functional evaluation of anti-IFN-I AAbs by luciferase reporter assay

The blocking activity against IFN-α2 and IFN-ω at 10^2 pg/mL and 10^4 pg/mL, and IFN-β at 10^4 pg/mL were determined with a reporter luciferase activity assay as previously described.[4]

SARS-CoV-2 serological analysis

Serum levels of SARS-CoV-2-specific immunoglobulin G (IgG) antibodies were assessed using an ELISA specific for antinucleocapsid IgG (Euroimmun, France) or the Maverick SARS-CoV-2 Multi-Antigen Serology Panel (Genalyte, USA), according to the manufacturer's instructions, as previously described.[25] The latter is designed to detect antibodies specific for five SARS-CoV-2 antigens: nucleocapsid, spike S1 receptor-binding domain (RBD), spike S1S2, spike S2 and spike S1, within a multiplex format based on photonic ring resonance technology.

SARS-CoV-2 pseudoneutralisation assay

Lentiviral particles carrying the luciferase Firefly gene and pseudotyped with spikes of SARS-CoV-2 historical strain or variants of concerns (VOCs were produced by triple transfection of 293 T cells as previously described.[25]

Statistical analysis

Qualitative variables are expressed as number (%) and quantitative variables as the mean±SD or median (quartiles), as appropriate. The Mann-Whitney U-test or Student's t test for continuous data and Fisher's exact or χ^2 test for categorical data were used to compare independent groups. Spearman's correlation coefficients were computed for quantitative values. The diagnostic performance of the serum anti-IFN-α AAb levels as assessed by ELISA, to detect an IFN-α-neutralising capacity, was investigated by analysing receiver operating characteristic (ROC) curves, with the capacity to neutralise 10 IU/mL of IFN-α serving as the gold standard. The areas under the ROC curves (AUCs) to differentiate sera with IFN-α-neutralising capacity versus sera without were calculated. The optimal threshold was determined using a compromise among the minimum sensitivity–specificity difference and the Youden's index. We measured the statistical association between the occurrence of severe or critical COVID-19 pneumonia in patients with SLE and different sets

of neutralising anti-IFN-I capacities. Time to flare was studied by the mean of Kaplan-Meier method and compared using Log-Rank tests for patients in whom immunosuppressive and corticosteroid therapy were not increased on the day monitoring was initiated. We performed a sensitivity analysis also including patients in whom immunosuppressive or corticoid therapy was increased on the day monitoring was initiated. Crude HRs were calculated using the Log-Rank or Mantel-Haenszel estimate when appropriate. All tests were two sided and $p<0.05$ defined significance. Statistical analyses were performed using GraphPad Prism, V.8.0.1 software (GraphPad Software, San Diego, California), R software, V.3.6.3 and V.4.0.5 and the web tool easy ROC, V.1.3.1.[26]

RESULTS

High prevalence of neutralising and non-neutralising anti-IFN-α AAbs in SLE

The presence of serum anti-IFN-α AAbs was detected by ELISA in 71 (11.7%) of the 609 patients we analysed, with levels measured at least once above 500 ng/mL in 27 (38.0%) patients and were usually persistent, since they became undetectable in only 10 out of 63 (16%) patients followed for a median (IQR) time of 4.2 years (3.6–6.4) (online supplemental figure 1). There was no significant difference in terms of gender or median age between patients with ELISA-detectable anti-IFN-α AAbs (aIFN-α^+) or not (aIFN-α^-): 65 out of 71 aIFN-α^+ patients (91.5%) versus 509 out of 538 aIFN-α^- patients (94.6%) were women, p=0.28 and 34.6 (26.5–46.5) years versus 37.7 (29.5–49.4), p=0.06, respectively). We then assessed the biological activity of these AAbs. Only 20 (28.2%) of the 71 sera with ELISA-detectable anti-IFN-α AAbs significantly abolished MDBK cell protection by IFN-α2 against viral challenge. Neutralisation capacity was proportional to anti-IFN-α AAb levels (figure 1A,B), although some rare serum samples containing high AAb levels were not endowed with neutralising activity (figure 1A). The AUC for anti-IFN-α AAb serum levels, differentiating between IFN-α-neutralising and non-neutralising sera, was 0.90 (95% CI 0.85 to 0.96, figure 1C), the optimal ELISA threshold for prediction of neutralisation activity, as determined using the minimum sensitivity–specificity difference and the Youden's index, being 310 ng/mL. Proportions of patients with neutralising activity were similar in all age groups (figure 2A). In conclusion, not all anti-IFN-α AAbs have neutralisation potential. Although evaluation of serum-neutralising activity remains the gold standard, simple assessments with ELISA assays are informative since a strong correlation with biological activity was observed.

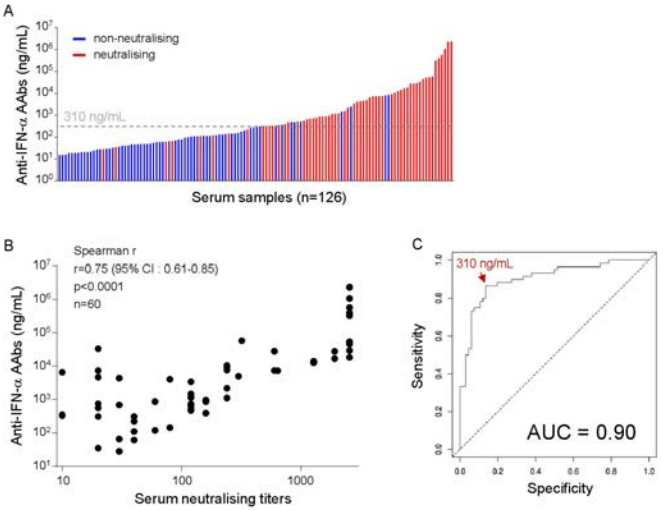

Figure 1 Neutralising and non-neutralising anti-IFN-α AAbs in SLE. (A) IFN-α neutralisation potential contained in 126 serum samples from 71 SLE patients with anti-IFN-α AAbs, measured using the MDBK antiviral activity cell assay. Each vertical bar represents a serum sample. Samples are distributed along the x-axis according to the increasing serum level of anti-IFN-α AAbs. Optimal cut-off point of anti-IFN-α AAb serum concentration, associated with IFN-α neutralising capacity (310 ng/mL), as determined using the minimum sensitivity—specificity difference and the Youden's index is indicated (*horizontal dashed grey line*). (B) Correlation between anti-IFN-α AAb serum concentrations and serum neutralisation titres. Each dot represents an individual. Only neutralising samples were analysed (n=60). Spearman's rank correlation coefficient was used. (C) Diagnostic performance of serum anti-IFN-α AAbs measured by ELISA to predict neutralisation of 10 IU/mL (50 pg/mL) of IFN-α2. Area under receiver operating characteristics (ROC) curve (AUC) is indicated. The optimal cut-off point (red arrow), determined using the minimum sensitivity–specificity difference and the Youden's index is represented. IFN, interferon; MDBK, Madin-Darby bovine kidney; SLE, systemic lupus erythematosus.

Anti-IFN-α-neutralising AAbs are associated with increased viral risk in SLE

We next searched for comorbidities associated with the presence of anti-IFN-α AAbs in SLE. In order to analyse the impact of anti-IFN-α AAbs on the risk of viral infection in SLE, we designed a retrospective cohort study in which all patients with SLE with anti-IFN-α AAbs (aIFN-α^+) were compared with patients without anti-IFN-α AAbs (aIFN-α^-) at a 1:2 ratio (see online supplemental patients, materials and methods). While none of the aIFN-α^- patients experienced a severe COVID-19 pneumonia, five patients (7%) out of the 71 aIFN-α^+ patients developed severe or critical COVID-19 pneumonia (table 1). The presence of anti-IFN-α-neutralising AAbs, unlike that of non-neutralising AAbs, was associated in a statistically significant manner with a history of severe or critical COVID-19 pneumonia, episodes of cutaneous herpes zoster and severe viral infection ($p=3.10^{-4}$, p=0.03 and $p=10^{-4}$, respectively, figure 2B and online supplemental table 2). Of note, the eight cases of severe viral infections in patients with anti-IFN-α-neutralising AAbs included five cases of COVID-19 pneumonia, two cutaneous herpes zoster and one varicella pneumonia. Importantly, patients had samples collected before SARS-CoV-2 infection, and anti-IFN-α AAbs were detected in all cases, prior to infection, further suggesting that they are a cause, rather than a consequence, of severe viral infection. On the other hand, aIFN-α^+ patients were not at higher risk to suffer from warts and human papillomavirus (HPV)-induced cervical lesions, as suggested by previous genetic studies on predisposition to HPV infection.[27]

Combined neutralisation of different IFN-I subtypes is associated with severe COVID-19

Given that in the general population, as well as in SLE patients, anti-IFN-α AAbs are frequently associated with the presence of antibodies against other IFNs-I, such as IFN-β and IFN-ω,[3 4 9 11] we tested whether their coexistence was associated with an increased infectious risk. Serum sampled as close as possible to the COVID-19 pandemic onset were assessed for their neutralisation capacity against IFN-α, and IFN-ω at 10^2 pg/mL and IFN-β at 10^4 pg/mL using a luciferase assay, as previously described.[4] None of the 134 sera lacking detectable levels of anti-IFN-α AAbs was able to neutralise IFN-α2 or IFN-β, and

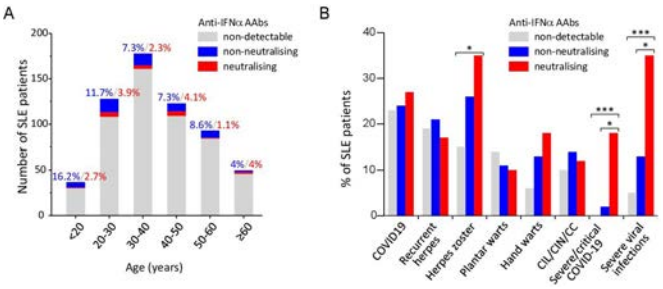

Figure 2 Anti-IFN-α AAbs and viral infections in SLE. (A) Serum anti-IFN-α AAb levels, as determined by ELISA, in SLE patients (n=609) according to age. Indicated proportions of IFN-α neutralisation activity were assessed using the MDBK cell assay. (B) History of viral infections in relation with neutralisation activity of serum anti-IFN-α AAbs. P values were calculated using the Fisher's exact test. p<0.05 was considered significant. *p<0.05 and ***p<0.001. CIL/CIN/CC, cervical intraepithelial lesions or cervical intraepithelial neoplasia or cervical cancer; IFN, interferon; SLE, systemic lupus erythematosus.

only 4 (3%) neutralised IFN-ω. In contrast, neutralising activities against IFN-α2, IFN-β and IFN-ω were more frequently detected (18 (25%), 12 (17%) and 15 (21%) sera, respectively) in the 71 sera with ELISA-detectable anti-IFN-α AAbs. A total of 30 (42%) of the 71 aIFN-α^+ sera tested neutralised at least one IFN-I, while 9 (13%) and 3 (4%) neutralised two and three IFNs-I, respectively. A high concentration of anti-IFN-α AAbs was associated with an increasing number of IFN-I neutralising abilities. Indeed, anti-IFN-α AAb concentrations in serum which neutralised at least two IFNs-I (median (Q1–Q3); 5592 (837–70 175) ng/mL) were significantly higher than those in serum which neutralised a single IFN-I (350 (72–2485) ng/mL; p=0.009) and in serum which did not neutralise IFN-I (53 (32–154) ng/mL; $p<10^{-4}$). Anti-IFN-α AAb concentrations in serum of these latter groups also differed significantly (p=0.008).

Importantly, the occurrence of severe or critical COVID-19 was significantly associated with the neutralisation of IFN-α2 or IFN-ω (p=0.013 and p=0.005, respectively, table 2). Finally, the analysis confirmed that severe or critical COVID-19 in SLE was very significantly associated with combined neutralisation of both IFN-α2 and IFN-ω subtypes ($p<10^{-4}$, table 2), as recently observed in the general population.[28] Of note, the only patients with SLE in this cohort who deceased of COVID-19 had AAbs that neutralised all three IFN-I subtypes tested, suggesting that the severity of COVID-19 pneumonia is even higher in individuals neutralising several IFN-I.[28] It should also be noted that two of the five patients who experienced a severe COVID-19 presented comorbidities conditions such as obesity, immunosuppressive therapy and renal allograft (table 1).

Anti-IFN-α-neutralising AAbs are associated with reduced SLE disease activity

We compared the clinical course of SLE in the presence or absence of anti-IFN-α AAbs (see online supplemental patients, materials and methods). Patients with neutralising anti-IFN-α AAbs had reduced disease activity, less flares and less clinically active SLE, were more likely to be in remission or in lupus low disease activity states compared with patients who lacked neutralising anti-IFN-α AAbs (figure 3A). Biological markers of SLE disease activity, such as elevated antidouble-stranded DNA Ab serum levels (ie, Farr assay), decrease in complement component C3 and increase in serum IFN-α levels were also reduced in patients with neutralising anti-IFN-α AAbs compared with patients without (figure 3A). Other characteristics of lupus disease were similar between the two groups (online supplemental table 3). Non-neutralising anti-IFN-α AAbs were associated with higher IFN-α serum levels and the presence of anti-RNP and anti-Sm Abs. Of the 18 patients with neutralising anti-IFN-α AAbs in whom immunosuppressive and corticosteroid therapy were not increased, none experienced a lupus flare during the following year (figure 3B). Log-Rank test analysis showed a significantly higher risk of relapse in patients with non-neutralising anti-IFN-α AAbs, as compared with patients with neutralising anti-IFN-α AAbs (HR 4.78 (95% CI 1.02 to 22.40), p=0.047). The results from a sensitivity analysis, including patients in whom immunosuppressive or corticoid therapy was increased at the beginning of the follow-up, showed that only one patient out of 20 with neutralising anti-IFN-α AAbs experienced a lupus flare during the following year. In summary, non-neutralising anti-IFN-α AAbs are more prevalent and are typically associated with both unstable disease and high IFN-α serum levels. In contrast, the presence of neutralising AAbs in patients with SLE was associated with a concomitant reduction in levels of serum IFN-α and disease activity.

Anti-IFN-α AAbs do not interfere with COVID-19 vaccine efficacy

Vaccination currently represents the best option to prevent serious infections in patients with SLE. We reasoned that neutralisation of IFN-α signalling might possibly dysregulate IFN-dependent B cell responses[29] and limit vaccine-induced antibody production. In order to determine whether anti-IFN-α AAbs could interfere with COVID-19 vaccine efficacy, we performed a subanalysis of the results we recently obtained in a cohort of patients with SLE,[30] evaluating their SARS-CoV-2-specific immune responses after BNT162b2 vaccination in presence or absence of these AAbs. IFN-I-neutralising activity was confirmed in 50% of the 10 vaccinated aIFN-α^+ patients tested, whereas demographics and main bioclinical characteristics were similar in aIFN-α^+ and aIFN-α^- patients (online supplemental table 4). Vaccine-induced anti-SARS-CoV-2 spike RBD IgG levels, and serum-neutralising capacity of SARS-CoV-2 and its major variants, were similar in both groups, thus confirming that aIFN-α^+ patients are able to mount an efficacious anti-SARS-CoV-2 humoral vaccine response, similar to that of aIFN-α^- patients (figure 3C). In conclusion, although only a limited number of vaccinated patients with SLE could be analysed, the results nevertheless show that anti-IFN-α AAbs do not seem to interfere with COVID-19 humoral vaccine response.

DISCUSSION

The COVID-19 outbreak has illustrated the fact that a previously poorly recognised form of autoimmunity underlies some severe forms of COVID-19 disease,[3–7] although the mechanisms driving the appearance of the anti-IFN-I AAbs and their potential broader medical impact remain unknown. Besides reported SLE-associated cases,[9–12 31] these AAbs have also been found in patients with thymoma,[32] myasthenia gravis[33 34] or affected by various primary immune deficiencies.[35–39] However, their potential inflammatory disease-ameliorating effects until now remained unexplored.

Here, we analysed a longitudinal cohort of 609 patients with SLE, a disease driven by IFN-α, evolving by successive phases of relapses and remissions affecting from 29 to 367 per 100 000 individuals in North America and Europe.[40] We show that the prevalence of anti-IFN-α antibodies is particularly

Table 1 Demographics, IFN-I neutralising capacities and severity of SARS-CoV-2 infection in 17 patients with SLE tested positive for circulating serum anti-IFN-α AAbs

			Daily treatment				Pre-COVID-19 anti-IFN humoral immunity†							
							IFN neutralisation capacities¶							
							IFN-α			IFN-ω				
Pts	Gender/ age (years)	Chronic medical illness	HCQ	Pred (mg/d)	Is	Maximal aIFN-α AAbs (ng/mL)*	aIFN-α AAbs (ng/mL)§	10^2 pg/mL	10^4 pg/mL	IFN-β 10^4 pg/mL	10^2 pg/mL	10^4 pg/mL	Description of COVID-19 signs or symptoms	Severity‡
30	F/61	APS, CKD, Hyp, CVD	+	5	MTX BMB	49	0	–	–	–	–	–	Headache, nausea, vomiting and cough	1
32	F/26	Ren Al	+	5	MMF TAC	108	0	–	–	+	–	–	Asymptomatic	1
29	F/48	Ob	+	–	–	98	35	–	–	–	–	–	Myalgia and fever	1
64	F/36	–	+	–	–	37	37	–	–	–	–	–	Anosmia, myalgia and fever	1
42	F/46	–	+	6	–	51	51	–	–	–	–	–	Asymptomatic	1
16	H/57	Hyp, CKD	+	–	MMF	75	55	–	–	–	–	–	Headache, myalgia and fever	1
63	F/39	CKD	–	5	MMF	368	198	–	–	–	–	–	Asymptomatic	1
55	F/61	–	+	–	–	241	241	–	–	–	–	–	Pneumonia ROT (NC 3 L/min)	3
52	F/41	–	–	–	–	520	260	–	–	+	–	–	Asymptomatic	1
8	F/41	Hyp, Ren Al, Ma Tu (CR)	+	5	MMF TAC	600	600	–	–	+	–	–	Asymptomatic	1
24	F/38	–	–	10	–	8968	625	–	–	+	+	+	Asymptomatic	1
26	F/45	Ob, Ren Al	+	40	MMF TAC RTX	1.1×10^4	763	+	–	+	+	–	ARDS (ECMO)	5
58	F/29	CKD	+	5	MMF	3.0×10^4	1060	–	–	+	+	–	Anosmia, cough, myalgia and fever	1
3	F/54	Ow, Hyp	+	–	–	2.8×10^4	1.2×10^4	+	+	–	–	–	Pneumonia requiring monitoring	2
40	F/29	Ob	+	9	–	8.8×10^4	8.8×10^4	+	+	–	+	+	Pneumonia ROT (HCM 12 L/min)	4
25	F/44	–	+	–	–	5.7×10^5	3.2×10^5	+	+	+	+	+	Pneumonia ROT (NC 5 L/min)	3
34	M/47	Thymoma (CR since 17 years)	+	–	–	3.2×10^6	2.3×10^6	+	+	–	+	+	Pneumonia ROT (non-invasive ventilation)	4

*Corresponds to the maximum level of serum anti-IFN-α AAbs assessed by ELISA during the follow-up of SLE.
†Tested on a serum collected during the COVID-19 pandemic or the 6 months preceding its onset.
‡Categorisation of COVID-19 severity (see online supplemental table 1). Encoding: 1 for asymptomatic infection, mild or moderate illness; 2 for moderate hospitalised illness; 3 for severe illness; 4 for critical illness and 5 for death.
§Assessed by ELISA.
¶The capacity of the serum with anti-IFN-α AAbs to neutralise 10^2 pg/mL of IFN-α or -ω and 10^4 pg/mL of IFN-α,-ω or -β were evaluated in a neutralisation assay developed in HEK293T cells using a luciferase system in the presence of serum 1:10 from patients.
aIFN-α AAbs, anti-interferon-alpha autoantibodies; APS, antiphospholipid syndrome; ARDS, acute respiratory distress syndrome; BMB, belimumab; CKD, chronic kidney disease; CR, complete remission; CVD, chronic vascular disease; ECMO, extracorporeal membrane oxygenation; F, female; HCM, high concentration mask; HCQ, hydroxychloroquine; Hyp, hypertension; IFN, interferon; Is, immunosuppressant; M, male; Ma Tu, malignant tumour; MDBK, Madin Darby Kidney cells; MMF, mycophenolate mofetil; MTX, methotrexate; NC, nasal canula; Ob, obesity; Ow, overweight; pred, prednisone; Pts, patients; Ren Al, renal allograft; ROT, requiring oxygen therapy; RTX, rituximab; SLE, systemic lupus erythematosus; TAC, tacrolimus; yrs, years.

elevated in this population. As expected, we confirm that this novel form of autoimmunity is associated with a greater risk to contract severe COVID-19 disease. We also highlight its association with herpes zoster. It should be emphasised that AAbs directed to human IFN-α were first observed in a patient with varicella-zoster disease,[41] but that link had been not confirmed as yet. More recently, the administration of anifrolumab, a human monoclonal antibody that binds IFN-I receptor subunit, was associated with an increased incidence of herpes zoster,[42] which confirms that IFN-I blockade impairs varicella-zoster recurrences control. Unlike others,[43] we did not observe reactivation of either type 1 and 2 herpes simplex virus or cytomegalovirus in patients with anti-IFN-I AAbs. We also show that IFN-α autoimmunity appears to have a beneficial effect on inflammatory disease activity.

The analysis of this cohort of patients with SLE might provide some clues regarding the mechanism underlying the development of anti-IFN-I AAbs. Overall, the results suggest that abnormally elevated IFN-I levels elicit an AAb response that eventually matures from non-neutralising to neutralising in some patients with SLE. This evolution might be predicted from our observation of two distinct clinical presentations associated with anti-IFN-I AAbs; either, (1) elevated IFN-I levels, instable SLE disease and non-neutralising anti-IFN-I AAbs or (2) low IFN-I levels, quiescent SLE disease and neutralising anti-IFN-I AAbs. This interpretation is in line

Table 2 Risk of severe or critical COVID-19 pneumonia in patients with SLE, carrying different sets of neutralising IFN-I activities

		Severe /critical COVID-19		
Neutralising		**n (%)**	**OR (95% CI)**	**P value**
Anti-IFN-α2	No (n=47)	1 (2)	15.3 (2.1 to 190.3)	0.013
	Yes (n=16)	4 (25)		
Anti-IFN-β	No (n=51)	3 (6)	3.2 (0.5 to 17.0)	0.239
	Yes (n=12)	2 (17)		
Anti-IFN-ω	No (n=50)	1 (2)	21.8 (2.8 to 269.5)	0.005
	Yes (n=13)	4 (31)		
Anti-IFN-α2 and anti-IFN-β	No (n=58)	3 (5)	12.2 (1.6 to 75.4)	0.046
	Yes (n=5)	2 (40)		
Anti-IFN-β and anti-IFN-ω	No (n=57)	3 (5)	9.0 (1.2 to 52.2)	0.067
	Yes (n=6)	2 (33)		
Anti-IFN-α2 and anti-IFN-ω	No (n=58)	1 (2)	228.0 (11.2 to 2726)	$<10^{-4}$
	Yes (n=5)	4 (80)		

Serum samples carrying anti-IFN-α AAbs as detected by ELISA were assessed for their neutralisation capacity against 10^2 pg/mL IFN-α and IFN-ω and 10^4 pg/mL IFN-β using a luciferase assay. Patients tested for anti-IFN-I activity more than 6 months before the onset of the COVID-19 pandemic and/or lost to follow-up on May 10 2021 were excluded from the analysis.
The numbers and proportion of patients with severe or critical COVID-19 pneumonia are shown for each neutralising IFN-I subgroups.
P values were calculated using the Fisher's exact test.
anti-IFN-α AAbs, anti-interferon-alpha autoantibodies; IFN, interferon; n, number of patients; SLE, systemic lupus erythematosus.

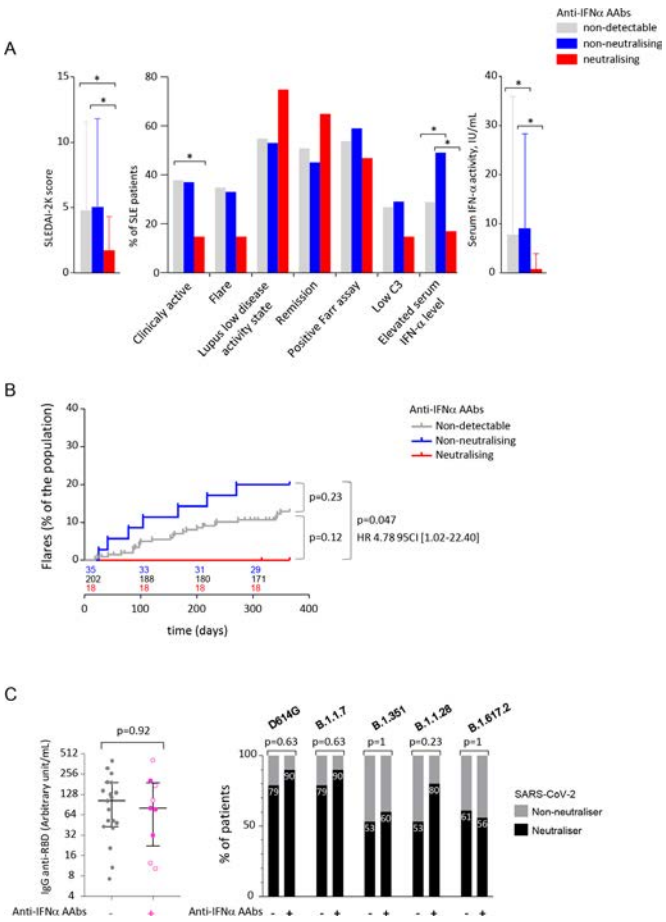

Figure 3 SLE disease activity and BNT162b2 vaccine immunogenicity. (A) SLE activity assessed with the SLEDAI-2K score (left), clinical and biological markers of SLE disease activity (middle) and IFN-α serum levels (right) according to anti-IFN-α AAb status. *Left and right,* columns represent the mean values of disease activity and IFN-α serum levels and vertical lines show positive SD. (B) Kaplan-Meyer analysis of the risk to develop SLE flares in relation to baseline anti-IFN-α AAb status. Red, neutralising aIFN-α+; blue, non-neutralising aIFN-α+ (positivity ELISA threshold: 15 ng/mL); grey, aIFN-α–. Vertical ticks indicate patients who remained flare-free but did not have a full year of clinical follow-up (censored data). Curves were compared using Log-Rank tests. Crude hazard ratios (HR) were calculated. P<0.05 was considered significant. (C) BNT162b2-vaccinated patients (two injections) evaluated at day 42 after first injection. *Left,* comparison of anti-RBD IgG serum levels measured by photonic ring immunoassay in patients with (n=9) and without (n=19) serum anti-IFN-α AAbs. Pink solid circles and empty circles represent IFN-I-neutralising and IFN-I-non-neutralising aIFN-α+ patients, respectively. Median values, first and third quartiles, are indicated. P values were calculated using the Mann-Whitney U test. *Right*, serum with (n=10) or without (n=19) anti-IFN-α AAbs tested for neutralisation of D614G SARS-CoV-2 and variants B.1.1.7 (alpha), B.1.351 (beta), B.1.1.28 (gamma) and B.1.617.2 (delta). Patients were defined as 'non-neutralisers' or 'neutralisers' according to the absence or presence of neutralising activity at first serum dilution (1/30). The Mann-Whitney U test for continuous variables and the Fisher's exact test for categorical variables were used for bivariable analysis. p<0.05 was considered significant. *p<0.05. IFN-α AAbs, anti-interferon-alpha autoantibodies; SLE, systemic lupus erythematosus.

with the observation that patients treated with IFN-α or IFN-β are also prone to develop AAbs targeting these cytokines.[44–46] Future longitudinal studies will be necessary to explore the relationship between neutralisation activity and somatic hypermutation-driven molecular evolution that may underlie in vivo promotion of neutralising anti-IFN-I AAbs.

Our study also has immediate implications in terms of medical management: (1) considering their prevalence in SLE, affected patients should be screened for the presence of anti-IFN-I AAbs, (2) because the biological activity of these AAbs, is correlated with their serum concentration, their mere titration might, in most instances, inform on their clinical relevance, (3) since anti-COVID-19 vaccination is well tolerated in SLE[30] and since its efficacy is not impaired by anti-IFN-I AAbs, patients with SLE carrying these AAbs should be vaccinated against COVID-19 as a priority and (4) preventive and/or early curative antiviral treatment[47] should also be considered in cases of SARS-CoV-2 infection in patients with SLE with serum anti-IFN-I AAbs. Finally, our results have also implications regarding innovative therapeutic options that are currently being tested in SLE.[48] Because viral risk seems likely associated with the neutralisation of more than one IFN-I subtype, we would argue that anti-IFN intervention in SLE and other diseases might not concomitantly target all IFNs. Long-term placebo-controlled assessment of patients treated with anifrolumab, that interferes with all IFNs-I besides IFN-α, was recently reported.[49] A total of seven deaths were attributed to infections (four pneumonia and three COVID-19) in anifrolumab-treated subjects, as compared with none in the group of patients receiving placebo.[49] The interpretation of these data should, however, take into account the large number of patients treated with anifrolumab, compared with those receiving placebo as well as the fact that the observation period spanned the first year of the pandemic prior to vaccination and implementation of effective treatments for severe COVID-19. Our own study also dates back to the prevaccination era of the pandemic and none of the patients who developed severe or critical COVID-19 in our cohort had been vaccinated against SARS-CoV-2. The forthcoming

anifrolumab safety data collected in patients vaccinated against SARS-CoV-2 should provide more important insights.

The main limitation of our study is associated with its design that was limited to a retrospective analysis of clinical data. However, there is arguably no reason to expect that clinical flares would tend to be better recorded in one group of patients or the other, characterised by the presence or absence of anti-IFN-α AAbs, because this biomarker was never recorded prior to the present study, and, therefore, had no impact on medical care. An additional limitation, pertaining to the estimation of viral risk, was study size. Even in a study that comprised several hundred patients affected by a rare disease, cases that present both anti-IFN-α AAbs and a history of COVID-19 constitute only a small subset. As a result, only few severe or critical COVID-19 cases were recorded, but it was nevertheless possible to establish a significative link between presence of AAbs against IFN-I and COVID-19 severity, furthermore taking into account that the majority of patients with SLE are women, often young, and, therefore, at lower risk of severe infection. It should also be underlined that the link between anti-IFNs-I and COVID-19 has been confirmed in different studies, including a cohort of 3595 patients hospitalised with critical COVID-19 pneumonia.[4 5 50–59] Our study setup was not designed to estimate the prevalence of anti-IFN-α AAbs among patients with SLE with severe COVID-19 pneumonia. Other factors will obviously contribute to an enhanced risk of developing a severe COVID-19, as suggested by the presence of associated comorbidities in two out of the five patients with anti-IFN-α AAbs who developed a severe COVID-19 in the cohort.[60] Finally, although we report that the presence of neutralising anti-IFN-α AAbs did not interfere with the induction of vaccine-induced antibody responses, we could not analyse the effect of these AAbs on the development of SARS-CoV-2-specific T cell immunity, and this point will, therefore, require further study since it was recently reported that a small proportion of individuals with such AAbs might not be fully protected by the vaccine.[61] A final limitation, which is not addressed here, is associated with the genetic evolution of SARS-CoV-2, which may alter its IFN-I sensitivity.

In summary, while neutralising anti-IFN-I AAbs seem to confer increased viral susceptibility, they are also associated with reduced SLE disease activity. It is tempting to not only speculate that immunisation against IFN-α could be a consequence of elevated levels of this cytokine recurrently observed in patients with SLE with active disease, but also that neutralising anti-IFN-I autoimmunity is progressively acquired in these patients.

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TRANSLATIONAL SCIENCE

Dissecting the histological features of lupus nephritis highlights new common patterns of injury in class III/IV

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ABSTRACT

Objective The International Society of Nephrology/Renal Pathology Society classification is the gold standard for the characterisation of lupus nephritis (LN) on renal biopsy, with therapeutic repercussions. Its recent revision simplified the current class subdivisions, eliminating the S/G forms of class IV, although data on a possible pathogenetic/clinical value of this subdivision are still contradictory.

Methods 353 renal biopsies from Belimumab International Study in LN were assessed through central pathology review. Univariate logistic models and a decision tree were performed on 314 adequate biopsies to evaluate the impact of histological features on focal/diffuse classes. Removing class I/II (n=6) and 'pure' class V (n=34), principal component analysis (PCA) and heatmap were used to explore similarities among III, IVS and IVG biopsies either incorporating or not the mixed classes (+V, n=274). Finally, a method aimed at partitioning the cases into k clusters based on their similarity (KMeans), was used to study features from the cohort of 'pure' class III/IVS/IVG cases (n=214) to determine alternative subdivisions based on phenotypic data.

Results Segmental endocapillary hypercellularity (EH) was prevalent in class III, global EH, wire loops, hyaline thrombi and double contours were hallmarks of class IVG, with IVS cases showing intermediate characteristics. Heatmap and PCA confirmed the segregation of these features among classes, showing better segregation for focal/diffuse LN as compared with the mixed classes (+V). KMeans revealed the presence of two main clusters, membranoproliferative-like (n=83) or vasculitis-like (n=131).

Conclusions This study reveals new phenotypic forms of LN surpassing the traditional classes as determined by the current classification. Future validation and confirmation are required to confirm these findings.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The International Society of Nephrology/Renal Pathology Society classification is the gold standard for the evaluation of renal biopsies with lupus nephritis (LN).
⇒ The LN classification is an important tool for classifying various forms of renal involvement in SLE, and consequently, an important tool for therapeutic decisions.

WHAT THIS STUDY ADDS

⇒ By cluster analysis, two main groups were distinguished labelled as membranoproliferative-like and vasculitis-like.
⇒ The clusters found here share similarities with previously diagnosed class III and IVG.
⇒ Cases from previously diagnosed class IVS may be reassigned to one of the two clusters.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The proposed clusters may have significant pathogenetic meaning, considering that the patterns of injury (MPGN vs vasculitic) are known to be linked to different underlying disease processes.
⇒ The simplification of the current classification (MPGN-like vs vasculitis-like) could potentially increase interobserver reproducibility.
⇒ This may be the starting point of further investigations focused on how to incorporate new subdivisions into a classification system that would better guide patient- tailored therapies in the near future.

INTRODUCTION

Renal biopsy is a cornerstone for the management of patients with lupus nephritis (LN), as their treatment is strongly guided by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification system.[1] The classification system was recently modified and updated. It was advised to more actively report on the Activity (AI) and Chronicity Index (CI), the definitions of lesions were updated,[2–4] and the subclassification of class IV into segmental and global forms was abandoned.[5] By doing so, it was hoped to make the classification scheme more useful for international usage in studies and to decrease the degree of interobserver variability that had been described previously.[6]

Although lupus classification is a useful tool in the guideline of therapeutic decisions of patients with LN, it does not exemplify possible pathogenetic mechanisms behind the lesions as evaluated in the renal biopsy. By re-evaluating the histological findings in detail, we have tried in this study to work towards new categories hitherto unknown. This may give new insights into a possible subdivision of

Table 1 Clinical characteristics of the cohort

Clinical characteristics	Overall (n=306)
Age	
Median (IQR)	37 (31–47)
Range	24–70
Sex (n, %)	
Male	39 (13)
Female	267 (87)
Ethnicity (n, %)	
African-American	52 (17)
Asian	150 (49)
Caucasian	104 (34)
Creatinine	
Mean (SD)	0.92 (0.43)
Range	0.31–3.20
UPCR	
Mean (SD)	4.06 (3.30)
Range	0.58–20.81
Activity	
Mean (SD)	6.57 (4.18)
Range	0–17
Chronicity	
Mean (SD)	2.45 (2.27)
Range	0–10
LN classes (n, %)*	
Class I/II†	6 (2)
Class III	83 (27)
Class IVS	104 (34)
Class IVG	82 (27)
Class V	31 (10)

*Integrating each class with the mixed +V, LN classes only in patients with complete clinical characteristics.
†Renal biopsy samples were initially analysed locally to confirm eligibility (biopsy-proven class III, IV, V or combination of these, as per BLISS-LN enrollment criteria[7]). Additional evaluation of the available renal biopsy samples was performed by a central pathology review board at the University of Milano-Bicocca, during which six cases have been assigned to class I/II.
BLISS-LN, Belimumab International Study in LN; LN, lupus nephritis; UPCR, urine protein/creatinine ratio.

LN that goes by unnoticed by using the classical scheme in terms of lupus classes I–VI.

The Belimumab International Study in LN (BLISS-LN)[7] collected 353 renal biopsies, an invaluable opportunity to test whether data obtained through systematic assessment of histological lesions would reveal new levels of LN phenotypes, other than those already known through the traditional classification criteria.

METHODS

Patients

This multicentre study included 353 cases, enrolled in the setting of the clinical trial BLISS-LN,[7] with ISN/RPS classes certified by a central pathology review board (IB, JAB, HTC, FF, L-HN). From the initial cohort, cases with less than five evaluable glomeruli[8] as per experts judgement were excluded (n=39), leading to a final cohort of 314 cases. Baseline clinical data (age, sex, ethnicity, serum creatinine and urinary proteine/creatinine ratio (UPCR)) were provided by the BLISS-LN study GSK team for 306/314 cases.[7]

Digital histopathology

All the original renal biopsy glass slides were received from July 2012 to July 2017 at the Pathology Unit, Department of Medicine and Surgery, University of Milano Bicocca, Monza, Italy. H&E, periodic acid–Schiff, Masson trichrome and periodic acid methenamine silver stains were available. A subset of cases (16%, n=59/353) were received as Whole-Slide Images (WSI). For the remaining cases, glass slides were scanned to obtain WSI through the Aperio CS2 scanner (Leica Biosystems, Nussloch, Germany) at ×40 magnification (0.247μm per pixel) and cases were uploaded on the online platform Spectrum after a careful quality control of each virtual slide.[9 10] Each biopsy was independently evaluated by two renal pathologists of the board, after a randomisation process that assigned two pathologists to each case. A detailed histology scoring was performed. Biopsies were classified according to the ISN/RPS classification[1] with class III defined as the presence of active/inactive glomerular focal (<50% of biopsy glomeruli) segmental or global lesions, class IV as the presence of active/inactive diffuse (>50% of biopsy glomeruli) lesions, either segmental (IVS) or global (IVG) if less or more of 50% of the tuft was involved, and class V when a membranous pattern was noted (holes and spikes of the glomerular basement membrane at the Jones stain along with reported diffuse/global granular positivity of the capillary walls in immunofluorescence). Mixed classes were defined as the coexistence of a class V with either class III or IV LN. Moreover, subsequent evaluation of AI and CI was performed, as per recent recommendations.[2] To minimise interobserver variability and facilitate critical discussion, glomeruli were numbered on the WebScope plugin for WSI visualisation.[11] After a first review, discordances in terms of final ISN/RPS class was recorded in 88 out of 314 cases between the two panel nephropatologists assigned to each case. In these cases, regular consensus meetings of the board were organised to reach consensus.[12] Detailed glomerular, tubulointerstitial and vascular parameters were systematically recorded case by case in a dedicated scoring sheet (multipage Excel file, Microsoft, Redmond, Washington, USA) with pull-down menus for data entry, in order to maintain data uniformity throughout the database (online supplemental table 1). The AI and CI were automatically calculated from the histological variables scored by the pathologists. In online supplemental material 1 are reported exemplificative WSI that recapitulates the class III, IVS and IVG cases with systematic annotation of the principal lesions that have been scored by the renal pathologists during the review process.

Statistical analysis

Relative frequencies of histological features were calculated in order to standardise by the number of glomeruli evaluated by the pathologist. For continuous variables, mean and SD or quartiles (Q1, median and Q3), were calculated, as appropriate, while qualitative variables were reported as count and frequency. For the descriptive analysis, histological data from all biopsies were included, while subsequent analyses focused on the subgroup of 274 cases from III (focal) or IV (diffuse) LN class (ie, excluding 6 cases from class I/II and 34 'pure' class V patients).

Univariate logistic models were performed to evaluate the impact of each histological feature on classes (ie, III vs IVS, III vs IVG, IVS vs IVG, incorporating the mixed classes+V to each group). Multiple tests were adjusted by Holms at 5% level of significance.

A decision tree was applied on histological components to explore their relevance in differentiating classes. Exploratory

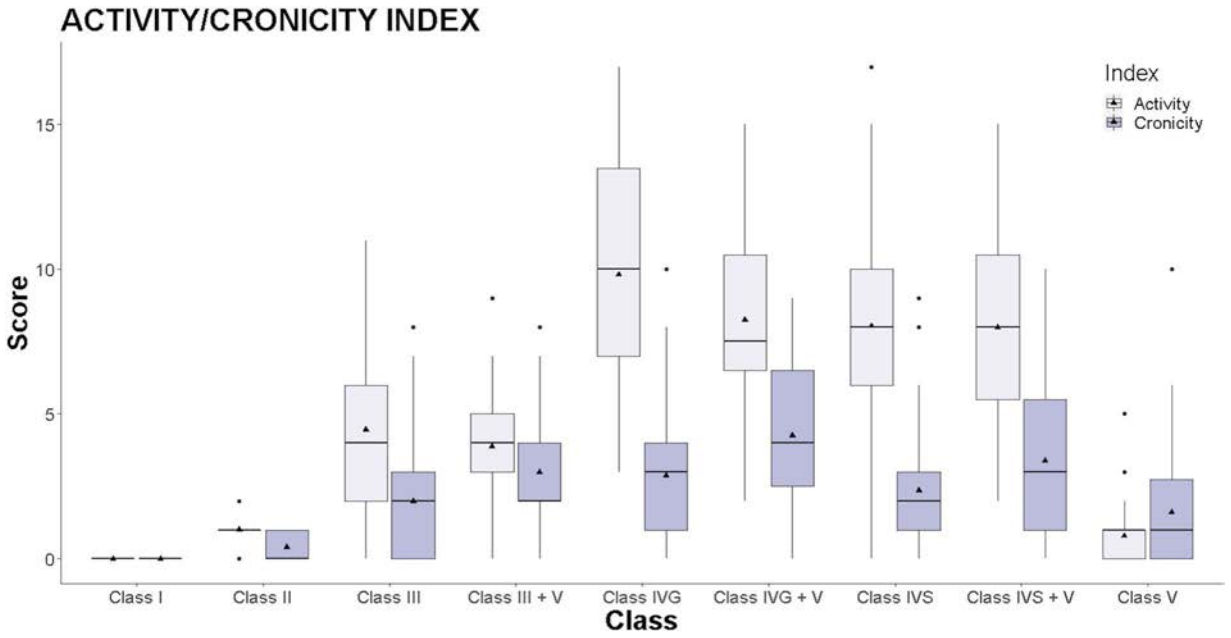

Figure 1 Box plot showing the distribution of median values of AI and CI among the different classes and subclasses. A difference can be noted in mixed as compared with 'pure' focal/diffuse classes in terms of median values of CI. Class I n=1, class II n=5, class III n=61, class III+V n=25, class IVG n=70, class IVG+V n=12, class IVs n=83, class IVS+V n=23, class V n=34. AI, activity index; CI, chronicity index.

analyses were performed to assess similarities among III, IVS and IVG biopsies either incorporating or not the mixed classes (+V) through principal component analysis (PCA) and heatmap. To investigate the putative presence of different clustering with respect to LN classes among biopsies, a method aimed at partitioning the observations (renal biopsy cases) into two groups in which each observation belongs to the group with the nearest mean (KMeans), has been performed on histological features. The internal validation of the cluster analysis was based on the silhouette index and the t-test was used to compare the two groups identified by the KMeans procedure in terms of AI and CI mean values. Statistical analyses were performed using the open-source R software V.3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Clinical characteristics

The clinical and demographic characteristics of the broader cohort of patients enrolled in the BLISS-LN trial have already been reported elsewhere.[7] In this study, 87% of patients were female, with a median age of 37 years (IQR 31–47). Ethnicity was Caucasian (34%), Asian (49%) and African-American (17%). The median number of glomeruli was 15 (IQR 11–23). Details on creatinine and UPCR are reported in table 1. No significant association was found among serum creatinine and UPCR with either AI or CI (ranging between 0.13 and 0.36). The distribution of AI and CI among classes and subclasses showed slightly higher values of CI in mixed as compared with 'pure' focal/diffuse classes (figure 1).

Histological lesions in LN

The distributions of a subset of histological features according to LN classes are shown in figure 2. Global glomerulosclerosis was prevalent in mixed classes, while no significant differences were noted for segmental glomerulosclerosis. Endocapillary hypercellularity (EH) was mostly segmental (<50% of the tuft) in class III, and typically involved more than 50% of the tuft in class IVG, with class IVS demonstrating a variable representation of segmental/global EH. A similar trend was noted for other histological features, such as wire loops (WL), double contours (DC), hyaline thrombi (HT) and tuft necrosis, the presence of which was more typical of class IVG as compared to class III, while IVS cases showed intermediate characteristics. The results of the univariate analysis identified a subgroup of histological features, mainly related to glomerular active lesions, which showed a statistically significant difference among class III, IVG and IVS (table 2). Results were confirmed by decision tree analysis (online supplemental figure 1). Heatmap and PCA on histological features, excluding those mainly related to glomerular chronic lesions based on the results of univariate analysis, revealed that focal and diffuse LN demonstrated a good segregation (figure 3B–D) as compared with the mixed classes (figure 3A), suggesting that the presence of an underlying class V could partially 'contaminate' the histological data. Results confirmed that the most impactful features were reconducible to those with a statistically significant value in univariate analysis (EH, WL, DC and HT).

Clustering cases from histological features

A different unsupervised clustering analysis (KMeans) proposed a better grouping for all 'pure' cases starting from their histological similarities (in online supplemental figure 2, the silhouette indices and the two identified clusters are shown). Translating this subdivision to the heatmap, a new grouping was proposed by the algorithm, as can be appreciated by the heatmap in figure 4A: the majority of class IVG is reassigned to the 'violet' group (global EH, WL, DC, the so-called membranoproliferative (MPGN)-like pattern) and the majority of class III cases to the 'orange' group (segmental HE, so-called vasculitis-like pattern). The IVS cases demonstrated a slight prevalence in clustering with the 'orange' cases (vasculitis-like, similar to class III), although there was a subset assigned to the 'violet' group (MPGN-like, similar to class IVG). A comparison of cases assigned to new groups through the PCA (figure 4B) with the ISN/RPS classes

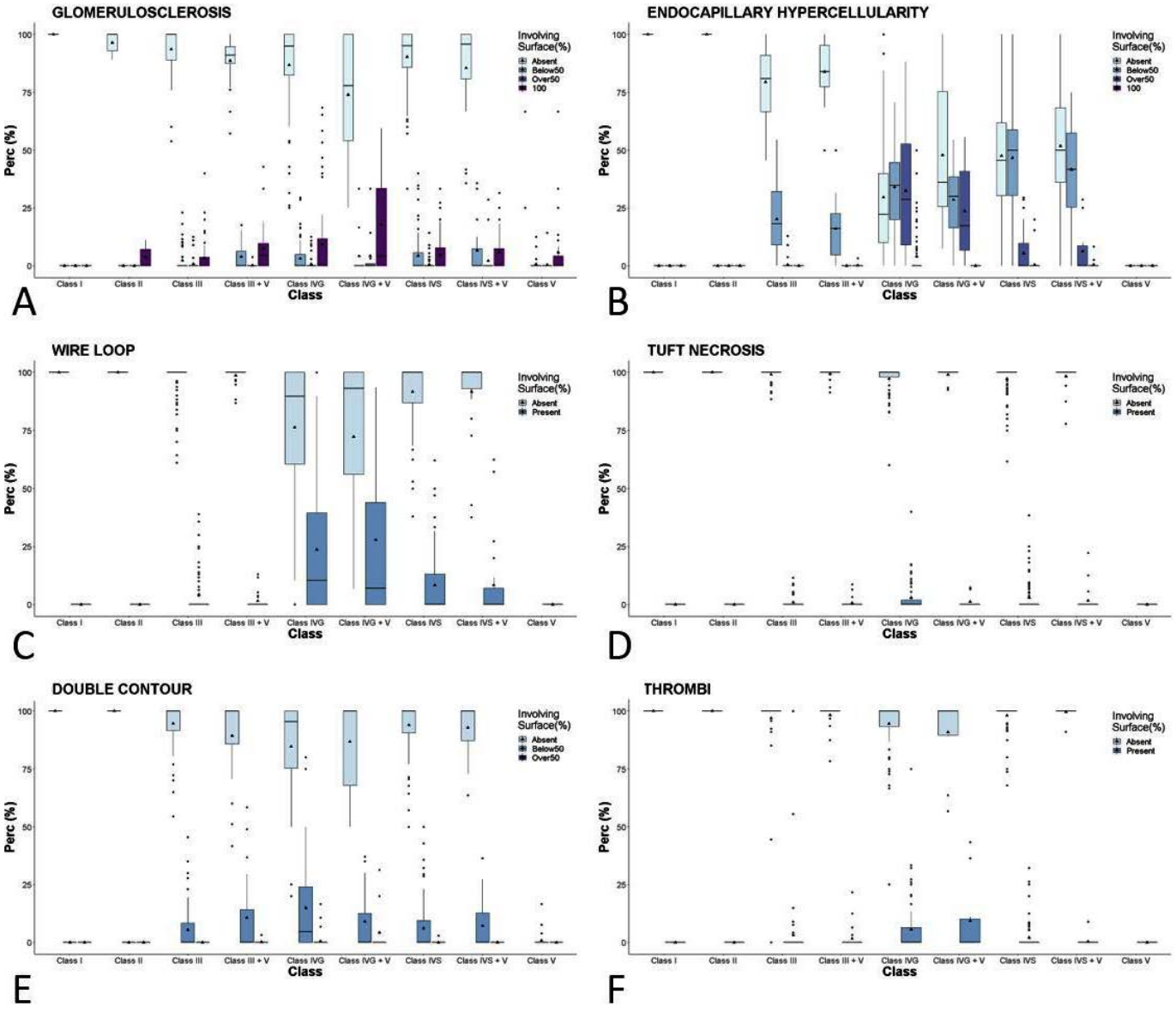

Figure 2 Box plots depicting the distribution of some of the histological features demonstrating substantial graphical differences among the different classes, especially class III, IVs and IVG cases: glomerulosclerosis (A), endocapillary hypercellularity (B), wire loops (C), tuft necrosis (D), double contours (E) and hyaline thrombi (F). Features are expressed either in a four tiered way (absent, involving less, more of the 50% or the 100% of the tuft) or as present/absent (see wire loops, double contours, tuft necrosis and hyaline thrombi). Class I n=1, class II n=5, class III n=61, class III+V n=25, class IVG n=70, class IVG+V n=12, class IVS n=83, class IVS+V n=23, class V n=34.

(figure 4C) demonstrates better segregation of cases with the newly proposed clustering. The revision of WSI from cases assigned to the 'vasculitis-like' group and classified as class III by two independent renal pathologists (VL'I and FP) highlighted the recurrent presence of segmental EH, eventually associated with segmental sclerosis or EH/crescents without DC, WL and HT. On the other hand, the analysis of WSI from cases clustered as 'MPGN-like' and belonging to the IVG group demonstrated a completely different histology, with global EH, often devoid of crescents and with invariable presence of DC, WL and HT. To further validate these observations, WSI belonging to pure class IVS were revised blindly from the new assignment to the 'MPGN-like' or 'vasculitis-like' group (figure 4D). Finally, to corroborate the proposed partition, we compared the AI and CI mean values among these two groups and found a statistically significant difference in terms of AI ($p<0.001$; 10.52 ± 3.18 vs 5.72 ± 2.85, for the 'MPGN-like' and 'vasculitis-like' group, respectively), but not for CI ($p=0.088$; 2.11 ± 1.74 vs 2.60 ± 2.39) (figure 4E).

DISCUSSION

LN is characterised by a wide spectrum of lesions, and historically, the LN classification has been used to distinguish various patterns of renal involvement. The LN classification provides an important tool in patient management and therapeutic decisions. Its usage has become so intricately linked to therapeutic decisions, that the basis on which it was created originally, is almost lost. The subdivision of class III and IV, for instance, was originally based on a difference in histology that later on was converted into a complex system of multiple glomerular lesions that were either active/chronic; segmental/global; present in more or less than 50% of the glomeruli in the biopsy. Although useful for clinical purposes, it may be questioned whether other ways of

Table 2 Univariate analysis depicting the impact of each histopathological feature in discriminating class III, IVS and IVG in the cohort

	Classes*			Univariate analysis†		
Histological characteristics (n=274)	**III (n=86)**	**IVS (n=106)**	**IVG (n=82)**	**III versus IVS**	**III versus IVG**	**IVS versus IVG**
Endocapillary hypercellularity						
Absent	80.77 (13.87)	48.40 (24.05)	32.24 (27.42)	<0.001	<0.001	0.003
<50%	18.90 (13.69)	45.54 (20.54)	33.14 (18.13)	<0.001	<0.001	0.003
≥50%	0.30 (1.73)	5.62 (8.12)	31.13 (25.17)	0.002	<0.001	<0.001
100%	0.04 (0.36)	0.44 (2.57)	3.48 (9.05)	1	0.6264	0.1862
Glomerulosclerosis						
Absent	92.10 (10.16)	89.20 (15.39)	84.89 (20.93)	1	0.1404	1
<50%	2.73 (5.07)	4.90 (9.37)	3.29 (6.98)	1	1	1
≥50%	0.61 (2.46)	0.89 (3.82)	1.25 (4.57)	1	1	1
100%	4.56 (8.45)	5.02 (8.48)	10.58 (17.88)	1	0.1734	0.178
Extracapillary hypercellularity (cellular)						
Absent	93.31 (9.39)	78.82 (19.72)	79.49 (20.59)	<0.001	<0.001	1
≤25%	5.26 (7.67)	12.10 (10.66)	10.04 (9.93)	<0.001	0.0273	1
>25%; <50%	0.94 (2.71)	6.22 (10.21)	6.05 (7.66)	<0.001	<0.001	1
≥50%	0.36 (1.71)	2.32 (5.24)	2.90 (6.91)	0.1276	0.056	1
100%	0.12 (0.83)	0.54 (1.89)	1.52 (5.16)	1	0.5642	1
Extracapillary hypercellularity (fibrous)						
Absent	94.49 (8.57)	90.97 (13.96)	93.38 (9.53)	0.8517	1	1
≤25%	3.47 (5.11)	5.04 (8.88)	3.39 (5.89)	1	1	1
≥25%; <50%	1.26 (3.57)	2.39 (5.09)	1.87 (4.57)	1	1	1
≥50%	0.78 (2.46)	1.30 (4.22)	1.22 (2.94)	1	1	1
100%	0.00 (0.00)	0.29 (1.62)	0.14 (0.64)	1	1	1
Wire loop						
Absent	96.49 (8.12)	91.67 (14.63)	75.67 (31.01)	0.2394	<0.001	0.003
Present	3.51 (8.12)	8.33 (14.63)	24.33 (31.01)	0.2394	<0.001	0.003
Thrombi						
Absent	97.26 (12.61)	98.35 (5.40)	93.91 (12.37)	1	1	0.0858
Present	2.74 (12.61)	1.65 (5.40)	6.09 (12.37)	1	1	0.0858
Double contour						
Absent	93.01 (12.96)	93.67 (11.32)	84.92 (21.29)	1	0.1026	0.024
≤50%	6.95 (12.87)	6.30 (11.33)	14.04 (20.59)	1	0.1856	0.0621
≥50%	0.04 (0.36)	0.03 (0.28)	1.04 (4.62)	1	1	1
Tuft necrosis						
Absent	99.11 (2.49)	97.37 (6.46)	97.37 (6.08)	0.5966	0.3375	1
Present	0.89 (2.49)	2.63 (6.46)	2.63 (6.08)	0.5966	0.3375	1
Karyorrhexis						
Absent	90.52 (15.14)	80.43 (19.92)	72.98 (25.39)	0.01	<0.001	0.4932
Present	9.48 (15.14)	19.57 (19.92)	27.02 (25.39)	<0.001	<0.001	0.4932

Mean (SD) and p value resulting from univariate logistic models were reported.
*Integrating each class with the mixed +V.
†P value with Holms adjustment for multiple comparisons are reported.

categorisation that better reflect pathogenic mechanisms, could be useful or even surpass the classification as we know it. In this study, we investigated whether an analysis of detailed histopathology data obtained from a large cohort of cases enrolled in the BLISS-LN trial would reveal new differences among classes and subclasses of LN.

Our results showed higher values of CI in mixed as compared with isolated III/IV classes, suggesting that the presence of a concurrent class V can worsen the chronic changes of the kidney. This is in line with the most recent evidence in the literature pointing out a worse outcome of mixed as compared with 'pure' cases.[13 14] Most interestingly, an alternative clustering for pure focal/diffuse classes was found after supervised and unsupervised analysis, with a dichotomous subdivision in MPGN-like and vasculitis-like forms based on morphological features. This goes back to an original division which could never be substantiated in terms of clear-cut histological parameters, but is characterised by a prevalence of global lesions, lower frequency of segmental lesions and higher presence of hyaline deposits on the one hand (MPGN-like pattern) and the prevalence of more segmental lesions, crescents and fibrinoid necrosis (vasculitis-like) on the other hand. In the LN classification, a subtle distinction by light microscopy described as IVG as compared with IVS, alludes to this difference.[15] Also other studies have alluded to this distinction, for instance by reporting more commonly WL in the IVG group.[16] Conversely, combined lesions with segmental endocapillary proliferation and fibrinoid necrosis were reported more frequently in class IV-S LN, and the percentage of glomeruli with cellular crescents was also reported to be more common in the IVS group.[16] However, in these studies the prevalence of cellular

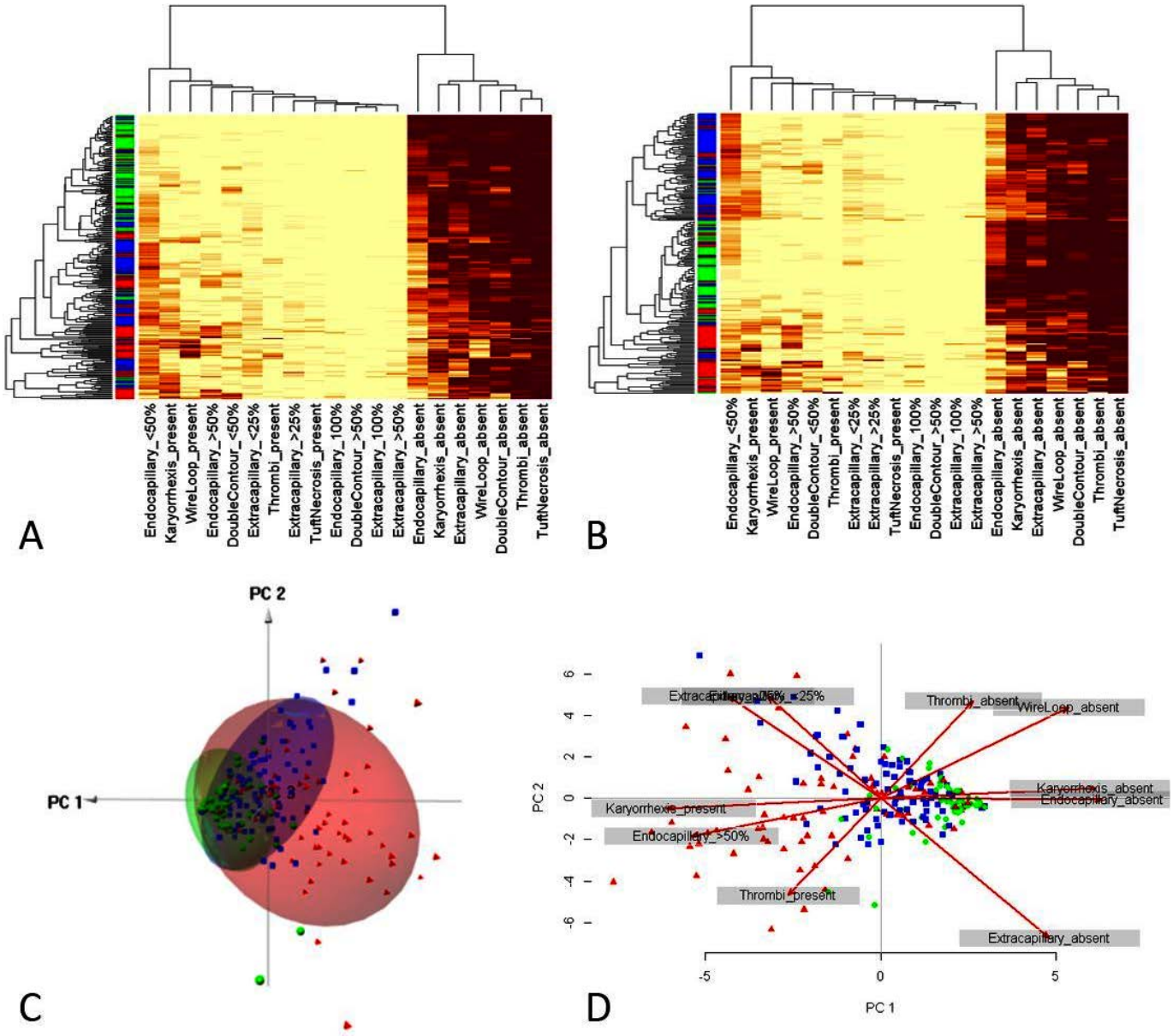

Figure 3 Heatmap of the different histological variables evaluated in the subset of cases with a final diagnosis of class III (green, n=86), IVS (blue, n=106) and IVG (red, n=82) incorporating (A) or not (B–D) the mixed classes (+V). In this last group, the distribution of the cases in PCA analysis demonstrated a compartmentalisation of green dots (pure class III, n=61) and blue (pure class IVS, n=83) with a spread distribution of red dots (pure class IVG, n=70), with some cases intersecting among classes, especially for class IVS (C). The same PCA analysed for the variables that mainly dictates the distribution of the cases shows that the most impactful histological features overlap with those that better differentiated the class III, IVS and IVG in the univariate regression analysis (D). PCA, principal component analysis.

crescents and EH did not reach a statistical relevance. The notion of different morphological aspects in LN with focal and segmental lesions led some authors to postulate a more 'vasculitic' nature of LN[17] in comparison to an MPGN-like pattern. In this setting, a re-evaluation of the classification in 2015 by the ISN/RPS working group stressed the need of new data to shed light on potential differences between either vasculitic-like or MPGN-like patterns, in order to propose eventual modifications in the classes and subclasses.[18] In this study, we found evidence through a cluster analysis for such a division, which was confirmed by re-evaluation of the biopsies. Moreover, as compared with the currently used ISN/RPS classification that did not show significantly different distribution of the AI among classes/subclasses, the application of the newly proposed clusters demonstrated a better segregation of cases with highly active lesions (MPGN-like), suggesting a better capability of this proposal to identify patients with potentially better responses to immunosuppressive therapies. However, cluster analysis per se cannot investigate whether such a division has clinical relevance, which is an important limitation of this study. Moreover, there is increasing evidence pointing to an active role for tubulointerstitial lesions in LN as crucial for the final prognosis, as already demonstrated by other large and independent cohorts,[19] which is also reflected by the revised 2018 ISN/RPS classification emphasising the employment of AI/CI for every biopsy in the report. It should be taken into account, however, that in this study, none of the tubulointerstitial histological features evaluated by the board demonstrated its relevance under both supervised and unsupervised analysis, maybe due to a relative lack of detail in the scoring process. For this reason, in the

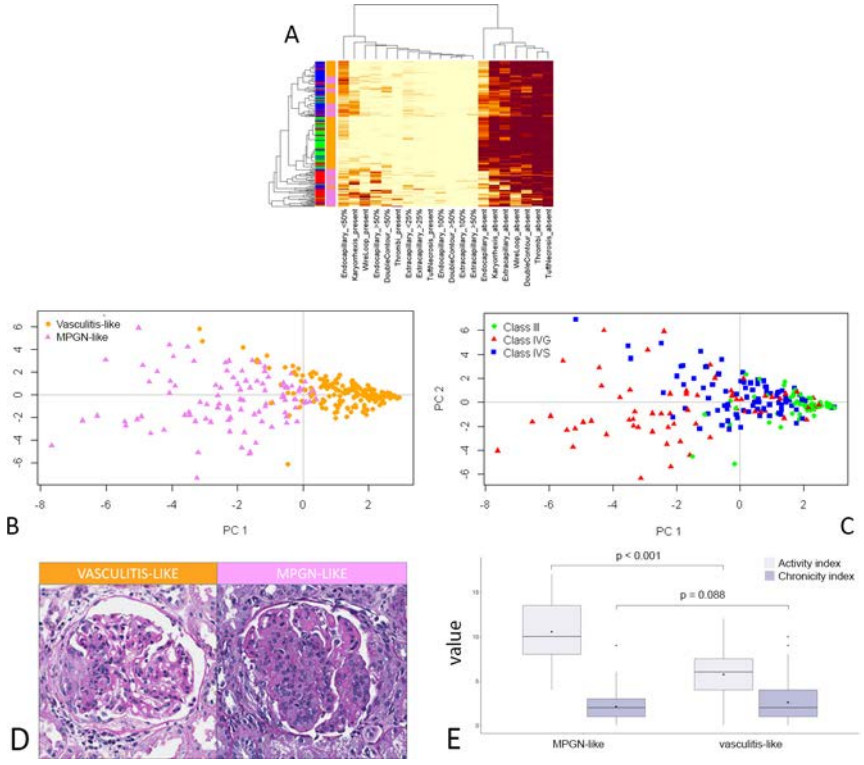

Figure 4 The heatmap (A) shown recapitulate the findings reported in figure 3B with a further subdivision of cases following the clustering suggested by KMeans analysis, for which the majority of 'pure' class III reclassified in orange, class IVG in violet and class IVS being containing a mixture of orange/violet cases. The comparison of the PCAs with the new clustering (B) and ISN/RPS classification (C) demonstrates a better segregation of cases with the former grouping. The histological aspects that characterise the newly defined group are depicted in (D), with the orange group characterised by mainly segmental EH in the absence of WL, DC and HT (vasculitis-like aspects, n=131) as opposite to the violet group, characterised by the presence of global EH, WL, DC and HT (MPGN-like aspects, n=83). Finally, a comparison of the AI and CI between the newly discovered clusters is reported in (E). DC, double contour; EH, endocapillary hypercellularity; HT, hyaline thrombi; ISN/RPS, International Society of Nephrology/Renal Pathology Society; PCA, principal component analysis; WL, wire loops.

future, a further and more detailed additional analysis of this compartment could allow a more in depth understanding of the pathobiological relationship between interstitial infiltrates, their composition and the clusters found here.

For its potential introduction in the routine assessment of LN renal biopsies, a validation involving clinical data including clinical follow-up data is required, to further look into the superiority/non-inferiority of the traditional LN classes. Moreover, the application of the proposed clustering on an alternative and independent cohort of cases by other expert renal pathologists would further strengthen the value of the proposed subdivision. This is of paramount importance, since such a change in the current paradigm would certainly imply a modification of the mindset in clinical practice, moving towards a specific study of different phenotypes. Finally, this would significantly impact on the design of future LN clinical trials, stressing the need for a more 'pathogenesis oriented' look at the therapeutic approach.

CONCLUSIONS

This study gives new insights into different phenotypic forms of LN thereby challenging the traditional classification scheme. This may be the starting point of further investigations focused on how to incorporate new subdivisions into a classification system that would better guide patient-tailored therapies in the near future.

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Contributors MMB and VL'I conceptualised the manuscript, writing a first draft, and coordinated the data collection during the renal biopsy scoring process. GCap and SG performed the statistical analysis. GCat and FP organised the local biopsy images acquisition and scoring activities and provided their support during the project. IB, JAB, HTC, FF and L-HN performed the renal biopsy scoring in the context of BLISS-LN trial. MWT critically revised the manuscript and provided English grammar revision. VL'I is the guarantor, coordinated the project and revised the final version of the manuscript. All the authors revised and approved the present form of the manuscript.

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

Patient consent for publication Not applicable.

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TRANSLATIONAL SCIENCE

Loss-of-function variants in *SAT1* cause X-linked childhood-onset systemic lupus erythematosus

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ABSTRACT

Objectives Families that contain multiple siblings affected with childhood onset of systemic lupus erythematosus (SLE) likely have strong genetic predispositions. We performed whole exome sequencing (WES) to identify familial rare risk variants and to assess their effects in lupus.

Methods Sanger sequencing validated the two ultra-rare, predicted pathogenic risk variants discovered by WES and identified additional variants in 562 additional patients with SLE. Effects of a splice site variant and a frameshift variant were assessed using a Minigene assay and CRISPR/Cas9-mediated knock-in (KI) mice, respectively.

Results The two familial ultra-rare, predicted loss-of-function (LOF) *SAT1* variants exhibited X-linked recessive Mendelian inheritance in two unrelated African–American families. Each LOF variant was transmitted from the heterozygous unaffected mother to her two sons with childhood-onset SLE. The p.Asp40Tyr variant affected a splice donor site causing deleterious transcripts. The young hemizygous male and homozygous female *Sat1*$^{p.Glu92Leufs*6}$ KI mice spontaneously developed splenomegaly, enlarged glomeruli with leucocyte infiltration, proteinuria and elevated expression of type I interferon-inducible genes. *SAT1* is highly expressed in neutrophils and encodes spermidine/spermine-N^1-acetyltransferase 1 (SSAT1), a rate-limiting enzyme in polyamine catabolism. Young male KI mice exhibited neutrophil defects and decreased proportions of Foxp3 +CD4+ T-cell subsets. Circulating neutrophil counts and proportions of Foxp3 +CD4+ T cells correlated with decreased plasma levels of spermine in treatment-naive, incipient SLE patients.

Conclusions We identified two novel *SAT1* LOF variants, showed the ability of the frameshift variant to confer murine lupus, highlighted the pathogenic role of dysregulated polyamine catabolism and identified *SAT1* LOF variants as new monogenic causes for SLE.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Monogenic lupus is a subset of lupus associated with highly penetrant single gene variants, which offer new insights into lupus pathogenesis and help unravel potential treatment strategies. To our knowledge, none of monogenic lupus and systemic lupus erythematosus (SLE) genome-wide association studies–defined risk loci have implicated polyamine metabolism in disease pathogenesis.

INTRODUCTION

Systemic lupus erythematosus (SLE or lupus) is a prototypic autoimmune disease with a multifactorial aetiology contributed to by genetic, epigenetic and environmental factors. SLE has a strong genetic component with more than 150 risk loci identified in genome-wide association studies (GWAS), and approximately 30 loci are associated with rare monogenic forms of lupus or lupus-like disease.[1 2] SLE is generally considered a polygenic trait contributed to by a large number of small-effect, non-coding, common GWAS-defined variants.[3 4] In a small proportion of patients with childhood-onset SLE, Mendelian forms of disease can develop caused by rare, damaging variants, mainly in innate immunity, including deficiency of early complement components (*C1Q, C1R/S, C2, C4A and C4B*), type I interferon (IFN-I) signalling, and nucleic acid sensing and degradation.[5–7] It appears that GWAS-defined common variants and rare monogenic causes of illness often disrupt the same biological processes that lead to disease. The highly penetrant rare cases of monogenic lupus continue to provide new insights into lupus pathogenesis and potential treatment targets.

In this study, we identified two rare *SAT1* loss-of-function (LOF) variants on the X chromosome using WES that segregate with SLE phenotype in two unrelated families. *SAT1* encodes the spermidine/spermine-N^1-acetyltransferase 1 (SSAT1), a rate-limiting enzyme that regulates the catabolism of polyamine and maintains cellular polyamine homeostasis. Dysregulated polyamine metabolism was previously described in patients with SLE.[8] Here, we present evidence for a causal role between LOF *SAT1* variants in the pathogenesis of SLE.

RESULTS

Patient history and genetic analysis

We performed WES in two unrelated American–African families that each had unaffected parents and two sons diagnosed with childhood-onset SLE (figure 1A and B, and table 1). After filtering, we identified two rare predicted pathogenic X-linked

WHAT THIS STUDY ADDS

⇒ We identified two previously undescribed, predicted loss-of-function (LOF) *SAT1* variants (p.Asp40Tyr and p.Glu92Leufs*6) by whole exome sequencing (WES) and in a X-linked recessive inheritance model in two unrelated African–American families. The p.Asp40Tyr variant caused aberrant splicing that resulted in deleterious transcripts assessed by in vitro assays. The p.Glu92Leufs*6 variant was introduced into the C57BL/6J mouse background (knock in, KI, by CRISPR/Cas9) to determine its role in lupus development.

⇒ Both young male and female *Sat1*$^{p.Glu92Leufs*6}$ KI mice spontaneously developed lupus-like autoimmune disease, including splenomegaly, glomerular infiltration of leukocytes, proteinuria and elevated type I interferon scores. Immune profiling showed functional neutrophil defects and decreased proportions of Foxp3 +CD4+ T-cell subsets in young KI mice. While nephritis did not progress up to 1 year of age in a specific pathogen-free environment, apoptotic cell treatment resulted in exacerbated glomerulonephritis in both 20-week-old male and female *Sat1*$^{p.Glu92Leufs*6}$ mice.

⇒ Compared with healthy controls, the treatment-naive, incipient SLE patients had decreased plasma levels of spermine that correlated with neutrophil counts negatively and with proportions of Foxp3 +CD4+ T cells positively. These correlates implicate a link of polyamine metabolites with defects in both innate and adaptive immune responses in patients with SLE.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings link dysregulated polyamine catabolism to the development of lupus manifestations and highlight potential monogenic contributions in multiplex families with childhood onset of SLE. Our findings support *SAT1* LOF variants as new monogenic causes for SLE.

SAT1 variants (c.118G>T, p.Asp40Tyr in family #1 and c.272_273dup, p.Glu92Leufs*6 in family #2; reference sequence transcript: NM_002970) (online supplemental figure 1A, B) that were confirmed by Sanger sequencing. The unaffected heterozygous mothers passed the putative *SAT1* LOF variant to the two hemizygous sons affected with SLE in each family and the wild-type (WT) *SAT1* allele to one unaffected son in family #2. These variants thus exhibit a X-linked recessive Mendelian inheritance pattern in which each familial *SAT1* LOF variant co-segregated with the SLE disease status of the affected sibling (figure 1A and B). The p.Asp40Tyr variant in exon 2, predicted to be deleterious by altering a splice donor site (figure 1C and online supplemental figure 2B), was confirmed using the minigene assay. Compared with a single normally spliced transcript generated from transiently transfected Asp40-containing minigene construct in 293T or HeLa cell lines, Tyr40-containing constructs yielded two aberrantly spliced transcripts (~30% exon 2 skipped, 30% intron 2 retention and 40% normally spliced Tyr40-containing transcripts) (figure 1D and E and online supplemental figure 3A, B). The exon 2 skipped transcript resulted in premature termination, the intron 2 retention transcript was modelled to impair SSAT1 functions due to extra protein domains and the normally spliced Tyr40-containing transcript was predicted to be deleterious (online supplemental figure 2B and figure 3C). The frameshift variant (p.Glu92Leufs*6) of *SAT1* is predicted to trigger nonsense-mediated mRNA decay (figure 1C). Both *SAT1* variants alter highly conserved residues (online supplemental figure 2A), are not previously known monogenic causes or GWAS-defined SLE-risk loci[7 9] and are extremely rare in reported populations (absent in the gnomAD,[10] TOPMed[11] and 1000 Genomes[12] databases).

To explore if *SAT1* coding variants were enriched in patients with SLE, we sequenced the coding and splice regions of *SAT1* in 562 patients with SLE (422 males and 140 females, online supplemental material table 2, 3), including 65 SLE probands from families containing multiple affected members, patients with sporadic pediatric-onset lupus (disease onset ≤18 years old, 107 males and 104 females) and patients with sporadic adult-onset lupus (disease onset >18 years old, 263 males and 23 females). The *SAT1* sequencing data showed 3 additional rare variants and 12 common variants (online supplemental material table 4), but none exhibited robust evidence for functional alterations based on HaploReg V.4[13] and Regulome database[14] (online supplemental material table 5).

Mice carrying the frameshift variant in *Sat1* have pathologies resembling SLE

SAT1 encoded SSAT1 is a rate-limiting enzyme that regulates polyamine catabolism to maintain many functions of cellular polyamine homeostasis.[15] Polyamines, putrescine, spermidine and spermine are cationic aliphatic amines that regulate macromolecule interactions affecting critical cellular functions, including growth, differentiation, apoptosis, mobility and resistance to oxidative and other stresses.[16 17] Considering that the *SAT1* gene and SSAT1-regulated polyamine catabolism are not previously associated with SLE, we constructed *Sat1*$^{p.Glu92Leufs*6}$KI mice in C57BL/6J background using CRISPR/Cas9 technology (online supplemental figure 4) to determine if this LOF p.Glu92Leufs*6 variant (online supplemental figure 5) could spontaneously cause lupus-like features.

Compared with 5-week-old to 10-week-old male WT littermates, hemizygous KI male mice spontaneously developed lupus-like features, including splenomegaly, increased ratio of spleen weight to body weight, increased levels of IgG anti-dsDNA, proteinuria, and blood urea nitrogen (BUN), and displayed glomerular enlargement with leucocyte infiltration and glomerular deposition of IgG and complement C3 (figure 2A, C and D and online supplemental figure 6). The KI male mice also exhibited increased levels of antinuclear antibodies (ANA) (online supplemental figure 6C) and the inflammatory cytokine IL-17A (figure 2D and online supplemental figure 6D). No sex differences were observed between 5-week-old hemizygous male and homozygous female KI mice (figure 2). The KI spleen cells exhibited elevated expression of IFN-I-stimulated genes (online supplemental figure 6E) presented as IFN scores (figure 2D), which positively correlated with quantities of IgG deposition in kidneys, proteinuria, serum anti-dsDNA and BUN (figure 2D). These spontaneously developed lupus-like features in young KI mice demonstrate causality of the *Sat1* p.Glu92Leufs*6 variant, supporting *Sat1*$^{p.Glu92Leufs*6}$ as a monogenic cause for lupus.

Despite early development of spontaneous lupus-like kidney disorder, nephritis did not progress by 1 year of age (figure 2A, C and D), leading us to test whether an increased systemic exposure of syngeneic apoptotic cells (ACs), a condition that mimics the defective AC clearance in patients with SLE, as an induced lupus model,[18 19] could exacerbate glomerulonephritis. Compared with AC-treated WT littermates, 20-week-old AC-treated KI mice developed robust lupus-like manifestations, including

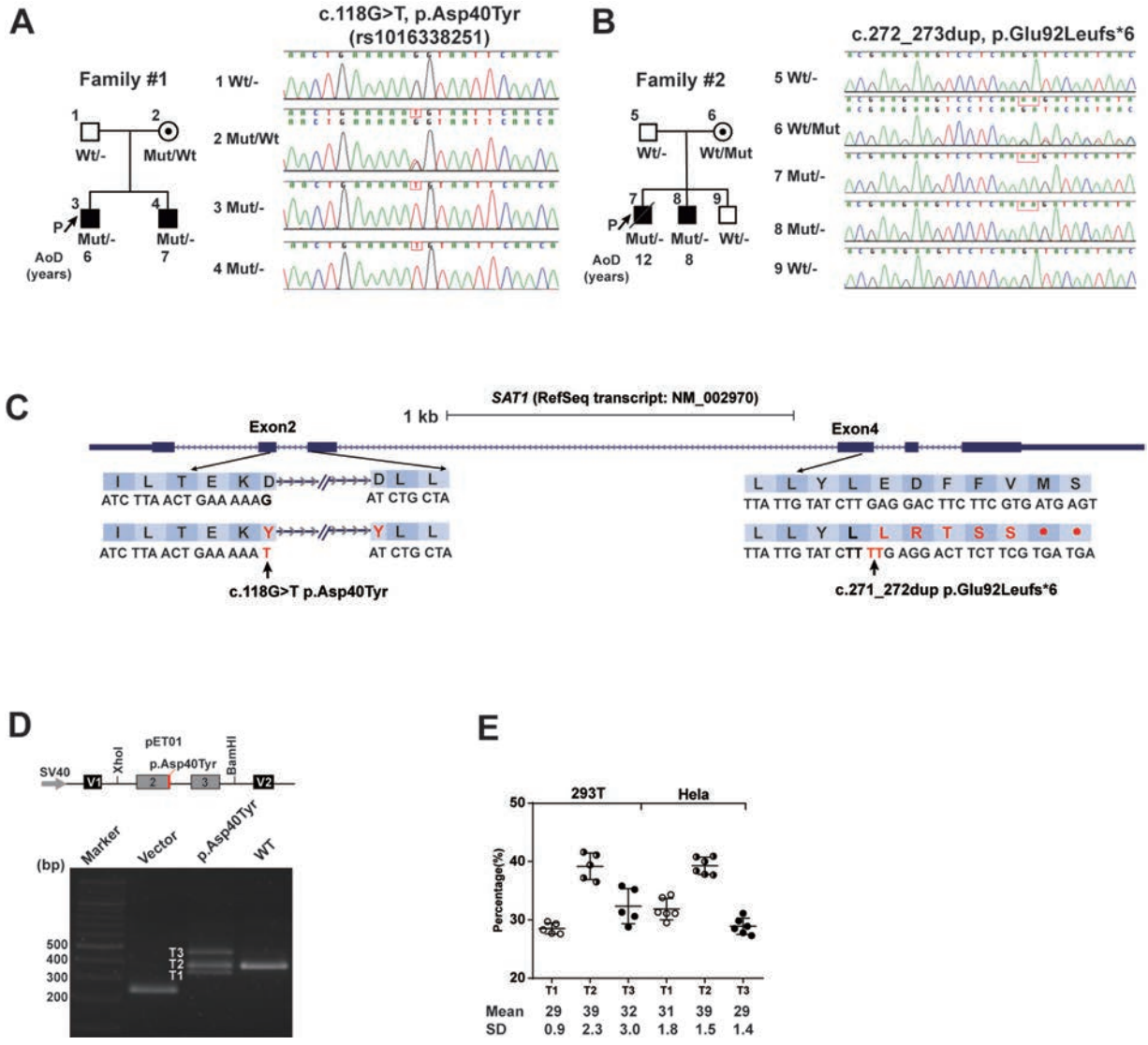

Figure 1 Identification of p.Asp40Tyr and p.Glu92Leufs*6 *SAT1* variants segregating with disease status in each family. (A and B) Pedigree information of two unrelated African–American families containing SLE-affected sibpairs and Sanger sequencing data that confirmed Mendelian inheritance of either p.Glu92Leufs*6 or p.Asp40Tyr variant of X-linked *SAT1* in each family. AoD, age of diagnosis; P, proband. p.Asp40Tyr is identified as rs1016338251 by human longevity company, but no annotation of any individual is available. (C) Locations of the p.Asp40Tyr (NM_002970: c.118G>T, ChrX(GRCh38):g.23783709G>T) and p.Glu92Leufs*6 (NM_002970: c.272_273dup, ChrX(GRCh38):g.23785397-23785398dup) *SAT1* variants and the corresponding sequences based on human reference genome build GRCh38/hg38. (D and E) The genomic segment containing p.Asp40Tyr cloned into the minigene assay vector resulted in aberrantly spliced transcripts in transfected 293T and HeLa cell lines. A schematic representation of the p.Asp40Tyr minigene plasmid and electrophoresed RT-PCR products from 293T cells transfected with a minigene plasmid with either Asp40-containing or Tyr40-containing genomic segment (D). The percentage of each spliced transcript from three independent transfection experiments is depicted. Data are mean±SD (E). WT, wild type.

elevated BUN, anti-dsDNA antibodies, ANA antibodies, proteinuria, increased serum creatinine levels, accelerated glomerulonephritis and increased glomerular deposition of IgG and complement C3. These lupus manifestations were also positively correlated with increased IFN-I scores (figure 2E). *Sat1*$^{p.Glu92Leufs*6}$ shifted immune phenotypes in spleen cells. Changes in the myeloid compartment included elevated macrophages ($CD3^-CD19^-CD11b^{hi}F4/80^+$) in 10-week-old male KI mice (figure 2B) and elevated proportions and cell numbers of plasmacytoid dendritic cells (pDCs, $CD3^-CD19^-CD11c^{int}B220^{low}$). These changes likely contribute to the upregulated IFN-I scores in 5- and 10-week-old male KI mice (figure 2B and D).

While the AC-treated *Sat1*$^{p.Glu92Leufs*6}$ male mice had elevated Th17, Tph and age-associated B cells (ABCs) indicative of extrafollicular activation, the female mice showed elevated T follicular helper cells (Tfh), Tfh/T follicular regulatory (Tfr) ratio, marginal zone B cells (MZ), follicular B cells (FO) and germinal centre (GC) B cell responses characterised by activated adaptive immunity and follicular humoral responses (online supplemental figure 8).

Bone marrow (BM)-isolated *Sat1*$^{p.Glu92Leufs*6}$ neutrophils show spontaneous NETosis and autophagy defect

While *SAT1* is fairly ubiquitously expressed, it is enriched in neutrophils of both human and mouse immune systems[20 21] (online supplemental figure 10). We hypothesised that *SAT1* expression is important in neutrophil functions. We observed

Table 1 Summary of clinical features in affected males from two families with *SAT1* mutations

Patient (gender)	Family (ethnic origin)	Age of onset	Brief clinical symptoms	Renal disorder	Immunological disorder
3 (M)	Family #1 (AA)	6 y	Malar rash, non-erosive arthritis, serositis, pleuritis and pericarditis	Cellular casts	ANA, anti-dsDNA, anti-Smith, anti-CL, anti-Ro, anti-RNP; low C3, C4
4 (M)	Family #1 (AA)	7 y	Malar rash, photosensitivity, leucopenia, lymphopaenia	Proteinuria	ANA, anti-dsDNA, anti-Smith, anti-CL, anti-Ro, anti-RNP; low C3
7 (M)	Family #2 (AA)	12 y	Non-erosive arthritis, anaemia, fatigue	Proteinuria; renal biopsy: membranous proliferative GN (Class V)	ANA, anti-dsDNA; low C3, C4
		14 y		Renal failure	
8 (M)	Family #2 (AA)	8 y	CNS lupus, non-erosive arthritis	Proteinuria; renal biopsy: focal proliferative GN (Class III)	Anti-dsDNA; ANA>1:1280
		14 y	Vasculitis affecting the eyes, 2/2	Diffuse lupus glomerulonephritis with crescents	Anti-dsDNA, anti-Smith, anti-RNP, anti-β2GPI and anti-CL

AA, African American; ANA, anti-nuclear antibody; anti-CL, anti-cardiolipin; anti-dsDNA, anti-double-stranded DNA; anti-RNP, anti-ribonucleoprotein; anti-β2GPI, anti-β2 glycoprotein I ; CNS, central nervous system; GN, glomerulonephritis; y, years old.

that BM-isolated neutrophils from 5-week-old male KI mice had decreased cell numbers and percentages compared with the WT littermates (figure 3A). These KI neutrophils undergo spontaneous NETosis without phorbol myristate acetate (PMA) stimulation (figure 3B). They released oxidised mitochondrial DNA (mtDNA) into culture supernatants indicated by an increased ratio of mitochondrial (*16s*) to chromosomal (*18s*) DNA in anti-8-OHdG immunoprecipitated total oxidised DNA (figure 3B). These observed phenotypes resemble manifestations in patients with SLE, including neutropaenia, spontaneous NETosis and release of oxidised DNA from mitochondria that elicit anti-dsDNA responses.[22 23]

BM-isolated *Sat1*$^{p.Glu92Leufs*6}$ neutrophils showed decreased AC ingestion at two time points using flowcytometry and confocal assays (figure 3C). Next, we performed immunoblot analysis to evaluate relative levels of LC3B, p62 and LAMP1 in BM-isolated neutrophils from 5-week-old WT and KI mice, with or without PMA administration. Compared with upregulated autophagy in PMA-stimulated WT neutrophils, p62 (an autophagosome cargo protein) was increased, and LC3-II (activated form of LC3) was reduced in KI neutrophils together with decreased LAMP1 levels (figure 3D). Using Autophagy RFP-GFP-LC3B Tandem Sensor, PMA-stimulated WT neutrophils exhibited elevated ratios of RFP (acid-insensitive) to GFP (acid-sensitive) indicative of autophagic flux with LC3B accumulation in acidic autophagolysomes compared with KI (figure 3E).

Mice with the p.Glu92Leufs*6 variant exhibit perturbed Foxp3-related T-cell subsets

Recent publications report the critical role of polyamine metabolism in maintaining fidelity of T-cell lineage via epigenome regulation.[24–26] Given that the p.Glu92Leufs*6 LOF variant likely disturbs cellular polyamine homeostasis, we tested the differentiation of Foxp3-related T cell subsets from thymocytes and spleen cells. Compared with their WT counterpart, KI thymocytes had significantly decreased proportions of natural Treg cells at 5 weeks, a decreased trend at 10 weeks (online supplemental figure 9B) and a trend towards increased percentages of CD4 +CD25+ T cells at 5 weeks (online supplemental figure 9A). In spleen cells, the percentage of CD4 +Foxp3+ including T regulatory (Tregs) and Tfr subsets was decreased in 10-week-old KI male mice (online supplemental figure 9C), resulting in an increased ratio of Tfh to Tfr compared with the WT littermates, a ratio that promotes humoral immune responses.

Plasma polyamine profiles of patients with SLE and correlated with elevated disease activities in patients with SLE

To assess if our findings are relevant to patients with SLE, we measured the levels of polyamine metabolites in treatment-naive, newly diagnosed patients with SLE. Figure 4A depicts polyamine metabolic pathways and intermediate metabolites. The percentage composition of nine polyamines was measured in plasma obtained from 26 patients with SLE and 20 healthy controls (HCs) (online supplemental material table 6, 7). In patients with SLE, putrescine, N^1-acetylspermidine and S-adenosyl-L-methionine were significantly increased, while levels of spermidine and spermine were significantly decreased compared with HCs (figure 4B). We explored potential links between proportions of polyamines and SLE manifestations. Plasma levels of putrescine acid were negatively correlated with cell-free DNA levels ($r=-0.6$, $p=0.01$) and positively correlated with proportion of Tph cells ($r=0.7$, $p=0.03$). Spermidine levels correlated with levels of anti-dsDNA antibody ($r=-0.42$, $p=0.04$), proportion of CD4+Foxp3+ cells ($r=0.48$, $p=0.02$) and Tfh cells ($r=0.53$, $p=0.04$). Spermine levels correlated with neutrophil count ($r=-0.66$, $p<0.01$), titres of anti-dsDNA antibodies ($r=-0.46$, $p=0.02$) and proportion of CD4+Foxp3+ cells ($r=0.60$, $p<0.01$) (figure 4C). Other correlations are shown in online supplemental material Table 8.

DISCUSSION

To the best of our knowledge, we show, for the first time, that LOF *SAT1* variants are likely monogenic causes of SLE by identifying two potential LOF variants (p.Asp40Tyr and p.Glu92Leufs*6) that segregate with SLE in two unrelated families following the X-linked recessive inheritance model. Functional studies demonstrated that the p.Asp40Tyr variant caused aberrantly spliced, deleterious *SAT1* transcripts. The hemizygous expression of the p.Glu92Leufs*6 variant in young C57BL/6J male mice induced glomerulonephritis, splenomegaly, the production of IgG anti-dsDNA antibodies, elevated type I IFN-scores, reduced numbers and proportions of BM-isolated neutrophils, decreased phagocytosis of ACs and autophagic flux by neutrophils, increased spontaneous NETosis and release of oxidised mitochondrial DNA, and decreased proportions of Foxp3 +CD4+ T cells. Given that these features are commonly found in patients with SLE, recapitulating human SLE by a Mendelian inherited single LOF *SAT1* variant on a non-autoimmune mouse background showed that it is a new monogenic cause for lupus. Similar to young

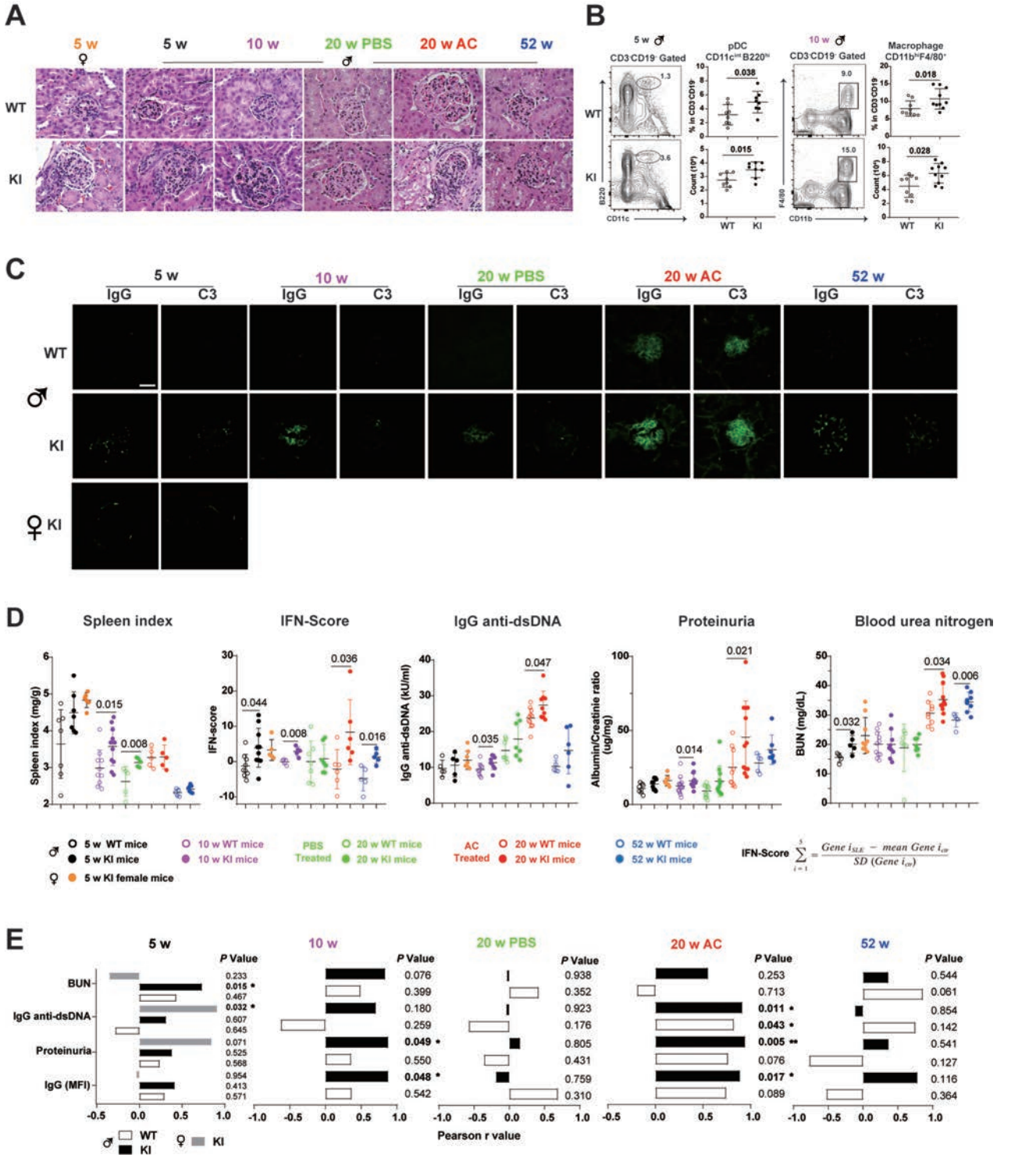

Figure 2 The young *Sat1*$^{p.Glu92Leufs*6}$ KI male and female mice spontaneously develop lupus-like autoimmune disorder. (A) Haematoxylin-eosin-stained kidney sections from female (5 weeks old) and male *Sat1*$^{p.Glu92Leufs*6}$ KI mice and WT littermates that were naive (5, 10 or 52 weeks old), or injected with either phosphate buffer saline (PBS) or apoptotic cells (ACs) starting at 10 weeks old and sacrificed at 20 weeks old. Bar: 50 µm. (B) Gating strategy and percentage of plasmacytoid dendritic cells (pDCs) ($CD3^-CD19^-CD11c^{int}B220^{hi}$) and macrophages ($CD3^-CD19^-CD11b^{hi}F4/80^+$) in spleen cells from 5-week-old and 10-week-old naive male mice, respectively. Open circle, WT mice; closed circle, KI littermates. Data are mean±SD. Mann-Whitney U test. (C) Immunofluorescent staining of mouse immunoglobulin G (IgG) and complement 3 (C3) depositions in the frozen kidney sections of *Sat1*$^{p.Glu92Leufs*6}$ female KI mice, male KI and WT littermates. Bar: 50 µm. (D) Levels of spleen index, type I IFN scores, serum IgG anti-dsDNA, proteinuria and blood urea nitrogen (BUN) in 5-week-old to 52-week-old female KI mice, male KI and WT littermates. Open circle, male WT mice; closed circle, male KI mice. Black, 5-week-old mice; purple, 10-week-old mice; green, 20-week-old mice injected with PBS; red, 20-week-old mice injected with ACs; blue, 52-week-old mice. Data are mean±SD. Yellow closed circle, 5-week-old female KI mice. Mann-Whitney U test. (E) Correlation analysis of levels of IgG deposition in the kidney, proteinuria, serum anti-dsDNA and BUN with type I IFN scores in splenocytes of 5-week-old to 52-week-old KI mice. Closed box depicts statistically significant correlation. White box, male WT mice; black box, male KI mice; grey box, female KI mice. KI, knock in; WT, wild type.

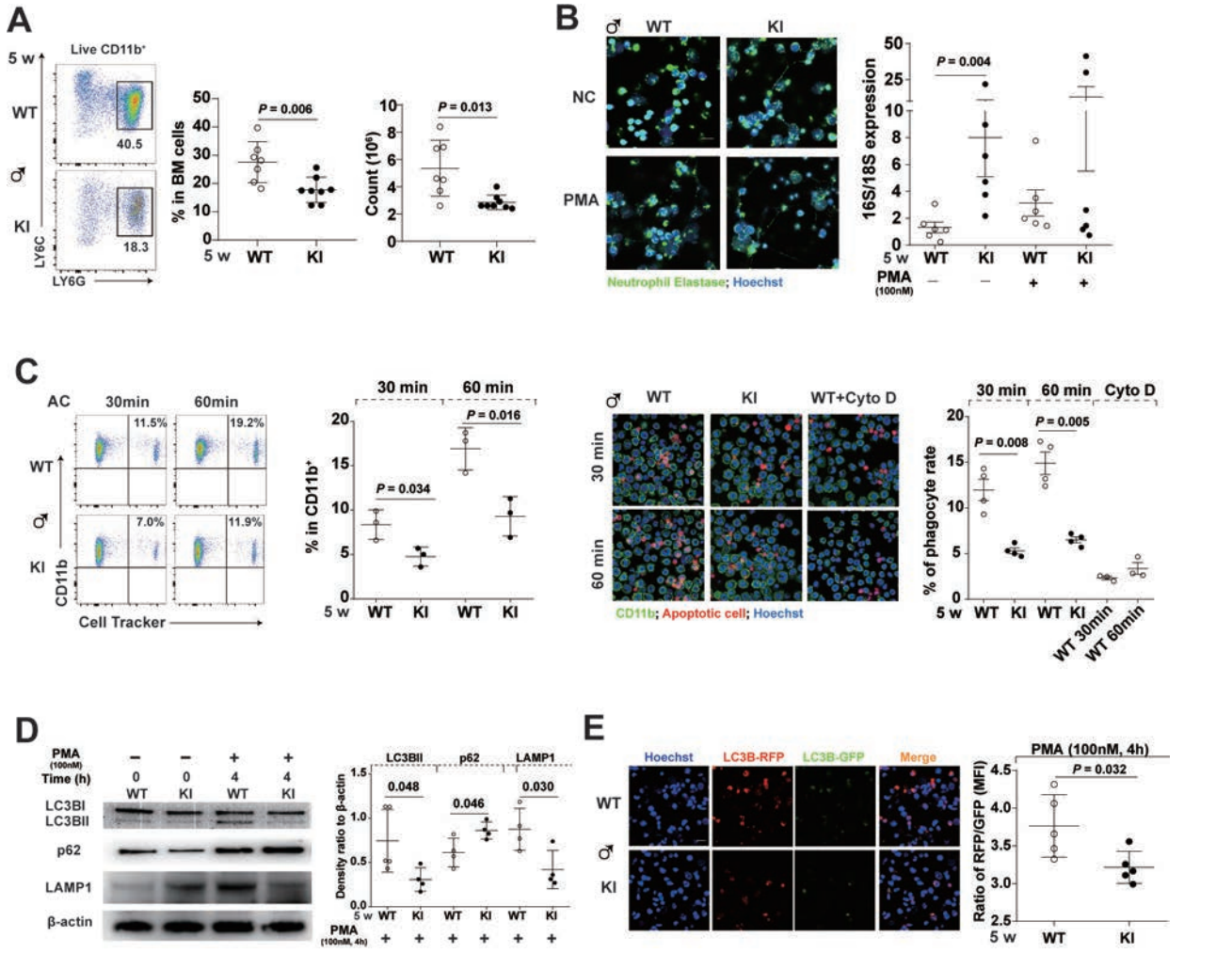

Figure 3 Decreased levels and defective functions of bone marrow (BM)-isolated neutrophils from young male *Sat1*$^{p.Glu92Leufs*6}$ mice. (A) Gating strategy, decreased percentage and cell numbers of BM-isolated neutrophils ($CD11b^+LY6G^+LY6C^{int}$) in 5-week-old male KI mice compared with their WT littermates. Data are mean±SD. Mann-Whitney U test. (B) (left) Representative images of the neutrophil extracellular traps (NET) induced by PMA in BM-isolated neutrophils. (right) Quantitation of mitochondrial (*16S*; officially known as *MT-RNR2*) and chromosomal (*18S;* officially known as *RNA18S5*) DNA in the immuno-precipitated total oxidised DNA from overnight culture supernatants of BM-isolated neutrophils of either WT or KI littermates incubated in the absence (spontaneous NETosis) or presence of PMA (induced NETosis). Green immunofluorescence represents neutrophil elastase and blue represents DNA (Hoechst_33342) of confocal images. Bar: 10 µm; PMA, phorbol myristate acetate, 100 nM; incubation time, 24 hours. Data are mean±SD. Mann-Whitney U test. (C) Defective engulfment of cell tracker-labelled apoptotic cells (ACs) by BM-isolated neutrophils from 5-week-old male KI mice after 30 or 60 min co-cultures assessed by either flow cytometry or confocal microscopy; BM-isolated neutrophils: AC=1:5. Cyto D, cytochalasin D, an inhibitor of actin polymerisation, at 10 µM; bar: 10 µm. Data are mean±SD. Unpaired t-test. (D) Representative Western blot of LC3B, p62 (an autophagosome cargo protein), LAMP1 and β-actin, and quantification of relative levels of LC3B-II to LC3B-I, and relative levels of p62 or LAMP1 to β-actin in PMA-stimulated groups. PMA, 100 nM; bar: 10 µm; incubation time, 4 hours. Data are mean±SD. Unpaired t-test. (E) Decreased levels of autophagic flux in PMA-treated BM-isolated neutrophils from 5-week-old male KI mice. The left panel depicts representative fluorescence images of autophagic flux assays using an RFP-GFP-LC3B tandem construct that only the GFP signal could be quenched by the acidic lysosomal pH, and the right panel depicts relative ratios of RFP: GFP in each group. Bar: 10 µm; PMA, 100 nM; incubation time, 16 hours. Data are mean±SD. Unpaired t-test. KI, knock in; WT, wild type.

hemizygous male KI mice, young homozygous female mice also exhibited lupus-like kidney features. By using both in vivo and in vitro studies, we showed defects in both innate (neutrophils) and adaptive immunity (T cells) induced by the p.Glu92Leufs*6 variant revealing a novel role of polyamine metabolism as a risk for SLE.

Monogenic forms of lupus or lupus-like syndromes are the most straightforward approach to unravel the molecular pathogenesis of childhood-onset SLE, especially in those with a more severe phenotype, with a family history of SLE, or from consanguineous marriages.[6 27 28] Recent advances in next-generation sequencing continues allow discovery of single gene rare variants that cause SLE/SLE-like syndromes, which are either inherited in an autosomal dominant/ recessive manner or occur due to de novo mutations.[2 7 29] While *SAT1* is an IFN-I-inducible gene,[30 31] the clinical characteristics of patients carrying the p.Asp40Tyr and p.Glu92Leufs*6 variants of the *SAT1* fulfil more than four ACR criteria of SLE (table 1), which differ from patients with some monogenic interferonopathies, who show clinical signs of lupus but do not fulfil classification criteria for SLE. The known causes for monogenic interferonopathies, including mutations in *TREX1*, *SAMHD1*, *ADAR*, *IFIH1*, *and RNASEH2A/2B/2C*, which disrupt functions of gene products participating in proteasome degradation and cytoplasmic RNA and DNA sensing

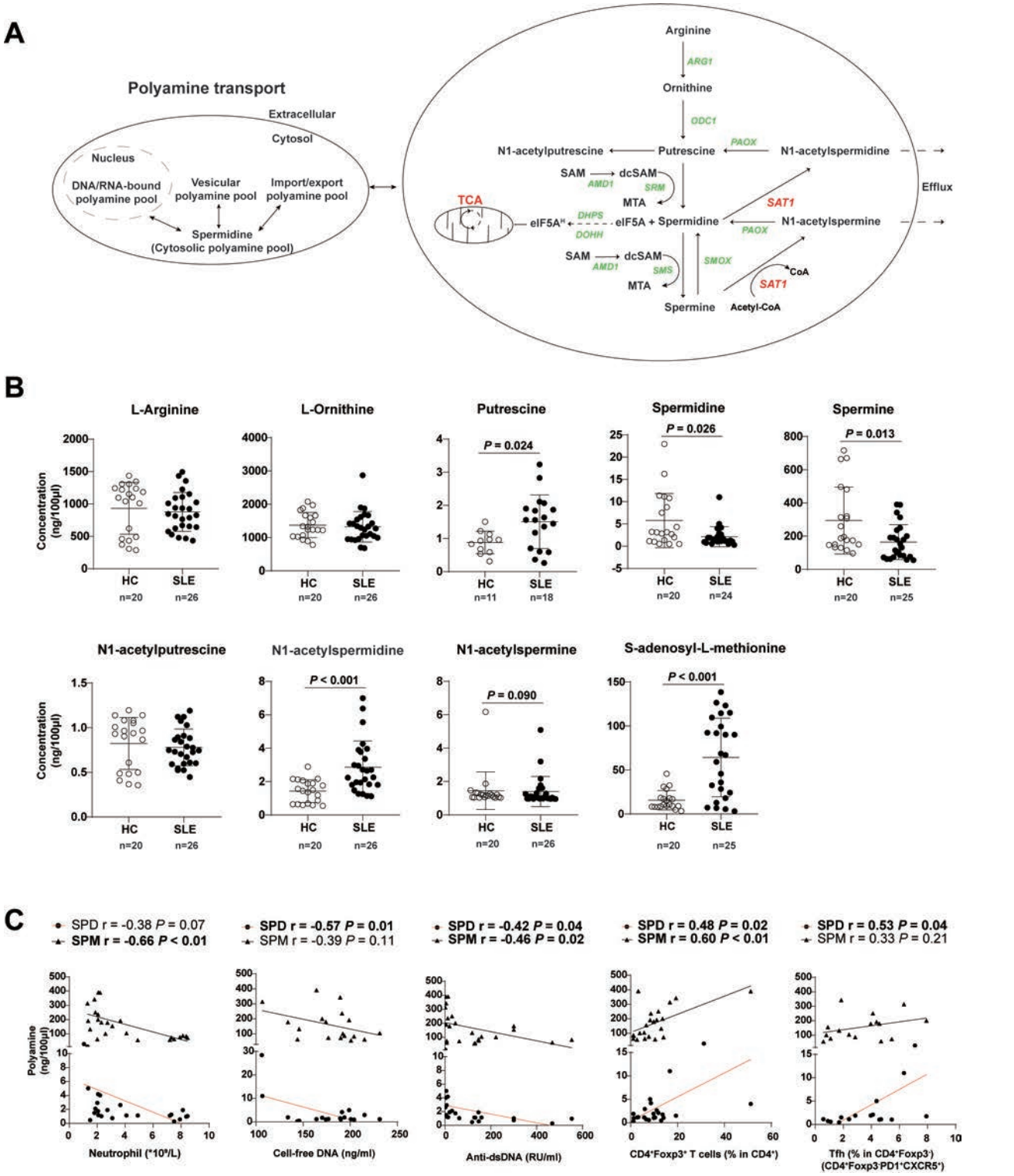

Figure 4 Plasma concentration of nine metabolites related to polyamine homeostasis in treatment-naive patients affected with SLE and in age-matched and sex-matched healthy controls (HCs). (A) The polyamine metabolism pathway. (B) Quantification of nine polyamine metabolites (ng/100 µL) found in fasting plasma samples of treatment-naive, newly diagnosed patients with SLE (n=26) and age-matched and sex-matched HCs (n=20). Open circle, health control; closed circle, patient with SLE. Data are mean±SD. Mann-Whiney U test. (C) Pearson correlations of polyamine concentrations (SPM and SPD, spermine and spermidine, respectively) with counts of blood neutrophils, percentages of Tfh and CD4 +Foxp3+T cells, serum levels of cell-free DNA and IgG anti-double-strand DNA antibodies in treatment-naive patients with SLE (n=26). Closed circle and orange line, spermidine; triangle and black line, spermine. Each symbol represents a sample from one individual subject. ARG1, arginase 1; CoA, coenzyme A; dcSAM, decarboxylated S-adenosylmethionine; DHPS, deoxyhypusine synthase; DOHH, deoxyhypusine hydroxylase; eIF5A, eukaryotic initiation factor 5A; eIF5AH, eukaryotic initiation factor 5A hypusination; PAOX, peroxisomal N(1)-acetyl-spermine/spermidine oxidase; MTA, 5′-Deoxy-5′-methylthioadenosine; ODC1, ornithine decarboxylase 1; SAM, S-adenosylmethionine; SAT1, spermine/spermidine N1-acetyltransferase 1; SLE, systemic lupus erythematosus; SMOX, spermine oxidase; SMS, spermine synthase; SRM, spermidine synthase; TCA, tricarboxylic acid cycle.

pathways.[32 33] Whole genome sequencing and WES applications in multi-case families with SLE identified a growing number of rare, likely pathogenic variants, but none clearly fulfilled a Mendelian inheritance pattern and/or demonstrated causality in vivo.[34–36] Higher genetic load for SLE, measured by individual polygenic risk scores (PRS; calculated from the effect size and number of common risk alleles), was reported in childhood-onset more than adult-onset patients with SLE and in non-European ancestry more than European ancestry.[37 38] Our study design that focused on the combined high genetic load of childhood-onset, familial SLE, male lupus and African–American ancestry[38–41] contributed to our identification of the two rare LOF variants of *SAT1* in these two unrelated families. These findings exhibit an X-linked recessive inheritance model, in that the single copy of a KI frameshift variant is sufficient to induce murine lupus in young male mice on a non-autoimmune C57BL/6J background.

To assess if *SAT1* variants were enriched in patients with SLE in addition to these two LOF rare variants, we sequenced *SAT1* coding regions in 562 SLE DNA samples enriched in male patients (422/562, 75.1%) and multiplex family cases (65/562, 11.6%). While we identified 3 additional rare variants and 12 common variants, none were predicted pathogenic, suggesting that the two identified LOF *SAT1* variants were unique (online supplemental material table 4 and 5). Most known LOF *SAT1* variants in the gnomAD database (with an average 50X sequencing depth) are located in coding regions of non-canonical transcripts that are expressed at low levels across all tissues[12] (online supplemental figure 1), implicating strong selection against LOF mutations in the highly expressed transcript. Given that both p.Asp40Tyr (in exon 2) and p.Glu92Leufs*6 (in exon 4) are located in highly conserved coding regions of *SAT1* and are not present in >200 000 individuals characterised in the gnomAD,[10] TOPMed[11] and 1000 Genomes database,[42] these two variants were ultra-rare and highly penetrant in families with patients, especially in male paediatric lupus.

How does this p.Glu92Leufs*6 variant of *SAT1* affect the autoimmune responses? In the normal C57BL/6J background, the young male and female KI mice spontaneously developed lupus-like autoimmune disorders. While *SAT1* has a broad spectrum of expression in many tissues and cell types, its expression in the immune system is mainly enriched in neutrophils in humans and C57BL/6 mice (online supplemental figure 10).[20 21] We extended this study and found neutrophil defects, including decreased cell numbers and aberrant functions in young KI mice. These KI BM-isolated neutrophils spontaneously released NETs enriched in oxidised mtDNA (figure 3B), which were features previously described in neutrophils from patients with SLE that could elicit IgG anti-dsDNA production and activate type I IFN responses (figure 2D).[43 44] While mechanisms underlying decreased neutrophil counts were not characterised, it is plausible that autoantibodies and type I IFN in young KI male mice could cooperatively induce neutrophil ferroptosis and spontaneous NETosis as shown in murine and human lupus individuals.[23 45 46] A well-established phagocyte defect in SLE is the impaired clearance of ACs (termed efferocytosis), which results in accumulated AC metabolites in various tissues as a source of self-antigens that could promote inflammation and the development of SLE.[47 48] We observed impaired KI neutrophil clearance of ACs via LC3-associated phagocytosis (LAP) (figure 3D and E) suggesting that a functional LAP is necessary to inhibit autoinflammatory, lupus-like responses to dying cells.[49] Additionally, it is plausible that dysfunctional SSAT1 encoded by *Sat1*$^{p.Glu92Leufs*6}$ could perturb polyamine import and accumulation resulting in diminished polyamine-mediated anti-inflammatory responses during efferocytosis.[50]

To our knowledge, none of monogenic lupus and SLE GWAS-defined risk loci have pointed to a role of polyamine metabolism in lupus pathogenesis.[1 2] Early connections between polyamines and lupus pathogenesis were shown using an inhibitor of polyamine synthesis that could reduce T-cell proliferation and prolong survival of lupus-prone MRL-lpr/lpr mice[51 52] and could reduce pokeweed mitogen-induced cell proliferation and production of IgM and IgG in PBMC cultures.[53] Emerging evidence supports inverse correlations between polyamine levels and the extent of autoimmunity and inflammation.[54] Our findings of decreased plasma levels of spermidine and spermine in untreated, newly diagnosed patients with SLE (figure 4) confirmed reduced levels previously reported in Korean patients with SLE.[8] Recently, polyamine spermidine was shown to mediate metabolic and epigenetic regulation through translation factor eukaryotic translation initiation factor 5A-1 (eIF5A) hypusination governing the ability of CD4 + T cells to develop into specific functional subsets.[24] Additionally, the polyamine pathway is required for Th17 induction and Treg suppression.[26] These pivotal roles of polyamine metabolism in the differentiation of CD4 + T cells could help explain our observed decreased proportions of Foxp3 + T cells (including Treg and Tfr cells) in young naive KI mice and increased proportions of Tfh and Tfh/Tfr ratios in AC-induced exacerbated autoimmune disease of KI mice (online supplemental figure 9).

Interestingly, even though young, naive, B6 KI mice of both sexes developed spontaneous lupus-like kidney disorder, it did not progress into proliferative lupus-like kidney disease as they aged (figure 2). As bioactive polycations, polyamines bind nucleic acid and proteins and promote cell proliferation, it is plausible that the rapid growth phase early in life activates *Sat1* expression. The maintenance phase of the adult mice kept on a normal chow diet confers relative low activation of SSAT1 enzyme activity, and B6 mice are a relatively lupus-resistant strain bred in a specific pathogen-free environment.[30 55–57] Administration of syngeneic apoptotic thymocytes is established as an immune challenge that can induce mild lupus features in non-autoimmune mice and robust lupus-like autoimmunity in genetically predisposed mice.[18 19] When we perturbed the 10-week-old male and female KI mice with increased exposure to syngeneic ACs, robust production of IgG anti-dsDNA diffuse proliferative glomerulonephritis, and proteinuria ensued, demonstrating the capability of the *Sat1*$^{p.Glu92Leufs*6}$ variant to confer lupus-like disease on immune activation. Of note, male KI mice appeared to have elevated extrafollicular immune activation and female KI mice more robust follicular humoral responses (figure 2 and online supplemental figure 8). The sex difference effect of *Sat1* on activated B cells is consistent with the mechanisms that fine-tuned B-cell physiology imparts on sexual dimorphism in humoral responses and autoimmunity.[58]

Limitations of this study include the followings: (1) The highly penetrant LOF *SAT1* variants are ultra-rare as we found only two familial variants in our study sample of 562 patients with SLE enriched in male, childhood-onset and family history of SLE. (2) The lack of cell sources from the two families and insufficient BM-isolated neutrophils from *Sat1*$^{p.Glu92Leufs*6}$ mice prevented direct measurements of intracellular polyamine metabolites.

In summary, we showed, for the first time, *SAT1* is a novel candidate causative gene of SLE by identifying two potentially LOF variants (p.Asp40Tyr and p.Glu92Leufs*6), which segregated with the SLE disease status in two unrelated African–American families. Functional studies demonstrated that mice carrying

the p.Glu92Leufs*6 variant exhibit lupus-like features, including immune cell dysplasia and dysfunction using both in vivo and in vitro studies. Our findings support LOF *SAT1* variants as new monogenic causes for SLE and highlight the pathogenic role of disturbed polyamine metabolism in developing SLE.

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Ethics approval This study involves human participants and was approved by human DNA samples obtained from subjects enrolled in IRB-approved longitudinal cohorts from the University of California, Los Angeles (IRB number 10-001489), the Medical University of South Carolina (MUSC) (HR#10852), the MUSC Clinical and Community Core of the Core Center for Clinical Research (Pro00021985) and the Oklahoma Medical Research Foundation (95-12 and 06-12). Human participants were recruited with informed consents approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (2020-SR-044). Participants gave informed consent to participate in the study before taking part. Animal subjects were approved by the Medical University of South Carolina Institutional Animal Care (ACORP 682).

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CLINICAL SCIENCE

Continued treatment with nintedanib in patients with systemic sclerosis-associated interstitial lung disease: data from SENSCIS-ON

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ABSTRACT

Objectives In the SENSCIS trial in patients with systemic sclerosis-associated interstitial lung disease (SSc-ILD), nintedanib reduced the rate of decline in forced vital capacity (FVC) versus placebo, with adverse events that were manageable for most patients. An open-label extension trial, SENSCIS-ON, is assessing safety and FVC decline during longer term nintedanib treatment.

Methods Patients who completed the SENSCIS trial or a drug–drug interaction (DDI) study of nintedanib and oral contraceptive on treatment were eligible to enter SENSCIS-ON. Adverse events and changes in FVC over 52 weeks of SENSCIS-ON were assessed in patients who received nintedanib in SENSCIS and continued nintedanib in SENSCIS-ON ('continued nintedanib' group) and in patients who received placebo in SENSCIS and initiated nintedanib in SENSCIS-ON or who received nintedanib for ≤28 days in the DDI study ('initiated nintedanib' group).

Results There were 197 patients in the continued nintedanib group and 247 in the initiated nintedanib group. Diarrhoea was reported in 68.0% and 68.8% of patients in these groups, respectively. Adverse events led to discontinuation of nintedanib in 4.6% and 21.5% of the continued nintedanib and initiated nintedanib groups, respectively. Mean (SE) changes in FVC from baseline to week 52 of SENSCIS-ON were −58.3 (15.5) mL in the continued nintedanib group and −44.0 (16.2) mL in the initiated nintedanib group.

Conclusions The safety profile of nintedanib over 52 weeks of SENSCIS-ON was consistent with that reported in SENSCIS. The change in FVC over 52 weeks of SENSCIS-ON was similar to that observed in the nintedanib group of SENSCIS.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The results of the randomised placebo-controlled SENSCIS trial showed that in patients with systemic sclerosis-associated interstitial lung disease (SSc-ILD), nintedanib reduced the rate of decline in forced vital capacity (FVC) over 52 weeks.

WHAT THIS STUDY ADDS

⇒ The results of this open-label extension study show that the safety profile of nintedanib over longer term use was consistent with that seen in the SENSCIS trial and that the change in FVC over 52 weeks of the open-label extension was similar to that seen in patients who received nintedanib in SENSCIS.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These findings suggest that nintedanib can be used over the long term to slow the progression of SSc-ILD and so improve patient outcomes.

INTRODUCTION

Systemic sclerosis is a heterogeneous autoimmune disease characterised by multiorgan vascular and fibrotic abnormalities.[1] Interstitial lung disease (ILD) is a common manifestation of SSc, which most frequently develops early in the disease course.[2] Systemic sclerosis-associated ILD (SSc-ILD) has a variable course and in some patients becomes progressive, characterised by an increase in fibrotic abnormalities on high-resolution CT (HRCT), a decline in forced vital capacity (FVC) and premature death.[3 4] A decline in FVC in patients with SSc-ILD is predictive of mortality.[3 5 6] There is no established algorithm to inform when pharmacotherapy for SSc-ILD should be initiated or which therapy should be used. Treatment decisions should be made on a case-by-case basis, taking into account the severity of ILD, risk factors for progression, other manifestations of SSc and the patient's preferences.[7 8]

Nintedanib, a tyrosine kinase inhibitor with anti-inflammatory and antifibrotic properties,[9] has been licensed for the treatment of SSc-ILD as well as for the treatment of idiopathic pulmonary fibrosis (IPF) and other chronic fibrosing ILDs with a progressive phenotype. The efficacy and safety of nintedanib in patients with SSc-ILD were investigated in the SENSCIS trial, in which patients were randomised to receive nintedanib or placebo until the last patient had reached week 52 but for a maximum of 100 weeks.[10] Over 52 weeks, nintedanib reduced the rate of decline in FVC (mL/year) by 44% compared with placebo, with an adverse event profile characterised predominantly by gastrointestinal events,

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particularly diarrhoea. Data collected over the whole SENSCIS trial (up to 100 weeks of treatment) suggested that nintedanib provided a sustained benefit on slowing the progression of SSc-ILD over 100 weeks, with adverse events that were manageable for most patients.[11] An open-label extension of SENSCIS, SENSCIS-ON, is assessing the safety and tolerability of nintedanib over the longer term. Exploratory data on FVC are also being collected. Here, we present data from the first year of SENSCIS-ON.

METHODS

Trial design

Patients in SENSCIS-ON (NCT03313180) came from two parent trials: SENSCIS (NCT02597933) and a drug–drug interaction (DDI) study (NCT03675581). SENSCIS enrolled patients with SSc-ILD with onset of first non-Raynaud symptom in the prior ≤7 years, extent of fibrotic ILD on HRCT ≥10% and FVC≥40% predicted.[10] Patients receiving prednisone ≤10 mg/day or equivalent and/or stable therapy with mycophenolate or methotrexate for ≥6 months were allowed to participate. Patients were randomised to receive nintedanib 150 mg two times per day or placebo, stratified by antitopoisomerase I antibody status, until the last patient had reached week 52 but for ≤100 weeks. Patients who completed SENSCIS on treatment and attended a follow-up visit 28 days later were eligible to participate in SENSCIS-ON. Per protocol, the off-treatment period between SENSCIS and SENSCIS-ON was ≤12 weeks.

The DDI study from which patients could enter SENSCIS-ON was an open-label study of nintedanib plus oral contraceptive (Microgynon; ethinylestradiol and levonorgestrel) in female patients with SSc-ILD.[12] Patients receiving prednisone ≤10 mg/day or equivalent and/or stable therapy with methotrexate for ≥6 months were allowed to participate. Treatment with mycophenolate ≤2 weeks prior to the start of the study was not permitted. Patients received nintedanib 150 mg two times per day over a period of ≥14 days to approximately 28 days. Per protocol, the off-treatment period between this study and SENSCIS-ON was ≤7 days.

In both SENSCIS and the DDI study, dose reductions to 100 mg two times per day were permitted to manage adverse events and dose could be increased back to 150 mg two times per day once the adverse event had resolved. Treatment could be interrupted for ≤4 weeks or ≤8 weeks to manage adverse events considered to be related to study drug, or not related to study drug, respectively. Patients receiving nintedanib or placebo at a dose of 150 mg two times per day at the end of the parent study received nintedanib 150 mg two times per day in SENSCIS-ON. Patients receiving nintedanib or placebo at a dose of 100 mg two times per day at the end of the parent study could receive nintedanib 100 mg two times per day or 150 mg two times per day in SENSCIS-ON. In SENSCIS-ON, nintedanib dose reductions from 150 mg two times per day to 100 mg two times per day were permitted, and treatment could be interrupted for ≤4 weeks or ≤12 weeks to manage adverse events considered to be related to study drug, or not related to study drug, respectively. FVC was assessed at baseline and at weeks 4, 12, 24, 36 and 52, using sponsor-supplied spirometers, in accordance with American Thoracic Society/European Respiratory Society guidelines.[13] FVC measurements were centrally reviewed.

SENSCIS-ON is being carried out in compliance with the protocol and in accordance with the principles of the Declaration of Helsinki, the International Council for Harmonisation Harmonised Tripartite Guideline for Good Clinical Practice, applicable regulatory requirements and standard operating procedures. Patients provided written informed consent prior to entry into the trial.

Exclusion criteria

Patients with aspartate aminotransferase or alanine aminotransferase >3 times the upper limit of normal (ULN) or bilirubin >2 times the ULN were excluded from SENSCIS-ON, as were patients at risk of bleeding and patients with major thromboembolic events following completion of the parent trial. A complete list of the exclusion criteria is provided in the supplemental material.

Endpoints

Adverse events, reported irrespective of causality, with onset from the first drug intake to week 52 (or to the last drug intake plus 7 days for patients who prematurely discontinued treatment) were coded using the Medical Dictionary for Regulatory Activities V.22.1. Serious adverse events were defined as adverse events that resulted in death, were life threatening, resulted in hospitalisation or prolongation of hospitalisation, resulted in persistent or clinically significant disability or incapacity, were a congenital anomaly or birth defect or were deemed serious for any other reason. Recommendations for the management of diarrhoea and liver enzyme elevations were provided to the investigators.[14] Efficacy endpoints assessed at week 52 included absolute change from baseline in FVC (mL); the proportions of patients with relative categorical increase and decline in FVC (mL); the cumulative distribution of patients by absolute change from baseline in FVC % predicted; and changes from baseline in the modified Rodnan skin score (mRSS), St. George's Respiratory Questionnaire (SGRQ) total score and University of California Los Angeles (UCLA) Scleroderma Clinical Trial Consortium Gastrointestinal Tract (UCLA SCTC GIT) V.2.0 instrument total score. The mRSS measures skin thickness based on palpation of 17 areas, each rated on a scale of 0–3, with higher scores indicating worse skin thickening.[15] The SGRQ is a measure of health-related quality of life (HRQL) in patients with respiratory diseases and comprises three domains: impact, symptoms and activity.[16] Each domain score and the total score are scaled from 0 to 100, with higher scores indicating worse HRQL. The UCLA SCTC GIT instrument V.2.0 comprises seven scales measuring the severity and impact of gastrointestinal symptoms: reflux, distension or bloating, faecal soilage, diarrhoea, constipation, emotional well-being, social functioning.[17] Each scale is scored from 0 to 3 except for diarrhoea (0 to 2) and constipation (0 to 2.5). The total score, derived as the mean of the scores for the scales except constipation, ranges from 0 to 2.83, with higher scores indicating worse symptoms.

Analyses

Analyses were conducted in patients who had received nintedanib in SENSCIS and continued nintedanib in SENSCIS-ON ('continued nintedanib' group), and in patients who had received placebo in SENSCIS and initiated nintedanib in SENSCIS-ON or who had received nintedanib for a brief period in the DDI study ('initiated nintedanib' group). All analyses were descriptive and conducted in patients who received ≥1 dose of trial medication. Changes from baseline in each endpoint were based on observed data available at the respective time point. The cumulative distribution of patients by absolute change from baseline in FVC % predicted was determined *post hoc* based on the worst observation carried forward method. In *post hoc*

Table 1 Baseline characteristics of patients at inclusion in SENSCIS-ON

	Continued nintedanib (n=197)	Initiated nintedanib (n=247)
Female, n (%)	148 (75.1)	187 (75.7)
Age, years, mean (SD)	55.8 (11.3)	54.4 (12.3)
Weight, kg, mean (SD)	68.0 (15.2)	70.7 (16.6)
Body mass index, kg/m^2, mean (SD)	25.4 (4.6)	26.1 (5.2)
Race, n (%)		
White	142 (72.1)	166 (67.2)
Asian	42 (21.3)	68 (27.5)
Black or African-American	9 (4.6)	9 (3.6)
Other	4 (2.0)	4 (1.6)
FVC, mL, mean (SD)	2379 (754)	2443 (814)
FVC, % predicted, mean (SD)	70.4 (18.1)	70.8 (17.9)
mRSS, mean (SD)	8.5 (7.7)	8.8 (7.8)
SGRQ total score, mean (SD)	41.5 (20.6)	37.8 (21.9)
UCLA SCTC GIT total score, mean (SD)	0.33 (0.33)	0.33 (0.34)
Taking mycophenolate, n (%)	105 (53.3)	127 (51.4)

FVC, forced vital capacity; SGRQ, St. George's Respiratory Questionnaire; UCLA, University of California Los Angeles; UCLA SCTC GIT, UCLA Scleroderma Clinical Trial Consortium Gastrointestinal Tract.

analyses, adverse events and absolute change from baseline in FVC (mL) at week 52 were analysed in subgroups by mycophenolate use at the start of SENSCIS-ON.

RESULTS

Patients

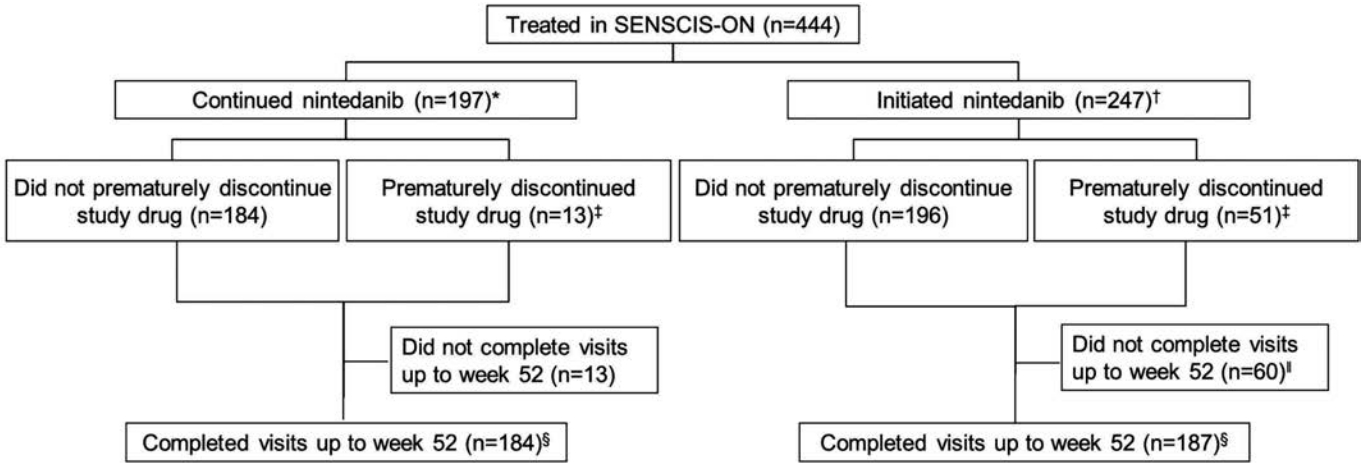
Of the 473 patients who completed SENSCIS (n=456) or the DDI study (n=17) on treatment, 444 (93.9%) entered SENSCIS-ON. There were 197 patients in the continued nintedanib group and 247 patients (231 from SENSCIS, 16 from the DDI study) in the initiated nintedanib group. Baseline characteristics at entry into SENSCIS-ON were generally similar between patients who continued and initiated nintedanib (table 1). The majority of patients were women (75.5%) and white (69.4%); mean (SD) FVC at baseline was 70.6 (18.0) % predicted; 232 patients (52.3%) were taking mycophenolate. Baseline characteristics at entry into SENSCIS-ON in subgroups by mycophenolate use are shown in online supplemental table S1. In the continued nintedanib and initiated nintedanib groups, respectively, 13 (6.6%) and 51 (20.6%) patients permanently discontinued nintedanib before week 52 (figure 1).

Exposure

Due to the trial design, the patients rolled over from SENSCIS into SENSCIS-ON had received different exposures to trial drug in SENSCIS (52–100 weeks). The median (minimum and maximum) off-treatment period between SENSCIS and SENSCIS-ON was 44 (26 and 88) days in patients who continued nintedanib in SENSCIS-ON and 49 (24 and 140) days in patients who initiated nintedanib in SENSCIS-ON. The median (minimum and maximum) off-treatment period between the DDI study and SENSCIS-ON was 8 (6 and 37) days. Median (minimum and maximum) exposure over 52 weeks in SENSCIS-ON was 13.8 (0.2, 13.8) months in the continued nintedanib group and 13.8 (0.0 and 13.8) months in the initiated nintedanib group. Total median (minimum and maximum) exposure to nintedanib across both SENSCIS and SENSCIS-ON was 29.5 (12.8 and 37.0) months. Among those in the continued nintedanib group, 54 patients (27.4%) had >36 months' exposure to nintedanib across both SENSCIS and SENSCIS-ON.

Adverse events and dose adjustments

Adverse events are shown in table 2. Diarrhoea was the most frequent adverse event, reported in 134 patients (68.0%) who continued nintedanib and 170 patients (68.8%) who initiated nintedanib. In the continued nintedanib and initiated nintedanib groups, respectively, the worst diarrhoea event was mild or moderate in intensity in 99.3% and 95.3% of the patients who had diarrhoea. Among patients who experienced diarrhoea, 3 (2.2%) and 17 (10.0%) patients who continued and initiated nintedanib, respectively, permanently discontinued nintedanib due to diarrhoea. Liver test



*Patients randomised to nintedanib in SENSCIS continued nintedanib in SENSCIS-ON.
†Patients from the drug–drug interaction study were pooled with patients randomised to placebo in SENSCIS.
‡Premature discontinuations recorded prior to the 52-week time window (i.e. up to day 310).
§Patients were considered as having completed visits up to week 52 if they had ≥1 visit during and/or after the 52-week time window (days 310–421).
‖11 patients had not reached this time point at the time of this interim analysis.

Figure 1 Disposition of patients in SENSCIS-ON.

Table 2 Adverse events (reported irrespective of causality) in SENSCIS and SENSCIS-ON

	SENSCIS		SENSCIS-ON	
	Nintedanib (n=288)	Placebo (n=288)	Continued nintedanib (n=197)	Initiated nintedanib (n=247)
Diarrhoea	218 (75.7)	91 (31.6)	134 (68.0)	170 (68.8)
Nausea	91 (31.6)	39 (13.5)	32 (16.2)	60 (24.3)
Vomiting	71 (24.7)	30 (10.4)	27 (13.7)	53 (21.5)
Skin ulcer	53 (18.4)	50 (17.4)	36 (18.3)	43 (17.4)
Nasopharyngitis	36 (12.5)	49 (17.0)	28 (14.2)	33 (13.4)
Upper respiratory tract infection	33 (11.5)	35 (12.2)	27 (13.7)	26 (10.5)
Cough	34 (11.8)	52 (18.1)	24 (12.2)	21 (8.5)
Weight decreased	34 (11.8)	12 (4.2)	14 (7.1)	26 (10.5)
Abdominal pain	33 (11.5)	21 (7.3)	6 (3.0)	33 (13.4)
Liver test abnormalities	40 (13.9)	9 (3.1)	22 (11.2)	48 (19.4)

Adverse events were coded according to preferred terms in the Medical Dictionary for Regulatory Activities (MedDRA). Adverse events are shown based on single preferred terms except for 'liver test abnormalities', which was based on the standardised MedDRA query 'liver related investigations, signs and symptoms' (broad definition). Data are n (%) of patients with ≥1 such event reported over 52 weeks (or until 28 days after last drug intake if earlier in SENSCIS or until 7 days after last trial drug intake if earlier in SENSCIS-ON). Events reported in >10% of patients in either group in SENSCIS-ON are shown.

abnormalities were reported in 22 (11.2%) and 48 (19.4%) patients who continued and initiated nintedanib, respectively. Bleeding and cardiovascular adverse events are summarised in online supplemental table S2.

Serious adverse events were reported in 42 (21.3%) and 60 (24.3%) patients in the continued nintedanib and initiated nintedanib groups, respectively. The most frequent serious adverse event was pneumonia, reported in 8 (4.1%) and 4 (1.6%) patients who continued and initiated nintedanib, respectively (online supplemental table S3). The adverse event profile of nintedanib was generally similar in subgroups by mycophenolate use at the start of SENSCIS-ON (online supplemental table S4). Among patients who continued nintedanib, upper respiratory tract infections were more frequent (17.1% vs 9.8%) and vomiting less frequent (10.5% vs 17.4%) in the subgroup taking mycophenolate. Among patients who initiated nintedanib, nasopharyngitis was less frequent in patients taking mycophenolate (10.2% vs 16.7%). Cough was more frequent in the subgroup taking mycophenolate both among those who continued (15.2% vs 8.7%) and initiated (11.8% vs 5.0%) nintedanib. Liver test abnormalities were less frequent in patients taking mycophenolate both among those who continued (3.8% vs 19.6%) and initiated (13.4% vs 25.8%) nintedanib.

Among patients who continued and initiated nintedanib in SENSCIS-ON, respectively, 36 (18.3%) and 122 (49.4%) had≥1 dose reduction and 55 (27.9%) and 104 (42.1%) had ≥1 treatment interruption. Among those who had ≥1 dose reduction, nine patients (25.0%) in the continued nintedanib group and eight patients (6.6%) in the initiated nintedanib group had ≥1 dose increase to 150 mg two times per day. Adverse events led to permanent discontinuation of nintedanib in nine patients (4.6%) who continued nintedanib and 53 patients (21.5%) who initiated nintedanib.

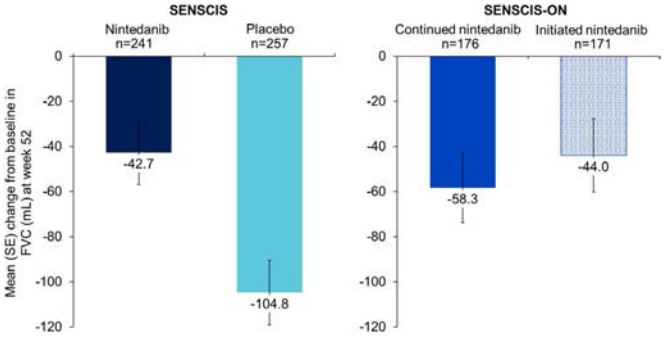
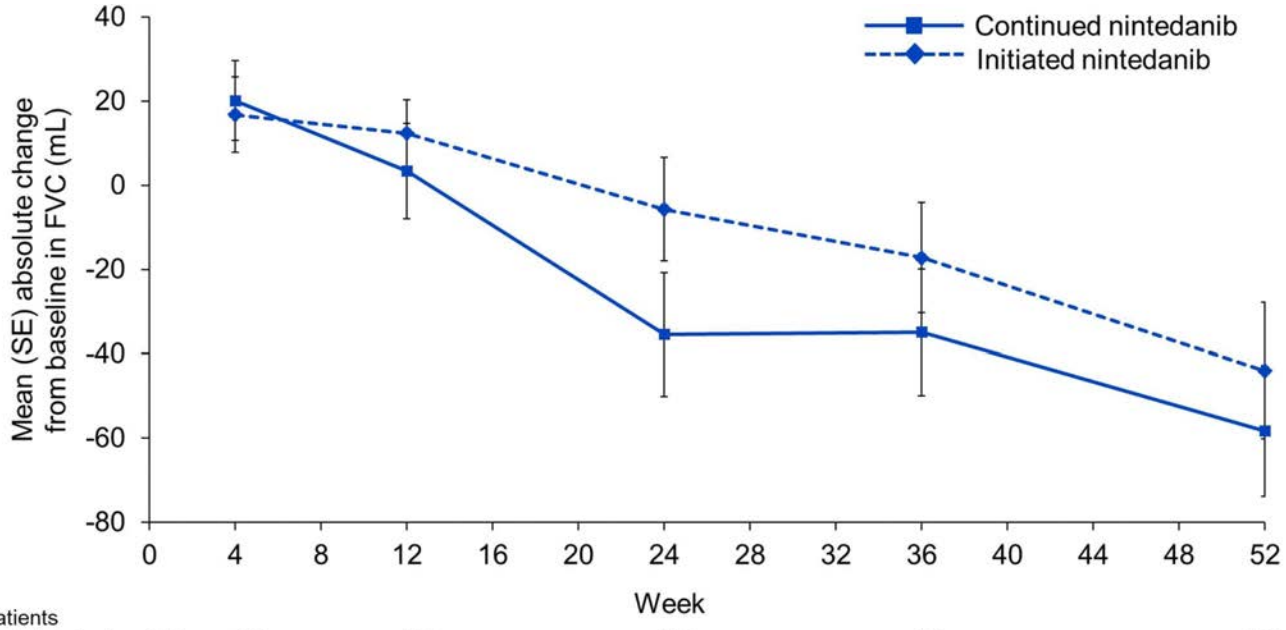


Figure 2 Change from baseline in FVC (mL) at week 52 in SENSCIS and SENSCIS-ON. Changes were based on data from patients with available data at baseline and at week 52. FVC, forced vital capacity.

Forced vital capacity

In total, 176 (89.3%) and 171 (69.2%) patients in the continued nintedanib and initiated nintedanib groups, respectively, had FVC data available at baseline and week 52. Mean (SE) changes in FVC from baseline to week 52 of SENSCIS-ON were −58.3 (15.5) mL in patients who continued nintedanib, −44.0 (16.2) mL in patients who initiated nintedanib and −51.3 (11.2) mL in all patients (figure 2). Changes in FVC over time in patients who continued and initiated nintedanib in SENSCIS-ON are shown in figure 3. Changes in FVC over time in SENSCIS and SENSCIS-ON are shown together in online supplemental figure S1. Changes in FVC over time based on pooled data from SENSCIS and SENSCIS-ON are shown in figure 4.

As patients remained in the SENSCIS trial until the last patient had reached week 52, the last few patients enrolled were treated for only 52 weeks in SENSCIS before transitioning into SENSCIS-ON. Thus, in the pooled analysis of changes in FVC over time, data after week 52 included data from patients treated with nintedanib or placebo in SENSCIS and patients treated with nintedanib in SENSCIS-ON. Of the patients who had FVC data available at baseline and at week 52, 13.6% of patients who continued nintedanib and 17.0% of patients who initiated nintedanib had an improvement in FVC (mL) ≥5% between baseline and week 52 of SENSCIS-ON (figure 5). A relative decline in FVC (mL) of >5% from baseline to week 52 of SENSCIS-ON was observed in 38.6% of patients who continued nintedanib and 29.2% of patients who initiated nintedanib; a relative decline in FVC (mL) of >10% occurred in 17.6% of patients who continued nintedanib and 12.9% of patients who initiated nintedanib. The cumulative distribution of patients by absolute change in FVC % predicted from baseline to week 52 of SENSCIS-ON is shown in online supplemental figure S2. Mean (SE) changes in



No. of patients						
Continued nintedanib	191	189	187	185	181	176
Initiated nintedanib	243	238	230	214	194	171

Figure 3 Absolute change from baseline in FVC (mL) over time in SENSCIS-ON. Baseline was the last measurement on or before the date of first trial drug intake in SENSCIS-ON. FVC, forced vital capacity.

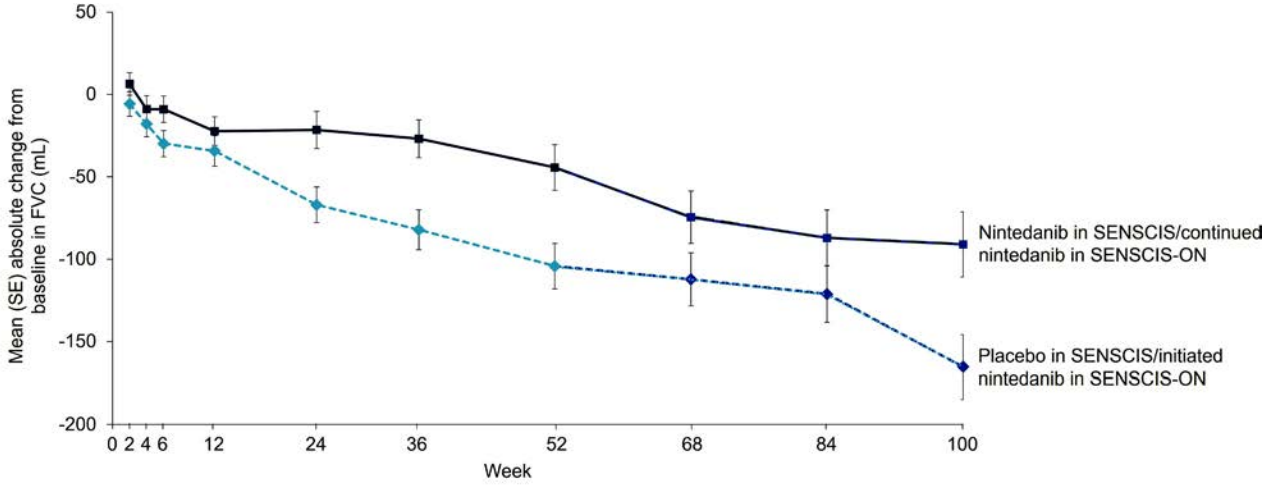
FVC in subgroups by mycophenolate use at the start of SENSCIS-ON are shown in online supplemental figure S3.

mRSS, SGRQ and UCLA SCTC GIT instrument

Mean (SE) change from baseline in mRSS at week 52 was −0.9 (0.2) in the continued nintedanib group (n=180) and −1.0 (0.3) in the initiated nintedanib group (n=174).

Mean (SE) change from baseline in SGRQ total score was 1.37 (0.87) in the continued nintedanib group (n=177) and −0.31 (0.91) in the initiated nintedanib group (n=183).

Mean (SE) change from baseline in UCLA SCTC GIT instrument total score was 0.28 (0.03) in the continued nintedanib group (n=168) and 0.18 (0.03) in the initiated nintedanib group (n=162).



No. of patients										
Nintedanib/continued nintedanib										
SENSCIS	288	283	280	271	271	255	246	232	154	110	64
Off treatment	0	0	1	2	7	10	16	24	17	12	11
SENSCIS-ON	0	0	0	0	0	0	0	0	61	101	119
Placebo/initiated nintedanib										
SENSCIS	288	283	281	279	281	274	260	256	169	107	69
Off treatment	0	0	0	1	2	6	8	11	10	10	6
SENSCIS-ON	0	0	0	0	0	0	0	0	72	120	130

Patients from the drug–drug interaction study were not included in this figure.
Patients remained in the SENSCIS trial until the last patient had reached week 52 but for ≤100 weeks. This meant that the last few patients enrolled in the SENSCIS trial were treated for only 52 weeks in SENSCIS before transitioning into SENSCIS-ON. In this pooled analysis, data from week 52 included data from patients treated with nintedanib or placebo in SENSCIS and patients treated with nintedanib in SENSCIS-ON. For patients who initiated nintedanib in SENSCIS-ON, the duration of placebo treatment in SENSCIS was 52 to 100 weeks and the duration of nintedanib treatment in SENSCIS-ON was 0 to 48 weeks.

Figure 4 Absolute change from baseline in FVC (mL) in SENSCIS and SENSCIS-ON (pooled). A digital version of this figure with a voiceover explaining the data is available at: https://www.globalmedcomms.com/respiratory/SENSCISandSENSCIS-ON. FVC, forced vital capacity.

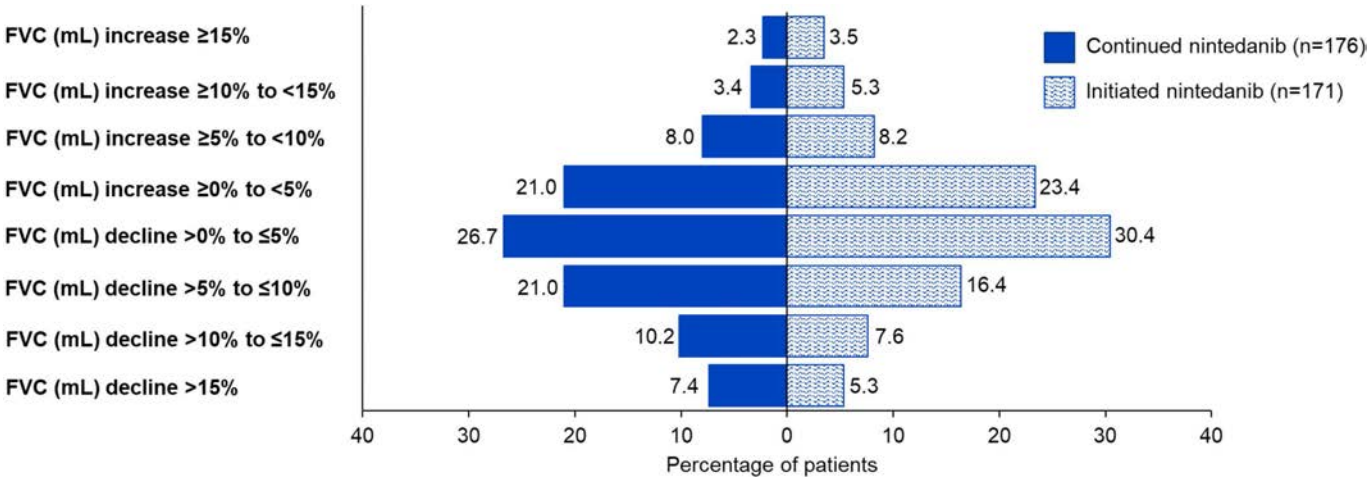


Figure 5 Proportions of patients with relative increases and declines in FVC (mL) from baseline to week 52 of SENSCIS-ON. Percentages were calculated using the number of patients with available data at baseline and at week 52 as the denominator. FVC, forced vital capacity.

DISCUSSION

Data from 52 weeks' follow-up in SENSCIS-ON showed that the adverse event profile of nintedanib over longer term use was consistent with that reported over 52 weeks in SENSCIS.[10 14] Among patients who initiated nintedanib in SENSCIS-ON, the proportions of patients who had a dose reduction or treatment interruption to manage adverse events over 52 weeks were similar to those observed in the nintedanib group of SENSCIS.[14] These dose adjustments were less frequent among the patients who continued nintedanib in SENSCIS-ON. Permanent discontinuations of nintedanib due to adverse events were also less frequent among patients who continued nintedanib in SENSCIS-ON than among those who initiated nintedanib in SENSCIS-ON or took nintedanib in SENSCIS. It is unclear whether the lower frequency of dose adjustments and discontinuations in the patients who continued nintedanib in SENSCIS-ON simply reflects that patients who were better able to tolerate the drug were more likely to have entered and continued in the trial, or whether there is improved tolerance to nintedanib with longer-term use.

Diarrhoea has consistently been shown to be the most frequent side effect of nintedanib in patients with ILDs.[10 18] Mild or moderate diarrhoea was the most frequently reported adverse event in SENSCIS-ON. Among patients who initiated nintedanib in SENSCIS-ON, 6.9% discontinued nintedanib due to diarrhoea over 52 weeks, consistent with the rate observed in patients who initiated nintedanib in SENSCIS. Discontinuation of nintedanib due to diarrhoea was less frequent among patients who continued nintedanib in SENSCIS-ON (1.5% over 52 weeks). Mean scores on the UCLA SCTC GIT instrument in both the continued nintedanib and initiated nintedanib groups suggested that most patients had no or mild gastrointestinal symptoms at the start of SENSCIS-ON.[17] A small worsening in mean UCLA SCTC GIT instrument total score was observed over 52 weeks. The adverse event profile of nintedanib was generally similar in patients who used nintedanib alone and in combination with mycophenolate, although the proportion of patients who had cough was higher in patients taking than not taking mycophenolate. This is consistent with the product label for mycophenolate, which reports cough as a side effect.

The change in FVC over 52 weeks of SENSCIS-ON was similar to the change in FVC over 52 weeks in the nintedanib group of SENSCIS (−51.3 and −42.7 mL, respectively) and much smaller than the change in FVC over 52 weeks in the placebo group of SENSCIS (−104.8 mL). Similar proportions of nintedanib-treated patients in SENSCIS and SENSCIS-ON had a decline in FVC from baseline of >5% and >10% over 52 weeks. These data, which suggest a sustained benefit of nintedanib on slowing the progression of SSc-ILD, are supported by data from the open-label extension of the INPULSIS trials, which suggested that the effect of nintedanib on slowing the progression of IPF persisted beyond 4 years.[19] The reduction in the rate of FVC decline provided by nintedanib in patients with SSc-ILD may be regarded as clinically meaningful given the disease trajectory and the known association between FVC decline and mortality in patients with SSc-ILD[3 5 6] and other ILDs.[20–22] Although the SENSCIS and SENSCIS-ON trials were not designed to investigate the effects of combination therapy, we note that the smallest decline in FVC over 52 weeks of SENSCIS-ON occurred in patients receiving both nintedanib and mycophenolate, consistent with observations in the SENSCIS trial.[23] Changes in the SGRQ total score in SENSCIS-ON were small, consistent with observations from SENSCIS[10] and from the INPULSIS trials in patients with IPF,[24] suggesting that the changes in FVC in SENSCIS-ON were not associated with a significant deterioration in respiratory symptoms.

Strengths of our analyses include the large cohort of patients who participated in SENSCIS-ON and the standardised collection of FVC measurements. About half of the patients who entered SENSCIS-ON were taking mycophenolate, increasing the relevance of our findings to clinical practice. Limitations of our analyses include the lack of a placebo group and the gradual loss of patients over the course of the trial. There may be selection bias among the patients who opted to participate in SENSCIS-ON, that is, these patients may have had fewer adverse events or better lung function; however, over 90% of patients who completed SENSCIS on treatment opted to participate in SENSCIS-ON. Although patients who participated in SENSCIS-ON were grouped according to their prior treatment, these are not randomised groups in SENSCIS-ON, so direct comparisons between patients who continued and initiated nintedanib should be approached with caution.

In conclusion, these data suggest that continued treatment with nintedanib, up to 3 years in duration, had a manageable safety and tolerability profile in patients with SSc-ILD. The adverse event profile of nintedanib over 52 weeks in SENSCIS-ON was consistent with that reported over the 52 weeks

of initial use in SENSCIS. The change in FVC in patients who received nintedanib over 52 weeks of SENSCIS-ON was similar to the change in FVC in patients who received nintedanib over 52 weeks in SENSCIS. These findings are consistent with a sustained clinically meaningful benefit of nintedanib in slowing the progression of SSc-ILD and support the prompt initiation of nintedanib in patients with SSc and pulmonary fibrosis.

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Patient and public involvement Patients were involved in the design and conduct of the SENSCIS trial and of its open-label extension, SENSCIS-ON (for example, by advising on the mouthpieces and blood draw needles that should be used). Patients' advice was also sought on the reporting of the results.

Patient consent for publication Not applicable.

Ethics approval The protocol was approved by an Ethics Committee or Institutional Review Board at all the participating centres (listed in the supplemental material). Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon reasonable request (see online supplementary material).

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TRANSLATIONAL SCIENCE

Distinct immune-effector and metabolic profile of CD8$^+$ T cells in patients with autoimmune polyarthritis induced by therapy with immune checkpoint inhibitors

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ABSTRACT

Objectives Rheumatic immune-related adverse events (irAE) such as (poly)arthritis in patients undergoing immune checkpoint inhibitor (ICI) treatment pose a major clinical challenge. ICI therapy improves CD8$^+$ T cell (CD8) function, but CD8 contributes to chronic inflammation in autoimmune arthritis (AA). Thus, we investigated whether immune functional and metabolic changes in CD8 explain the development of musculoskeletal irAE in ICI-treated patients.

Methods Peripheral CD8 obtained from ICI-treated patients with and without arthritis irAEs and from AA patients with and without a history of malignancy were stimulated in media containing ^{13}C-labelled glucose with and without tofacitinib or infliximab. Changes in metabolism, immune-mediator release, expression of effector cell-surface molecules and inhibition of tumour cell growth were quantified.

Results CD8 from patients with irAE showed significantly lower frequency and expression of cell-surface molecule characteristic for activation, effector-functions, homing, exhaustion and apoptosis and reduced release of cytotoxic and proinflammatory immune mediators compared with CD8 from ICI patients who did not develop irAE. This was accompanied by a higher glycolytic rate and ATP production. Gene-expression analysis of pre-ICI-treated CD8 revealed several differentially expressed transcripts in patients who later developed arthritis irAEs. In vitro tofacitinib or infliximab treatment did not significantly change the immune-metabolic profile nor the capacity to release cytolytic mediators that inhibit the growth of the human lung cancer cell line H838.

Conclusions Our study shows that CD8 from ICI-treated patients who develop a musculoskeletal irAE has a distinct immune-effector and metabolic profile from those that remain irAE free. This specific irAE profile overlaps with the one observed in CD8 from AA patients and may prove useful for novel therapeutic strategies to manage ICI-induced irAEs.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Immune checkpoint inhibition (ICI) therapies have a high success rate regarding progression-free and overall survival for patients with cancer. However, up to 20% of ICI-treated patients develop musculoskeletal immune-related adverse events (irAE) that are often associated with severely reduced quality of life.

⇒ To avoid precocious ICI treatment termination, strategies to treat rheumatic irAE must be simultaneously efficient in curbing musculoskeletal symptoms without interfering with the antitumor therapy.

⇒ CD8$^+$ T cells play a pivotal role both in arthritis pathogenesis and antitumor responses.

WHAT THIS STUDY ADDS

⇒ Immunofunctional and metabolic analysis of peripheral CD8$^+$ T cells from patients with musculoskeletal irAEs revealed that they share a common profile with those from patients with chronic autoimmune polyarthritis (AA) but are distinct from ICI-treated patients who remained irAE free.

⇒ CD8$^+$ T cells from patients with irAE treated in vitro with the Janus-kinase (JAK) pathway inhibitor tofacitinib and TNF-α blocker infliximab still maintained the capacity to release cytokines and cytolytic molecules, express immune-effector cell surface molecules and prevent the growth of a human lung cancer cell line.

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INTRODUCTION

Immune checkpoint inhibition (ICI) therapies that prevent cytotoxic T-lymphocyte-associated Protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) from blocking T cell activation are a milestone in cancer management. Their initial success in patients with advanced melanoma and non-small-cell lung cancer (NSCLC) has encouraged their use for other types of solid tumours.[1 2] However, the increase in the number of patients under ICI therapy is leading to a rise in the number of patients developing ICI-induced immune-related adverse events (ICI-irAE) resembling chronic autoimmune diseases,[3] including rheumatic musculoskeletal and systemic symptoms as well as flares of pre-existing inflammatory diseases.[4] De novo arthralgia, inflammatory arthritis, tendinitis/tenosynovitis, enthesitis and (poly-)myalgia have been reported in about 20% of ICI patients

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The specific immunofunctional and metabolic profile in rheumatic irAEs and its overlap to AA-CNT profile is a potential starting point for a better understanding of the pathogenesis and identification of patients with ICI at risk of developing an irAE.

⇒ JAK inhibitors may expand the, thus, far limited therapeutic armamentarium to cope with severe, refractory and/or chronic rheumatic irAEs.

in clinical trials, with a large variation in prevalence due to differing criteria and awareness of these side effects.[4 5] Intriguingly, the development of ICI-induced irAE has been associated with a better survival and clinical outcome,[6–8] including patients with rheumatic irAEs.[9–11] However, severe irAEs may force clinicians to terminate ICI therapy due to ICI-irAE-associated mortality for 0.5%–1.5% of patients.[12] Fortunately, except for myositis, rheumatic irAEs are seldom fatal but can cause considerable suffering and disability. In contrast to other ICI-irAEs, rheumatic irAEs regularly take a chronic course and require long-term medication.[13] While numerous severity-based treatment algorithms for rheumatic irAEs have been formulated to reduce inflammation and patient suffering,[4 5] there is an unmet need for evidence-based anti-inflammatory approaches without negative effects for the beneficial antitumor response in this population.[9 14–17]

In this context, data from our and other groups support the hypothesis that $CD8^+$ T cells (CD8) play an important role in maintaining chronic arthritis and their permanent proinflammatory effector phenotype is fuelled by an enhanced aerobic glycolysis.[18–20] While the use of therapies that reduce the CD8 cytotoxic proinflammatory potential such as Janus-kinase inhibitors (JAKi) may be beneficial to control autoimmune arthritis (AA), they might be inappropriate for irAE, since a fully functional CD8 antitumor response is crucial for long-term remission.[21] However, CD8 seems to play a role in the induction and/or propagation of irAE since patients with irAE present a clonal expansion of CD8 in the periphery prior to symptom development[22] and gene expression profiles of CD8 from patients with irAE are distinct from those who do not develop irAE.[8] Nonetheless, functional studies on CD8 in irAE patients that could provide information to evaluate this therapeutic target are largely missing. Therefore, the aim of the present study was to characterise the immunofunctional and metabolic phenotype of the peripheral CD8 pool in patients with rheumatic irAEs and compare these to the CD8-profiles from patients who did not develop irAE under ICI treatment (ICI-CNT), patients with AA (AA-CNT) and patients with AA and a clinical history of malignancy (AA-MAL). We also explored JAK inhibition as a potential therapeutic strategy in ICI-irAEs and AA-MAL by testing whether in vitro blockade of the JAK pathway in the CD8 of these patients results in a major loss of functionality and metabolic remodelling.

PATIENTS AND METHODS

A detailed description of the patient selection and the experimental and statistical methods are found in online supplemental information file 1.

RESULTS

Patient characteristics

Demographic and clinical data regarding malignancy and autoimmune characteristics are summarised in table 1. Further details on underlying rheumatic diseases, irAEs and malignancies of individual patients are listed in online supplemental table 1. Most ICI patients had a diagnosis of stage III or IV melanoma or NSCLC and all had at least a stable disease as best response. More than half (63.2%) of the patients with ICI-irAE and all in the ICI-CNT group were still under ICI treatment at sample collection. The ICI-CNT group had a shorter disease and ICI treatment duration and higher proportion of men. Musculoskeletal irAEs were verified and treated by a rheumatologist and were characterised by inflammatory arthralgia/arthritis, tenosynovitis and/or polymyalgia, including one patient with an overlap of polymyalgia and suspected mild myositis, another with overlap of spondylarthritis and acute gout, one with concomitant scar sarcoidosis as further irAE, and two with a flare of either pre-existing rheumatoid arthritis (RA) or psoriatic arthritis. Treatment consisted mainly of low-dosed glucocorticoids (GC) ≤10 mg prednisolone-equivalent with only one patient receiving a higher dose at sample collection. Two patients required methotrexate and one received leflunomide for GC sparing, none was previously treated with biologic (b) or targeted synthetic disease-modifying antirheumatic drugs (DMARDs). Three patients showed high disease activity as measured by a disease activity score (DAS28) and five had elevated C-reactive protein (CRP) at sample collection.

The AA-MAL patients had a longer duration and a larger spectrum of malignant diseases though most patients showed complete remission. Most of the AA-MAL group received conventional synthetic (cs) and/or biological disease-modifying antirheumatic drugs (bDMARDs) at sample collection, while GC were used in a lower dosage than for the ICI-irAE group. In contrast to the AA-MAL group, the AA-CNT and AA-JAK groups consisted of patients of younger age, male gender, slightly shorter duration of the rheumatic disease and higher rates of predominantly rheumatoid factor and/or anti-citrulinated protein antibodies (ACPA)-positive RA. CRP levels were low to normal across all AA groups.

Expression of cell-surface markers and release of immune mediators distinguishes the CD8 between patient groups

In vitro culture and T-cell receptor (TCR) stimulation of peripheral blood CD8 for 72 hours did not significantly affect the number of viable cells when compared with ex vivo analysis after cell isolation, and all groups had levels of live cells in excess of 85% (online supplementary figure SF1A). Thus, any subsequent differences observed in marker expression and immune mediator release could not be attributed to general alterations in cell viability. The expression differences in CD8 cell-surface molecules characteristic for activation and effector functions, homing and exhaustion and apoptosis on TCR-mediated stimulation were determined for the total CD8 pool and in its functional subsets defined by the expression of CCR7 and CD45RA. The distribution of naïve, effector (T_{EMRA}), effector memory (T_{EM}) and central memory (T_{CM}) subsets within the total CD8 population was similar for all study groups before culture (Ex vivo: $\chi^2=16.8$, $p=0.052$) and did not change after 72 hours in vitro culture (Nst: $\chi^2=5.007$, $p=0.83$) nor on in

Table 1 Clinical and demographic characteristics of the study participants

	AA-CNT	AA-JAKi	AA-MAL	ICI-irAE	ICI-CNT
Patients total	18	16	16	19	10
Females (%)	13 (72.2)	9 (56.3)	7 (43.8)	13 (68.4)	3 (30.0)
Age (y)±SD	57.4±12.1	58.0±8.6	70.6±12.5	60.8±11.2	63.4±13.5
CRP (mg/L)±SD	5.6±6.1	7.7±11.2	5.6±5.9	13.6±22.8	8.5±10.0
Malignancy characteristics					
Mean disease duration (y)±SD	–	–	12.5±8.3	6.0±4.9	2.0±1.0
Malignancy type: *					
Melanoma (%)	–	–	2 (12.5)	11 (57.9)	8 (80.0)
NSCLC (%)	–	–	0 (0)	4 (21.1)	0 (0)
Urogenital (%)	–	–	8 (50.0)	0 (0)	0 (0)
Haematological (%)	–	–	3 (18.8)	0 (0)	0 (0)
Others (%)	–	–	3 (18.8)	4 (21.1)	2 (20.0)
Malignancy stage:					
• I/II (%)	–	–	4 (25.0)	0 (0)	0 (0)
III (%)	–	–	0 (0)	6 (31.6)	4 (40.0)
IV (%)	–	–	1 (6.3)	13 (68.4)	6 (60.0)
Other classifications (%)	–	–	3 (18.8)	0 (0)	0 (0)
n/a (%)	–	–	8 (50.0)	0 (0)	0 (0)
Malignancy treatment:					
ICI ever (%)	–	–	0 (0)	19 (100.0)	10 (100.0)
Anti-PD-(L)1 only	–	–	–	13 (68.4)	8 (80.0)
Combined anti-CTLA-4 and anti-PD-1	–	–	–	6 (31.6)	2 (20.0)
ICI currently (%)	–	–	0 (0)	12 (63.2)	10 (100.0)
Mean ICI duration (months)±SD	–	–	–	20.8±16.3	10.7±4.3
Other immunotherapy (%)	–	–	3 (27.3)	3 (15.8)	1 (10.0)
Chemotherapy ever (%)	–	–	4 (25.0)	6 (31.6)	1 (10.0)
Chemotherapy currently (%)	–	–	1 (6.3)	4 (21.1)	0 (0)
Radiotherapy ever (%)	–	–	2 (12.5)	3 (15.8)	2 (20.0)
Primary excision (%)			8 (50.0)	0 (0)	0 (0)
Other (%)			1 (6.3)	1 (5.3)	0 (0)
Current remission status:					
CR (%)	–	–	14 (87.5)	6 (31.6)	2 (20.0)
PR (%)	–	–	0 (0)	3 (15.8)	3 (30.0)
SD (%)	–	–	2 (12.5)	9 (47.4)	4 (40.0)
PD (%)	–	–	0 (0)	1 (5.3)	1 (10.0)
Autoimmunity characteristics					
Mean disease duration (y)±SD	12.5±11.5	10.7±6.9	14.1±13.1	1.0±1.2	–
Serology					
Rheumatoid factor positive (%)	12 (66.7)	11 (68.8)	2 (12.5)	0 (0)	–
ACPA positive (%)	10 (55.6)	10 (62.5)	5 (31.3)	0 (0)	–
Autoimmune arthritis type:					
RA (%)	12 (66.7)	13 (81.3)	8 (50.0)	1 (5.3)	–
PsA (%)	6 (33.3)	3 (18.8)	5 (31.3)	1 (5.3)	–
Other SpA (%)	0 (0)	0 (0)	3 (18.8)	0 (0)	–
RA-like irAE (%)	0 (0)	0 (0)	0 (0)	7 (36.8)	–
SpA-like irAE (%)	0 (0)	0 (0)	0 (0)	6 (31.6)	–
Other irAE phenotype (%)	0 (0)	0 (0)	0 (0)	4 (21.1)	–
Autoimmune arthritis treatment:					
GC (%)	3 (16.7)	5 (31.3)	8 (50.0)	9 (47.4)	–
Mean GC dosage (mg/d)±SD	5.0±0.0	5.8±2.4	4.4±2.1	9.4±5.9	–
csDMARDs (%)	11 (61.1)	9 (56.3)	9 (56.3)	3 (15.8)	–
Methotrexate (%)	8 (44.4)	6 (37.5)	8 (50.0)	2 (10.5)	–
Leflunomide (%)	5 (27.8)	3 (18.8)	1 (6.3)	1 (5.3)	–
bDMARDs (%)	8 (44.4)	–	6 (37.5)	0 (0)	–
TNFi (%)	7 (38.9)	–	4 (25.0)	0 (0)	–
Others (%)	1 (5.6)	–	2 (12.5)	0 (0)	–

Continued

Table 1 Continued

	AA-CNT	AA-JAKi	AA-MAL	ICI-irAE	ICI-CNT
tsDMARDs (%)	–	16 (100.0)	1 (6.3)	0 (0)	–
Tofacitinib (%)	–	4 (25.0)	0 (0)	0 (0)	–
Baricitinib (%)	–	6 (37.5)	1 (6.3)	0 (0)	–
Upadacitinib (%)	–	5 (31.3)	0 (0)	0 (0)	–
Filgotinib (%)		1 (6.3)	0 (0)	0 (0)	
Current remission status:					
DAS28 available (%)	16 (88.9)	16 (100.0)	14 (87.5)	10 (52.6)	–
Remission: DAS28<2.6 (%)	8 (44.4)	8 (50.0)	9 (56.3)	6 (31.6)	–
Low disease activity: DAS28 2.6–3.19 (%)	2 (11.1)	3 (18.8)	1 (6.3)	1 (5.3)	–
High disease activity: DAS28≥3.2 (%)	6 (33.3)	5 (31.3)	4 (50.0)	3 (15.8)	–

*Only the main malignant diagnosis that led to ICI or other antineoplastic therapy is listed here. Patients with more than one malignant diagnosis are identified in online online supplemental table 1.
AA-JAKi, autoimmune arthritis Janus-kinase inhibitor; AA-MAL, autoimmune arthritis malignancy; ACPA, anti-citrulinated protein antibodies; CR, complete remission; CRP, C-reactive protein; csDMARDS, conventional synthetic disease-modifying antirheumatic drugs; DMARDs, disease-modifying antirheumatic drugs; GC, glucocorticoids; ICI-irAE, immune checkpoint inhibitor-immune-related adverse event; NSCLC, non-small-cell lung cancer; PD, progressing disease; PR, partial remission; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SD, stable disease; SpA, spondylarthritis; TNFi, tumour necrosis factor inhibitors.

vitro TCR-mediated stimulation (St: χ^2=9.772, p=0.3692; figure 1A). Significant positive fold changes from baseline in the frequency and expression of activation-related molecules (CD69 and CD25) and homing molecules (CD11a and CD49a) were observed in all CD8 subsets and the total CD8-pool in all groups. Additionally, $CD69^+$ and $CD25^+$ CD8 were significantly more enriched in the ICI-CNT total CD8-pool—but not in any particular subset—than in the ICI-irAE total CD8-pool (figure 1B–D and online supplemental figure SF1C). CD25 expression was higher on the surface of CD8 subsets and the total pool from ICI patients when compared with the ICI-irAE. A few other significant differences in the expression of cell-surface molecules were observed after TCR stimulation of AA-CNT compared with AA-MAL or ICI-irAE and between ICI-CNT and ICI-irAE.

Total CD8 increased the release of cytotoxic mediators and cytokines after TCR-mediated stimulation (figure 1E) across all groups. However, ICI-CNT CD8 overall presented a more robust secretion of immune mediators and, in particular, the release of cytolytic molecules perforin, granulysin and granzymes A and B higher than for ICI-irAE.

Next, we analysed whether these differences could distinguish AA-CNT from the AA-MAL and ICI-irAE groups (figure 1H). A higher expression of Granzyme A and PD-1 was characteristic for AA-MAL and ICI-irAE CD8 in comparison to AA-CNT, even though only Granzyme A reached a significant adjusted p value for irAE versus AA-CNT. Further molecules that separated these groups from AA-CNT were the cytolytic molecules sFasL and Granulysin (AA-MAL) and CTLA-4 (ICI-irAE). We observed that ICI-irAE CD8 were distinguished from ICI-CNT by a lower expression of activation and homing molecules, proinflammatory cytokines (IFN-γ, TNF-α) and several cytolytic mediators. When searching for molecules that were distinct between ICI-CNT and AA-CNT, we found that they mostly overlapped with the ones separating ICI-CNT from ICI-irAE. However, in both cases, only Perforin reached a significant adjusted p value.

To determine whether clinical or demographic characteristics could contribute to and explain any of the described differences, we correlated the continuous clinical (including treatment modalities) and demographic variables with the experimental data for both the whole study cohort and for each patient group. However, there were no clinical or demographic variables with a significant correlation with the CD8 phenotype across all study groups (online supplemental figure SF2). Moreover, comparison of anti-PD-1-monotherapy and combined anti-PD-1 and anti-CTLA-4 treatment within the ICI-CNT and ICI-irAE patient groups did not yield any significant differences in the CD8 subsets, expression of functional surface molecules or production of cytolytic mediators (online supplemental tables S2 and S3). Furthermore, these parameters also did not differ between ICI-irAE patients with continued versus stopped ICI therapy due to severe irAE in any organ prior to sample collection (online supplemental table S4).

Metabolic phenotype of CD8

Since the cell-culture media contained (U-^{13}C)-glucose, the in vitro glucose consumption and de novo (U-^{13}C)-lactate production could be precisely quantified by ^{1}H-NMR (figure 2A). CD8 from AA-CNT increased glycolysis on in vitro TCR-stimulation, characterised by a strong de novo (U-^{13}C)-lactate synthesis, which accounted for more than 60% of the total lactate pool. A similar behaviour was also observed for ICI-CNT CD8 (figure 2B and C). Interestingly, the unstimulated ICI-irAE CD8 had a significantly higher de novo (U-^{13}C)-lactate synthesis and a larger contribution of (U-^{13}C)-lactate to the total lactate pool than did the AA-CNT or ICI-CNT CD8. However, (U-^{13}C) lactate levels after TCR-mediated stimulation were comparable between all groups. The oxidative phosphorylation (OXPHOS) rate in all groups bar ICI-irAE dropped after stimulation (Figure 2D). Additionally, we evaluated glucose consumption against expression levels of the glucose transporter 1 (GLUT1) but did not find any significant correlation for any of the groups (data not shown).

To confirm that differences in de novo (U-^{13}C)-lactate synthesis and OXPHOS rate represented a preference for ATP-production through aerobic glycolysis, we calculated the percentage of cytoplasmatic ATP within the total cellular ATP (cytoplasmatic plus mitochondrial) by fluorescence microscopy (figure 2E). Without TCR-mediated stimulation, total ATP was significantly higher in ICI-irAE CD8 than in any other group (figure 2F) and directly correlated with an increase in (U-^{13}C)-lactate-enrichment (Spearman R=0.821, p=0.034). In

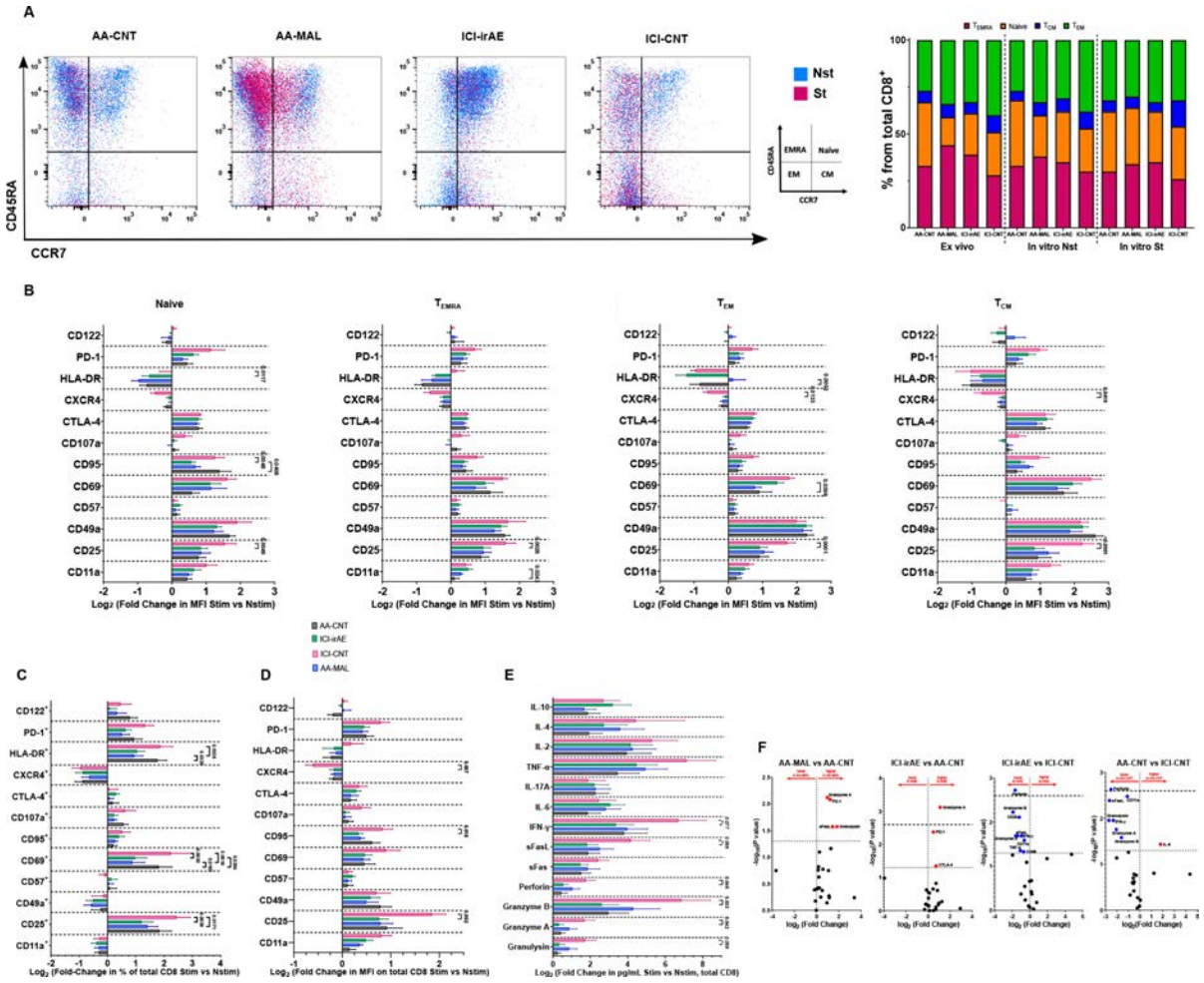

Figure 1 Immunophenotype and release of immune mediators is different between ICI-irAE and ICI-CNT CD8. (A) Representative overlay dot-plots of CD45RA versus CCR7 expression in unstimulated and TCR-stimulated CD8 and stacked-column graphs showing the distribution of the four main functional CD8 subsets based on CD45RA versus CCR7 expression (naïve: $CD45RA^+CCR7^+$; T_{EMRA}: $CD45RA^+CCR7^-$; T_{EM}: $CD45RA^-CCR7^-$; and T_{CM}: $CD45RA^-CCR7^+$) within each patient group. (B) Bar graphs showing the fold-change expression (MFI) of the different markers in the main functional CD8 subsets after TCR-mediated stimulation. (C–E) Bar graphs showing the fold-change in surface-marker frequency (C), surface marker expression (D) and cytokines, and cytotoxic molecules release (E) after TCR-mediated stimulation. (F) Volcano plots showing the differentially expressed molecules between the different patient groups. The horizontal dotted line represents p value<0.05; the horizontal dashed line represents adjusted p value<0.05. For all panels: AA-CNT n=18; AA-MAL n=16; ICI-irAE n=19; ICI-CNT n=10. Representative patients for panel A: #17 AA-CNT list; #14 from AA-MAL list; #16 from ICI-irAE list; and #10 from ICI-CNT list. AA-CNT, autoimmune arthritis; AA-JAKi, autoimmune arthritis Janus-kinase inhibitor; AA-MAL, autoimmune arthritis malignancy; GC, glucocorticoids; ICI-irAE, immune checkpoint inhibitor-immune-related adverse event; NSCLC, non-small-cell lung cancer; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SpA, spondylarthritis.

unstimulated cells, cytoplasmatic ATP was the major contributor to the total ATP-pool for all groups (figure 2G). TCR-mediated stimulation did not significantly change the contribution of cytoplasmatic ATP to the total ATP-pool in AA-CNT and ICI-irAE CD8, whereas AA-MAL and ICI-CNT CD8 obtained most of their ATP from the mitochondria.

The release of proinflammatory cytokines and cytolytic molecules positively correlated with increasing (U-^{13}C)-lactate concentrations, particularly the ICI-CNT CD8 (figure 2H). We did not find any general or group-specific correlation between clinical or demographic variables and GLUT1 expression or (U-^{13}C)-lactate production (online supplemental figure SF2).

Different baseline gene-expression profiles distinguish CD8 from ICI patients who develop musculoskeletal irAE

We retrieved the EGAS00001004081 gene expression data[8] obtained from peripheral CD8 isolated before the onset of ICI therapy and compared the profiles of patients who later developed specifically arthritis as a rheumatic irAE (13.5%) with those who did not develop any ICI-induced irAE (online supplementary table ST5). Twenty-two transcripts had a significant differential expression between the group of patients who developed an arthritis-irAE and those who did not, and pathway analysis revealed an enrichment of genes involved in cell-population proliferation, immune system development and response to TNF in the group that remained irAE free (figure 3A; online supplementary table ST6). Even though we did not find any significant differences in CD8 phenotype and immune-mediator production among patients with ICI receiving only anti-PD-1 or combined anti-CTLA-4 and anti-PD-1 therapy, it is well documented that patients receiving combined ICI therapy are more prone to develop ICI-induced irAEs in any organ, including arthritis.[23 24] Therefore, we conducted a subanalysis of the differences in gene expression at baseline between

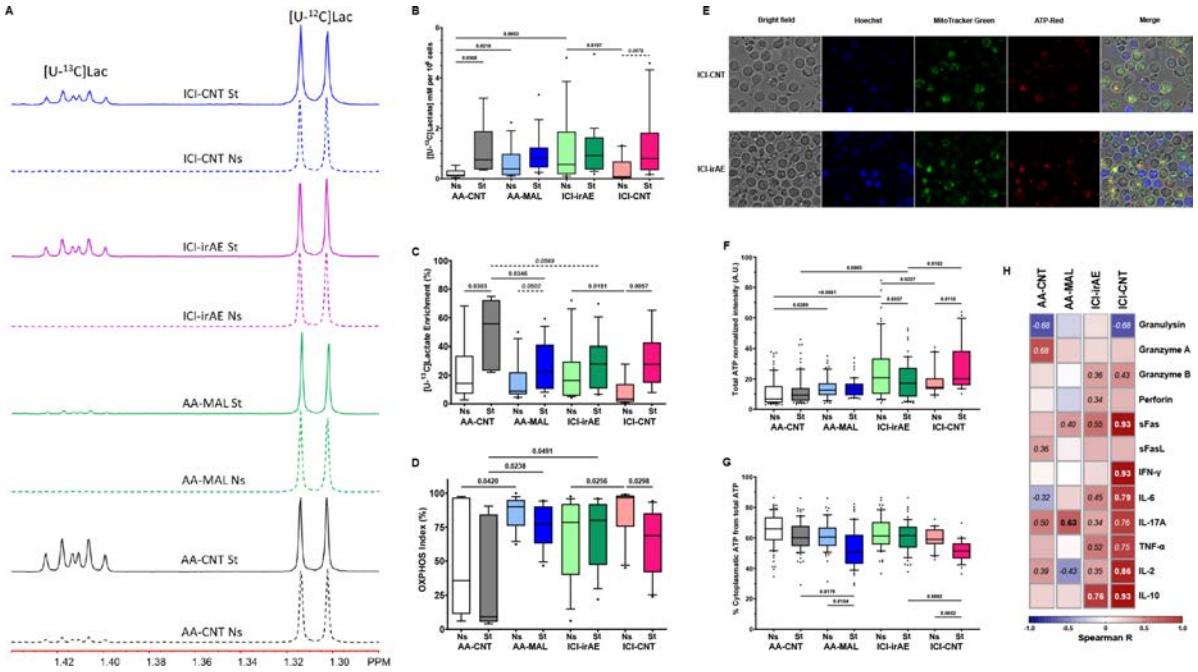

Figure 2 irAE CD8 present a Warburg effect-like phenotype when resting and on TCR-stimulation undergo a Crabtree effect-like metabolic shift. (A) Representative ^{1}H NMR sub-spectra of cell-culture media for each group of CD8, either unstimulated or TCR-stimulated. The region covers the [U-^{12}C]-lactate methyl signal and the ^{13}C satellite at higher frequency arising from [U-^{13}C]-lactate. Each spectrum has been normalised separately to its [U-^{12}C]-lactate methyl signal. (B–D) The concentration of [U-^{13}C]-lactate in the cell-culture medium (B), [U-^{13}C]-lactate enrichment (C) and OXPHOS-rate (D) before and after TCR–mediated stimulation for each group. Results are shown as box plots. Each box represents the 25th to 75th percentiles. Lines inside the boxes represent the median. Lines outside the boxes represent the 10th and 90th percentiles. Dots represent outliers. For all panels, AA-CNT n=18; AA-MAL n=16; ICI-irAE n=19; and ICI-CNT n=10. (E) Representative microscopy images of unstimulated ICI-irAE and ICI-CN CD8. (F) Total ATP produced by in vitro cultured CD8 without stimulation or with TCR-mediated stimulation, quantified by measuring the relative ATP-Red fluorescence. Each box represents the 25th to 75th percentiles of nine technical replicates for each patient (AA-CNT n=6; AA-MAL n=6; ICI-irAE n=7; and ICI-CNT n=3). Lines inside the boxes represent the median, lines outside the boxes represent the 10th and 90th percentiles, and dots represent outliers. (H) The correlation between [U-^{13}C]-lactate production and cytokines/cytotoxic molecules release on TCR-mediated stimulation. Numbers show correlations with Spearman R>|0.3|, bold numbers represent p<0.05. AA-CNT, autoimmune arthritis; AA-JAKi, autoimmune arthritis Janus-kinase inhibitor; AA-MAL, autoimmune arthritis malignancy; GC, glucocorticoids; ICI-irAE, immune checkpoint inhibitor-immune-related adverse event; TCR, T-cell receptor.

patients who later developed an arthritis-irAE and those who did not base on the type of ICI therapy. Before ICI treatment with only anti-PD-1, we identified 47 significant transcripts and an enrichment of pathways linked to ATP metabolism and immune response which were differentially expressed in those patients who later developed arthritis-irAE (figure 3B; online supplemental table ST7). No major significant gene-expression differences were observed within the patients who received a combination of anti-CTLA-4 and anti-PD-1 (online supplemental table ST8). Based on arthritis severity, although we identified 18 transcripts that had a significantly different expression (figure 3C, online supplemental table ST9), we could not define any significantly altered pathway. The data did not indicate which patients would require GC to curb arthritis-irAE (online supplemental table ST10). All these data indicate that CD8 differ even before the initiation of ICI.

In vitro inhibition of JAK-signalling pathway and TNF-α blockade does not induce major functional or metabolic changes in CD8

To evaluate the effect of JAKi, TCR-stimulated CD8 of all groups were compared with in vitro JAKi-treated CD8. Additionally, TCR-stimulated AA-CNTnbD CD8 were compared with in vitro TCR-stimulated CD8 from AA patients under in vivo JAKi therapy (AA-JAK group). Furthermore, since TNF-α blockade with infliximab is part of the current standard-of-care therapies for ICI-induced irAE, we ran parallel experiments with TNFi-treated CD8, plus compared in vitro TCR-stimulated AA-CNTnbD CD8 to in vitro TCR-stimulated CD8 from patients under in vivo TNFi therapy (AA-TNF group).

In vitro JAKi and TNFi of TCR-mediated stimulation led to a generalised reduction in the expression of most cell surface markers in all patient groups (figures 4A–B and 5A–B). The presence of JAKi significantly reduced the expression of surface HLA-DR in ICI-irAE and ICI-CNT CD8 and of CD11a in AA-MAL and ICI-CNT CD8. TNFi had a significant effect on CD95 expression in ICI-CNT and CD107a and CD57 in AA-nbDCNT CD8. A tendency for a decrease in the frequency of CD8 expressing activation and homing markers was observed for all groups, though not reaching statistical significance for any of the inhibitors (figures 4C and 5C). Since tofacitinib inhibits intracellular signal transduction of several cytokines by blocking JAK1 and JAK3,[25] while infliximab only blocks the binding of soluble or transmembrane TNF-α to its receptor,[26] we assessed whether in vitro they influenced the release of cytokines and immune mediators by TCR-stimulated CD8 (figures 4D and 5D). Probably due to the focused inhibition of TNF-α-signalling by infliximab, the release of most cytokines and cytotoxic molecules remained unchanged for all groups, and only the ICI-CNT

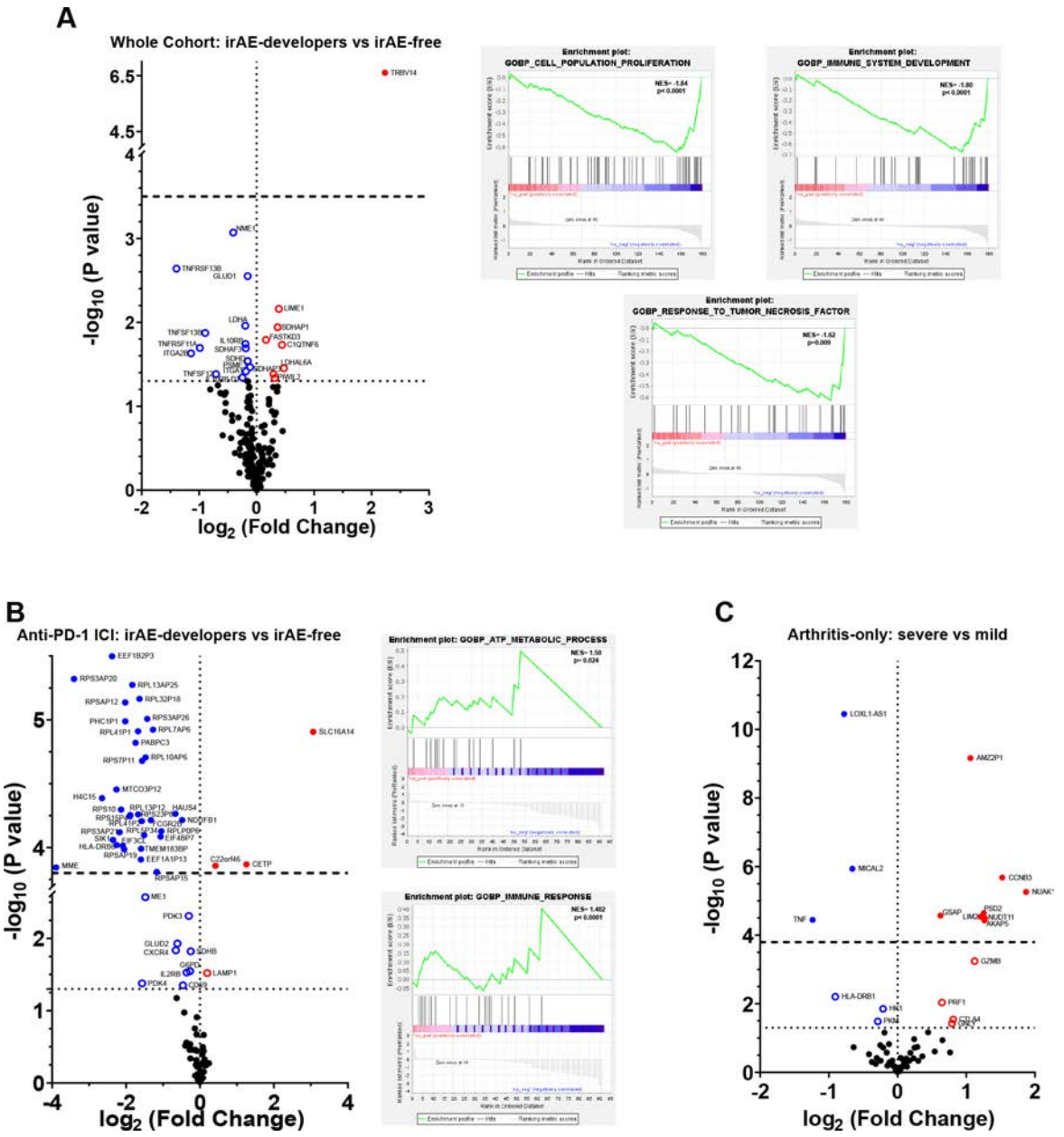

Figure 3 Before therapy begins, patients who later on develop ICI-induced irAE have a different gene expression than those who remain irAE-free. Volcano plots and pathway enrichment plots showing the gene-expression differences before ICI-therapy. (A) Total ICI-treated patients. Those who developed arthritis irAE (n=21) versus those who remained irAE-free (n=135). (B) Patients treated only with anti-PD-1 ICI. Those who developed arthritis irAE (n=7) versus those who remained irAE-free (n=73). (C) ICI-treated irAE patients who developed severe arthritis (grade 3–4; n=7) versus those who developed mild arthritis (grade 1–2; n=14). Horizontal dotted line represents p<0.05; horizontal dashed line represents adjusted p<0.05. ICI, immune checkpoint inhibitor; irAE, immune-related adverse event.

group presented a reduced release of cytolytic mediators. In contrast, in vitro JAKi treatment led to a generalised reduction in the concentration of soluble mediators in all groups, but only ICI-CNT CD8 released significantly less cytolytic mediators in the presence of JAKi. When comparing AA-nbDCNT CD8 to those from AA-JAK or AA-TNF patients after TCR-stimulation (online supplemental figure 3A), we observed a significant reduction in the pool of $CD69^+$ and/or $CD25^+$ in patients under in vivo JAKi or TNFi therapy. Reminiscent of the in vitro behaviour observed for the AA-nbDCNT CD8 under JAKi or TNFi, there was a reduction in the frequency of CD8 expressing homing markers (CXCR4, CD11a and CD49a) within the CD8 pool from AA-JAK and AA-TNF patients, though not significantly different from AA-nbD. No differences were observed in the capacity of TCR-stimulated AA-JAK and AA-TNF CD8 to release cytokines and cytotoxic molecules when compared with their AA-nbDCNT counterparts (online supplemental figure 3B).

The in vitro expansion of H838 cells could be inhibited for 5 days using conditioned medium from TCR-stimulated CD8 but not with medium containing only JAKi or TNFi (figure 4E; online supplemental figure SF3C-E). The conditioned media from AA-nbDCNT, AA-TNF and AA-JAK patients' CD8 presented the strongest inhibitory effect. In contrast to ICI-CNT and AA-MAL, conditioned media from ICI-irAE CD8 cultured in the presence of JAKi had comparable inhibitory capacity on H838 cell growth to media without JAKi. In vitro TNFi-treated media from all groups were able to inhibit H838 expansion. Significant negative correlations between H838 inhibition and the concentration

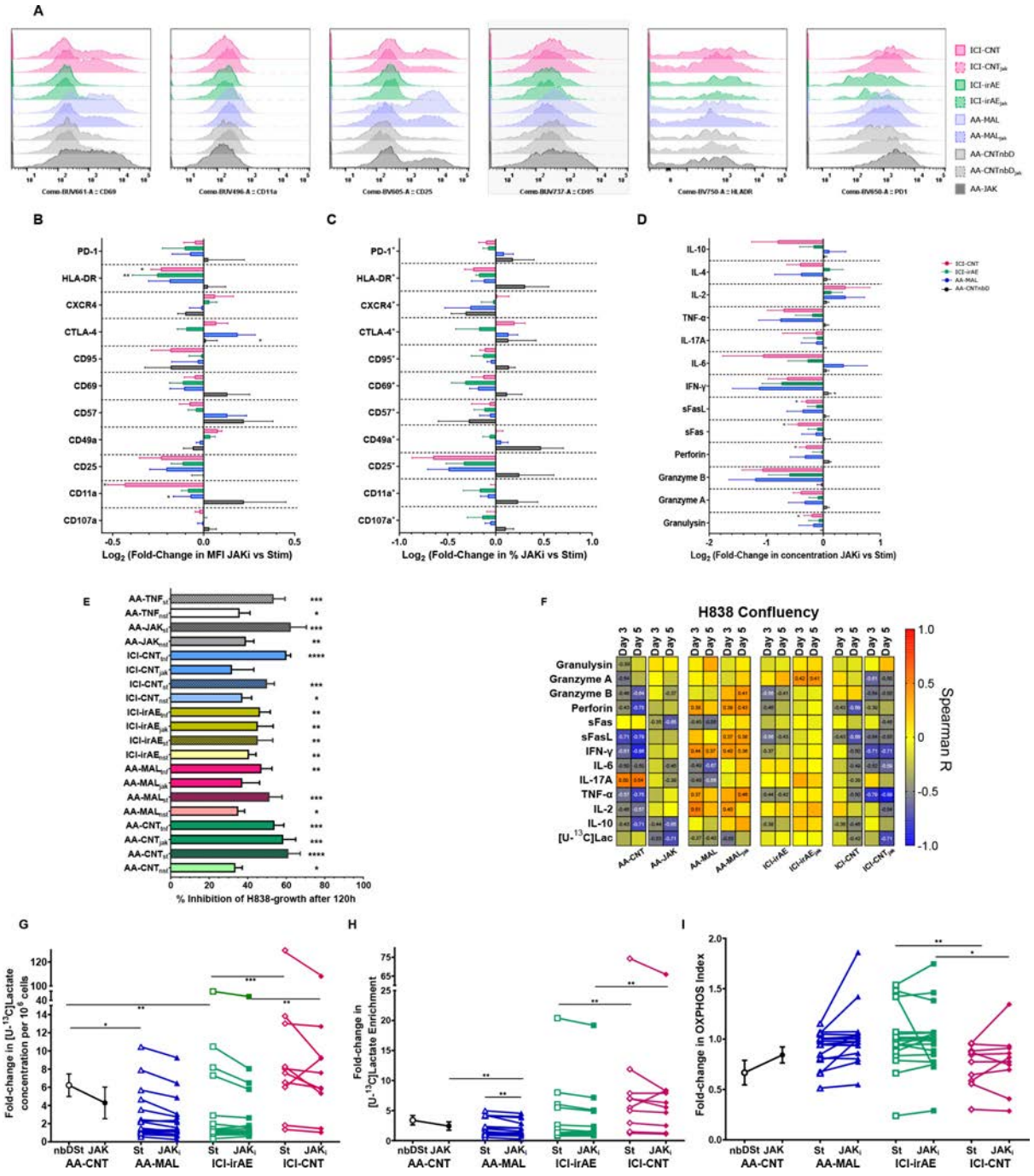

Figure 4 In vitro JAK-pathway inhibition with tofacitinib does not alter the immuno-metabolic profile of ICI-irAE CD8. (A) Representative histograms of changes in the expression of cell-surface molecules by TCR-stimulated CD8 after in vitro JAKi-treatment (AA-nbDCNT, AA-MAL, ICI-irAE and ICI-CNT) and AA-JAK patients. (B–D) Bar graphs showing the fold-changes in surface-marker expression (B), surface marker frequency (C) and cytokines and cytotoxic molecules release (D) of TCR-stimulated CD8 after in vitro JAKi treatment. *p<0.05, **p<0.01 changes between stimulated and JAKi conditions. (E) Inhibition of H838 growth by conditioned media from CD8 (unstimulated, and TCR-stimulated with and without JAKi or TNFi treatment) after 5 days. *p<0.05, **p<0.01; ***p<0.001, ****p<0.0001 between conditioned media versus H838 in medium only. (F) Correlations between H838 cell growth and the concentration of cytokines or cytotoxic molecules in the conditioned cell-culture media. Numbers show correlations with Spearman R>|0.35| and p<0.05. (G–I) Fold change relative to baseline in the concentration of [U-^{13}C]-lactate in the cell-culture medium (G), [U-^{13}C]-lactate enrichment (H) and OXPHOS rate (I) in TCR-stimulated CD8 with (solid symbols) or without (open symbols) in vitro JAKi treatment (AA-MAL, ICI-irAE, and ICI-CNT) or between AA-CNT and AA-JAK patients. *p<0.05, **p<0.01, ***p<0.001 between JAKi-treated and untreated cells. For (B–I): AA-nbDCNT n=10; AA-MAL n=16; ICI-irAE n=19; and ICI-CNT n=10. Representative patients for (A): AA-nbDCNT patient #17 from AA-CNT list; #16 from AA-JAK list; #14 from AA-MAL list; #16 from ICI-irAE list; and #10 from ICI-CNT list. AA-JAKi, autoimmune arthritis Janus-kinase inhibitor; AA-MAL, autoimmune arthritis malignancy; ICI-irAE, immune checkpoint inhibitor-immune-related adverse event; TCR, T-cell receptor.

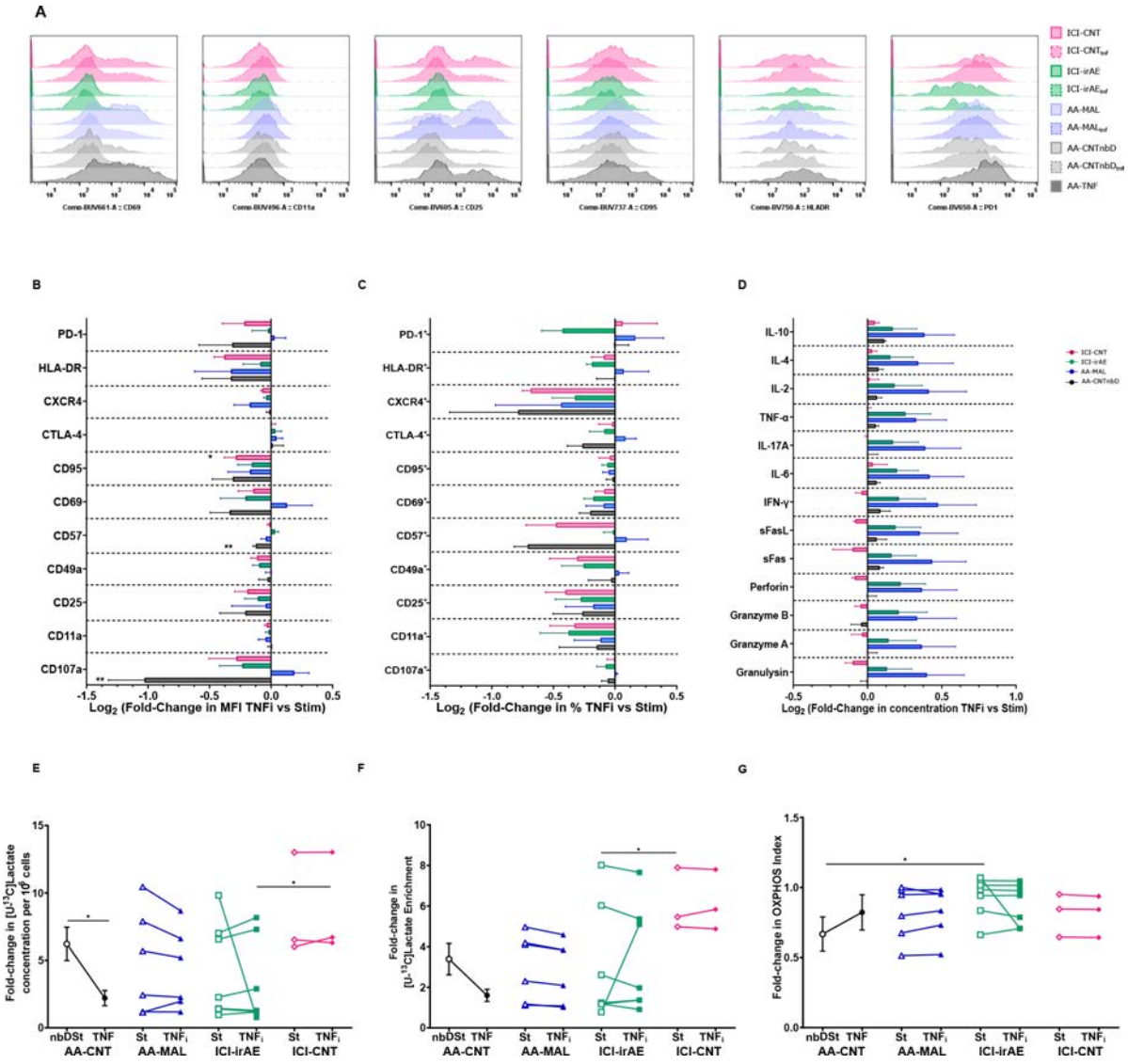

Figure 5 In vitro TNF-α inhibition with infliximab does not alter the immuno-metabolic profile of ICI-irAE CD8. (A) Representative histograms of changes in the expression of cell-surface molecules by TCR-stimulated CD8 after in vitro TNFi-treatment (AA-nbDCNT, AA-MAL, ICI-irAE and ICI-CNT) and AA-TNF patients. (B–D) Bar graphs showing the fold-changes in surface-marker expression (B), surface marker frequency (C) and cytokines and cytotoxic molecules release (D) of TCR-stimulated CD8 after in vitro TNFi treatment. *p<0.05, **p<0.01 changes between stimulated and TNFi conditions. (E–G) Fold change relative to baseline in the concentration of [U-^{13}C]-lactate in the cell-culture medium (G), [U-^{13}C]-lactate enrichment (H) and OXPHOS rate (I) in TCR-stimulated CD8 with (solid symbols) or without (open symbols) in vitro TNFi treatment (AA-MAL, ICI-irAE and ICI-CNT) or between AA-CNT and AA-TNF patients. *p<0.05 between TNFi-treated and untreated cells. For (B–G): AA-nbDCNT n=4; AA-MAL n=6; ICI-irAE n=7; and ICI-CNT n=3. Representative patients in (A): AA-nbDCNT patient #17 from AA-CNT list; AA-TNF patient #13 from AA-CNT list; #14 from AA-MAL list; #16 from ICI-irAE list; and #10 from ICI-CNT list. AA-JAKi, autoimmune arthritis Janus-kinase inhibitor; AA-MAL, autoimmune arthritis malignancy; ICI-irAE, immune checkpoint inhibitor-immune-related adverse event.

of cytokines or cytolytic molecules were evident in the CD8-conditioned media from AA-CNTnbD, AA-JAK and ICI-CNT patients (figure 4F), but none was observed for AA-TNF or in vitro TNFi (data not shown).

The metabolic profile of CD8 of AA-nbDCNT was generally more glycolytic than in AA-JAK and AA-TNF patients with higher (U-^{13}C)-lactate production and lower OXPHOS rate (figures 4G–I and 5E–G and online supplemental figure 3F). Except for a lower enrichment of (U-^{13}C)-lactate in AA-MAL CD8 under in vitro JAKi treatment, no other TNFi-induced or JAKi-induced changes were observed in the metabolic profile of ICI-irAE, ICI-CNT or AA-MAL CD8.

DISCUSSION

Functional and phenotypical changes in CD8 have been generally associated with the success of antitumor response and are, thus, the core of ICI therapy.[27 28] However, such changes are equally contributing to the pathophysiology of chronic AA.[19 20 29] This poses a major challenge in the treatment of ICI-induced arthritis as inhibition of CD8 would be required for sustained arthritis therapy, which would, however, limit the antitumor effects. To clarify some of these aspects, we have compared phenotype, functional and metabolic changes in peripheral CD8 from ICI patients who either developed or did not develop musculoskeletal irAEs with those from AA patients and tested the effects of in vitro JAKi and TNFi treatment on CD8 associated with

antitumor response. The fraction of cells expressing effector/activation and homing markers within the total CD8 pool and the different CD8 subsets, as well as the amount of released immune mediators, was similar between patients with AA-CNT and ICI-irAE, but lower than was observed for ICI-CNT. This AA-like profile was independent of arthritis symptom duration and remained in those ICI-irAE patients who had stopped ICI therapy. Thus, it suggests that ICI-induced arthritis imprints a lasting phenotype on peripheral CD8. Alterations in the phenotype of peripheral blood CD8 have equally been reported in the blood of thymic epithelial tumour and metastatic patients with NSCLC developing different forms of irAEs,[30] in the epidermis of melanoma, renal cell carcinoma, gastric cancer and lung cancer patients with ICI-induced psoriasis-like dermatitis[31] and in the colon epithelium of melanoma patients with ICI-induced colitis.[32]

Metabolic remodelling from OXPHOS towards aerobic glycolysis is a hallmark of CD8 activation,[33–35] as this allows cells to produce ATP much faster than through OXPHOS.[36] Patients with chronic AA display a permanent and exacerbated lactate production (Warburg effect), which is associated with a glycolytic profile and lower OXPHOS that maintains chronic cytotoxicity. On TCR stimulation, CD8 from AA patients further drop their OXPHOS rate and rely solely on aerobic glycolysis[19] in a process known as the Crabtree effect. Thus, it was not surprising to observe that unstimulated ICI-irAE CD8 were able to release large amounts of newly synthesised lactate, which correlated to a higher amount of total ATP. Moreover, and like what was observed in the AA-CNT CD8 but unlike ICI-CNT CD8, TCR stimulation might have triggered a Crabtree effect-like profile in ICI-irAE CD8 for they maintained glycolysis as their major source of ATP. Since resting ICI-irAE CD8 were more glycolytic than ICI-CNT but released less cytotoxic and proinflammatory mediators, it is possible that ICI-irAE CD8 have a more robust biosynthetic balance to sustain their effector/antitumor functions for longer periods when compared with ICI-CNT and might contribute to the better clinical outcomes observed in patients with ICI-irAE. However, the downside of keeping a steady proinflammatory and cytotoxic effector phenotype for longer periods is that it may render ICI-irAE CD8 with a RA-like profile, which favours the surge and relapse of irAE and may contribute to the chronic course of rheumatic irAEs.[13]

Gene-expression analysis has shown that the development of different irAEs has been associated with pre-ICH and post-ICI downregulation of CXCR1 on peripheral CD8 in melanoma patients.[8] Here, we reanalysed the same pre-ICI gene-expression data set focusing on those patients who developed specifically arthritis as a rheumatic irAE. Even though the number of available samples was sparse—which limits data interpretation—the results suggest a baseline impairment in the upregulation of TNF-signalling and proliferation pathways. These differences appear to remain after arthritis-irAE has developed, since CD8 from ICI-irAE patients released less TNF and expressed less CD25 than those from ICI-CNT. Collectively, these are relevant findings in the context of the lively discussion on the beneficial or detrimental effects of TNF inhibition as a treatment option for ICI patients with severe irAEs.[37–39] Since anti-PD-(L)1- rather than anti-CTLA-4-monotherapy is associated with a higher incidence of rheumatic irAEs,[24 40] it justified a separate gene-expression analysis comparing ICI patients who received only anti-PD-1 therapy. It was interesting to observe already at baseline the enrichment of the ATP metabolism-related pathway in those CD8 from patients who later developed arthritis irAE and which could be mirrored by the data obtained for ICI-irAE CD8 from patients with established arthritis. Therefore, this suggests that even before ICI therapy, CD8 from patients who later develop arthritis irAE, present a gene-expression profile that already indicates a different immunometabolic phenotype than those that remain irAE free.

Currently, therapeutic algorithms for irAE-arthritis rely on the defensive use of GC, csDMARDs and TNF- or IL-6-blockers.[4 5] However, the use of TNF-inhibition in irAEs is increasingly controversial[37–39] and our data suggest that CD8 from ICI-irAE patients present a downregulation of the TNF-signalling pathway and release less TNF than ICI-CNT. Thus, finding other therapeutic strategies to curb ICI-induced musculoskeletal inflammation and maintain antitumor activity will be required to meet current clinical needs in the management of ICI-irAEs. Preclinical studies on human cancer cell lines have shown that JAK-pathway inhibition impairs tumour growth.[41 42] Still, new data on increased risk of malignancy in patients with RA associated with tofacitinib use put the previously favourable assessment of JAKi in vitro and in vivo into question.[43 44] Clinicians remain hesitant in general to use JAKi in patients with a history of malignancy due to the shorter observational time in premarketing and postmarketing studies compared with most bDMARDs and, in line with this cautious attitude, only one JAKi-treated patient versus six patients with bDMARD therapy were present in our AA-MAL cohort. However, considering the limited treatment options, one needs to expand the therapeutic armamentarium to cope with severe and/or chronic ICI-irAEs, the latter being a frequent course of rheumatic irAEs.[13] Therefore, the beneficial effects, as well as potential risks of JAKi in musculoskeletal and other irAEs, should be further investigated, particularly when keeping in mind the increasing number of available JAKi with minor, but clinically relevant, differences in their modes of action. Of note, tofacitinib was previously successfully used for one ICI patient with arthritis-irAE[45] and a case series of GC-refractory myocarditis-irAE.[46] In view of this, we carried out in vitro experiments to explore the feasibility of using JAK-pathway inhibition by tofacitinib to control CD8 proinflammatory activity without severely compromising antitumor response and compared the data to TNF-α blockade. Our data suggest that in vitro tofacitinib, similarly to infliximab, did not significantly reduce the release of cytotoxic mediators by ICI-irAE CD8. Due to constraints imposed by the limited volume of collected blood, we could only indirectly measure the in vitro capacity of tofacitinib or infliximab-treated CD8 to inhibit tumour cell growth using conditioned media and not through a direct coculture system. Nevertheless, it seems that. in our experimental setting, the antitumor capacity of CD8-conditioned media from patients with ICI-irAE could be maintained in the presence of both drugs, even if it was lower than observed for the cancer-free AApatients. Additionally, in vitro tofacitinib and infliximab treatment did not reduce aerobic glycolysis, essential for maintaining antitumor functions in CD8.[47]

The lack of a group of AA-free patients with cancer with ongoing tumour activity and without ICI therapy and the use of only one type of JAK inhibitor for the in vitro studies (a constraint imposed by the reduced number of cells obtained from each patient) are potential limitations of our study. To counter this limitation, we included the patients AA-MAL who had simultaneously a clinical history of malignancy (some still with active tumours) and chronic arthritis, and AA-JAK patients with chronic AA receiving different types and doses of JAK inhibitors. Since the AA-MAL and the ICI-irAE CD8 presented similar profiles, even in their response to in vitro JAKi, we assume that ICI, other cancer therapies, or ongoing tumour activity did not

play a major role in the observed immune and metabolic profile changes. Since the CD8 phenotype was quite consistent among all AA-JAK patients, we considered that limiting the in vitro studies to one type of JAKi does not reduce the veracity of our findings.

Further potential limitations are the uneven distribution of anti-PD-1 monotherapy and combination treatment of anti-CTLA-4 and anti-PD-1 between ICI-irAE and ICI-CNT groups as well as the shorter duration of ICI therapy and higher proportion of ongoing treatment in the latter group. This bias is a result of the exclusion criterion of moderate to severe irAEs in any organ in the ICI-CNT and the fact that patients exposed to anti-CTLA-4 or the combination treatment generally show a higher incidence, increased severity and faster onset of irAEs[23 24] and, therefore, are less likely to remain irAE free over a longer period of ICI treatment. To address this problem, we compared the expression of cell-surface markers and release of immune mediators between ICI-irAE and ICI-CNT patients with monotherapy and combination treatment and ICI-irAE patients with ongoing and discontinued ICI treatment but did not observe any significant differences. Therefore, we hypothesise that previous anti-CTLA-4 exposure and/or ongoing ICI treatment at sample collection were not the driving factors behind the differences in metabolic and immune-effector profiles between ICI-irAE and ICI-CNT groups.

Overall, our study shows that CD8 from patients with cancer who develop musculoskeletal irAEs during ICI treatment have a distinct immune-effector and metabolic profile from those ICI patients that remain irAE free. The irAE profile is characterised by lower cytotoxic and proinflammatory activity and more aerobic glycolysis and overlaps with the profile observed in AA-CNT and AA-MAL CD8. This suggests that chronic inflammatory arthritis has a unique fingerprint that can be used to direct new therapeutic strategies for managing ICI-induced irAE. One such therapeutic approach may involve JAK pathway inhibition that does not interfere with the antitumor capacity of ICI-irAE CD8 in our experimental model. Thus, future trials on tumor-bearing mice with (poly)arthritis or controlled clinical trials on ICI-irAE patients using JAKi should be the next step to improve therapeutic outcomes while maintaining ICI efficacy together with simultaneous irAE control.

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EPIDEMIOLOGICAL SCIENCE

Temporal trends in COVID-19 outcomes among patients with systemic autoimmune rheumatic diseases: from the first wave through the initial Omicron wave

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ABSTRACT

Objectives To investigate temporal trends in incidence and severity of COVID-19 among patients with systemic autoimmune rheumatic diseases (SARDs) from the first wave through the initial Omicron wave.

Methods We conducted a retrospective cohort study investigating COVID-19 outcomes among patientswith SARD systematically identified to have confirmed COVID-19 from 1 March 2020 to 31 January 2022 at Mass General Brigham. We tabulated COVID-19 counts of total and severe cases (hospitalisations or deaths) and compared the proportion with severe COVID-19 by calendar period and by vaccination status. We used logistic regression to estimate the ORs for severe COVID-19 for each period compared with the early COVID-19 period (reference group).

Results We identified 1449 patients with SARD with COVID-19 (mean age 58.4 years, 75.2% female, 33.9% rheumatoid arthritis). There were 399 (28%) cases of severe COVID-19. The proportion of severe COVID-19 outcomes declined over calendar time (p for trend <0.001); 46% of cases were severe in the early COVID-19 period (1 March 2020–30 June 2020) vs 15% in the initial Omicron wave (17 December 2021–31 January 2022; adjusted OR 0.29, 95% CI 0.19 to 0.43). A higher proportion of those unvaccinated were severe compared with not severe cases (78% vs 60%).

Conclusions The proportion of patients with SARD with severe COVID-19 has diminished since early in the pandemic, particularly during the most recent time periods, including the initial Omicron wave. Advances in prevention, diagnosis and treatment of COVID-19 may have improved outcomes among patients with SARD.

WHAT IS ALREADY KNOWN ABOUT THIS TOPIC

⇒ Patients with systemic autoimmune rheumatic diseases (SARDs) may be at increased risk for severe COVID-19, defined as hospitalisation or death.

⇒ Previous studies of patients with SARD suggested improving COVID-19 outcomes over calendar time, but most were performed prior to the wide availability of COVID-19 vaccines or the initial Omicron wave that was characterised by high infectivity.

WHAT THIS STUDY ADDS

⇒ The proportion of patients with SARD with severe COVID-19 outcomes was lower over calendar time.

⇒ The adjusted OR of severe COVID-19 in the initial Omicron wave was 0.29 (95% CI 0.19 to 0.43) compared with early COVID-19 period.

⇒ The absolute number of severe COVID-19 cases during the peak of the Omicron variant wave was similar to the peaks of other waves.

⇒ Patients with SARD and severe COVID-19 were more likely to be unvaccinated than patients with SARD whose COVID-19 was not severe.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These findings suggest that advances in COVID-19 prevention, diagnosis and treatment have contributed to improved outcomes among patients with SARD over calendar time.

⇒ Future studies should extend findings into future viral variants and consider the roles of waning immunity after vaccination or natural infection among patients with SARD who may still be vulnerable to severe COVID-19.

INTRODUCTION

In the 2 years since COVID-19 was recognised as a global pandemic, significant strides have been made in the testing, prevention and treatment of COVID-19. The initial wave of infections caused by the 'Omicron" variant has been reported to cause less severe outcomes in the general population compared with prior waves.[1–4] Multiple factors including vaccinations, prior infection, increased testing and effective treatments as well as intrinsic features of the variant likely contribute to these observations. However, whether such improved outcomes have also been observed in people with systemic autoimmune rheumatic diseases (SARDs) remain unclear. Some people with SARDs have been found to have an increased risk of severe outcomes from COVID-19, such as hospitalisation

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and death. This has been attributed to altered underlying immunity, immunosuppression contributing to blunted responses to both natural infection and vaccination, and end-organ damage from the SARD.[5 6] Advances in vaccination, testing, and outpatient and inpatient treatments during the initial Omicron wave in the USA may have also contributed to improved temporal COVID-19 outcomes among people with SARDs.

Previous studies have investigated temporal trends in COVID-19 outcomes among patients with SARD prior to the initial Omicron wave. A Swedish study compared patients with inflammatory joint diseases to matched comparators and found worse outcomes, particularly early in the pandemic.[7] A small cohort study in Ireland found no improvement in hospitalisation or mortality rates in the first three waves of the pandemic.[8] Two other studies performed about 6 months into the pandemic showed that excess risk of severe COVID-19 among patients with SARD was similar to the general population.[9 10] It remains unclear whether outcomes have improved in recent time periods for patients with SARDs.

We aimed to investigate temporal trends in incidence and severity of COVID-19 among patients with SARD. We hypothesised that the proportion of patients with SARD experiencing severe COVID-19 has improved since early in the pandemic due to several factors including vaccinations, testing availability, as well as outpatient and inpatient treatments.

METHODS

Study design and population

We performed a retrospective cohort study investigating temporal trends of COVID-19 outcomes among patients with SARD throughout the pandemic (from 1 March 2020 to 31 January 2022) at the Mass General Brigham (MGB) HealthCare system in the greater Boston, Massachusetts area. MGB is composed of 14 hospitals including Massachusetts General Hospital and Brigham and Women's Hospital and affiliated community health centres.

Identification of COVID-19 cases and patients with SARD

As previously described in more detail we systematically identified all patients with SARD with PCR-confirmed SARS-CoV-2 infection using electronic query.[6 10–14] Our EHR also flags patients who had positive testing either at home (eg, patients who notified their rheumatologist about a positive rapid antigen test through the secure patient portal) or outside of the MGB system (eg, admitted patient with COVID-19 transferred from an outside hospital). These lists were filtered by the presence of at least one International Classification of Diseases 9th revision (ICD-9) or ICD-10 code for SARD as a sensitive screen. This was further supplemented by direct referrals to our study team from rheumatologists who learnt of patients' positive tests during a clinical encounter. Among these lists, the presence of prevalent SARD and SARS-CoV-2 infection was confirmed by medical record review. As in our previous studies, we excluded participants being treated for osteoarthritis, fibromyalgia, mechanical back pain, Raynaud's phenomenon, gout or pseudogout alone since these conditions are not typically treated with systemic immunomodulators and are often managed by non-rheumatologists.[6 10–14] Thus, all patients included had a confirmed SARD diagnosis with verified SARS-CoV-2 infection between 1 March 2020 and 31 January 2022 (See online supplemental figure 1 for flow diagram of analyzed sample).

Exposure variable: time periods throughout the pandemic

The date of COVID-19 onset was determined by the first date of SARS-CoV-2 test positivity for those with PCR tests in the MGB system, date of first COVID-19 flag or date of positive test from medical record review or referral. We a priori divided calendar time into periods based on changes in viral epidemiology and care advances. The 'Early COVID-19 period' was 1 March 2020 to 30 June 2020, corresponding to when Massachusetts experienced the first wave of COVID-19. The 'Early treatment period' was 1 July 2020 to 31 January 2021, corresponding to seminal advances in treatment of hospitalised patients with dexamethasone and remdesivir as well as a wave of cases in the fall/winter.[15 16] The 'Early vaccination' period was 1 February 2021 to 30 June 2021, corresponding to the initial roll-out of COVID-19 vaccines to high-risk populations starting on 1 February 2021 in Massachusetts, USA.[17–19] The 'Additional vaccination and Delta wave' period was 1 July 2022 to 16 December 2022, corresponding to recommendations for additional doses of vaccines to immunocompromised patients as well as a fall surge of cases due to the Delta variant.[20] The 'Omicron wave' period was 17 December 2021 to 31 January 2022, corresponding to the initial large local surge of cases due to the Omicron variant.

Outcome variables: severe COVID-19

As in previous studies, we defined severe COVID-19 as a composite of hospitalisation or death within 30 days from COVID-19 date.[5] Medical record review was performed to determine these outcomes and to identify patients who received mechanical ventilation. This was supplemented by electronic query and information from the referring rheumatologist for those who were hospitalised outside the MGB system. We also examined the individual outcomes of hospitalisation, mechanical ventilation and death from COVID-19.

Other characteristics

We collected information on demographics, comorbidities, vaccination status, and rheumatic disease characteristics and medications. Age, sex, race (white, black, Asian/Hawaiian/Pacific Islander or other/unknown) and Hispanic ethnicity were identified from electronic query. Comorbidities were collected from medical record review and included hypertension, diabetes mellitus, obesity, cardiovascular disease, obstructive lung disease (including asthma, chronic obstructive pulmonary disease, and obstructive sleep apnoea), and interstitial lung disease. We classified vaccination status as follows: (1) unvaccinated or prevaccine, (2) partially vaccinated (occurring between day 0 and day 13 of 2-dose mRNA vaccines or between day 0 and day 13 of the one-dose Johnson & Johnson-Janssen (J&J) vaccine), (3) two-dose mRNA or one dose J&J (14 days after completion of the initial series) and (4) additional doses (day 14 or later after third vaccine dose for those who initially received two-dose mRNA vaccines or day 14 or later after second vaccine dose for those who initially received one-dose J&J vaccine per US Centers for Disease Control and Prevention (CDC) recommendations for an additional dose)[21] (See online supplemental figure 2 for diagram illustrating vaccine status definitions).

SARD characteristics were obtained from medical record review. These included specific type of SARD, glucocorticoid use/dose (none, low dose (1–10 mg/day of prednisone-equivalent), moderate/high dose (>10 mg) and unknown dose), disease-modifying antirheumatic drug (DMARD) use (categorised by specific drug for conventional synthetic DMARDs or mechanism

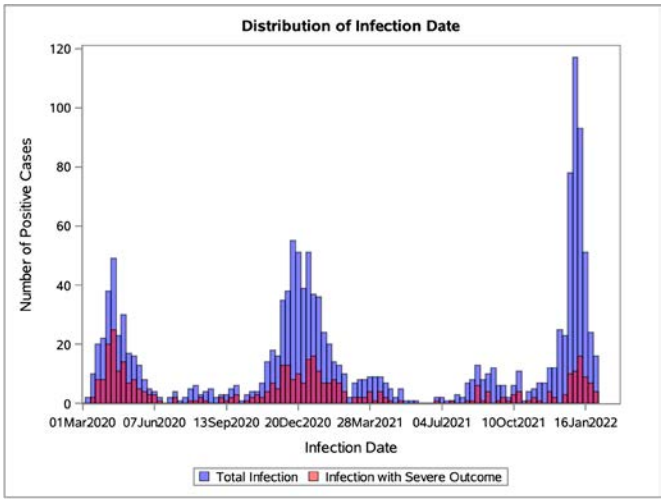

Figure 1 Total and severe COVID-19 case counts over time. Case counts of SARS-CoV-2 infections over calendar time with total infections shown in blue and infections with severe outcomes in red.

of action for biologic DMARDs) at time of COVID-19 diagnosis, and preceding SARD activity per review of notes from the medical record (categorised as remission/low, moderate/severe or unknown).

Treatment of COVID-19 was also collected by medical record review and supplemented by electronic query: neutralising monoclonal antibodies (typically given as outpatient for COVID-19 treatment rather than inpatient or as postexposure prophylaxis during our study period), remdesivir (only used as inpatient during our study period), convalescent plasma, dexamethasone (or other high-dose glucocorticoids used inpatient to treat COVID-19), tocilizumab (to treat inpatient COVID-19 rather than underlying SARD), baricitinib (used inpatient to treat COVID-19 rather than underlying SARD).[22] No patients with SARD were documented to have received nirmatrelvir/ritonavir (Paxlovid) or molnupiravir for COVID-19 treatment during the study period.[23]

Statistical analysis

Calendar time was the exposure of interest for our study. We graphed weekly counts of total COVID-19 cases and severe outcomes among patients with SARD throughout the entire study period. We reported frequencies and proportions of demographics, comorbidities, vaccination status, type of COVID-19 test and SARD characteristics overall and stratified by the five calendar periods. Since severe COVID-19 during the initial Omicron wave period has been reported to be relatively uncommon in the general population,[1] we also reported on characteristics of patients with SARD with severe versus not severe COVID-19 during the period. We also reported a case series of the patients with SARD with COVID-19 who died during the Omicron wave period.

We reported the proportion of severe COVID-19 outcomes that occurred during each calendar period and calculated a p for trend across the five calendar periods. We also stratified cases by severe versus not severe and calculated the proportion in each category by vaccination status. We performed logistic regression to estimate the ORs and 95% CIs) for severe COVID-19 for each period compared with the early pandemic period as the reference. The base model was unadjusted. The multivariable model adjusted for age, sex and race.

We performed several sensitivity analyses to assess the robustness of our findings with different definitions of time periods and alternative reference groups. Some of the COVID-19 advances were iterative, rather than discrete (eg, inpatient treatments for severe COVID-19), and may have had slow uptake (eg, monoclonal antibody and vaccine uptake). First, we divided the original period 'Early treatment' into two separate time periods, before and after 9 November 2020. This date was chosen as the date that monoclonal antibodies first received emergency use authorisation from the US Food and Drug Administration and coincided with the beginning of the second wave of COVID-19. Second, we considered the entire 'prevaccine era' (1 March 2020 to 31 January 2021) as a single reference group. Third, we changed the reference group to the 'Early treatment' time period (1 July 2020 to 31 January 2021) in case the original choice of the reference group influenced results.

Since monoclonal antibodies were the only effective outpatient treatment available during our study period, we performed two analyses to investigate how their availability may have impacted results. First, we removed all patients who received monoclonal antibodies from the primary analysis to investigate whether there was still improved outcomes over calendar time. Second, we investigated the association of monoclonal antibody use versus no use with severe COVID-19 in an exploratory analysis using logistic regression. For that analysis, we limited the sample to those diagnosed with COVID-19 on 9 November 2020 or later since monoclonal antibodies were not available through routine clinical care prior to then.

We considered a two-sided $p<0.05$ as statistically significant. SAS V.9.4 was used for all analyses.

RESULTS

COVID-19 cases among patients with SARD

From 1 March 2020 to 31 January 2022, we identified 1449 patients with SARD with confirmed COVID-19 (see flow diagram for the analysed sample in online supplemental figure 1). Figure 1 shows the weekly counts of total and severe COVID-19 cases. The tallest peak of cases occurred during the initial Omicron wave period. There were 261, 492, 123, 172 and 401 total cases in the early COVID-19, early treatment, early vaccination, additional vaccination and Delta wave, and initial Omicron wave periods, respectively.

Characteristics of patients with SARD with COVID-19

Table 1 shows the demographics, comorbidities, SARS-CoV-2 testing type and COVID-19 vaccination status. Mean age of the entire study sample was 58.4 years (SD 17.5). Cases in the early COVID-19 period tended to be older compared with cases in the initial Omicron wave period (mean age 63.1 years vs 54.2). The overall sample was 71.4% white, 11.2% black and 3.6% Asian/Hawaiian/Pacific Islander. The most common comorbidity was hypertension (43.8%). The vaccination status for the entire sample at the time of infection was 64.7% prevaccination/unvaccinated, 3.5% partially vaccinated, 15.7% two-dose mRNA or one-dose J&J and 16.1% additional doses. Breakthrough infections were particularly common in the initial Omicron wave: 136 cases (33.8% of cases in the period) occurred among those who received 2-dose mRNA or 1-dose J&J and 205 cases (51.1% of cases in this period) occurred among those with additional vaccine doses. Most cases were diagnosed with PCR testing. The initial Omicron wave was the only period where a substantial portion of cases were diagnosed with home rapid antigen testing (122/401 (30.4%) during this period).

Table 1 Demographics and clinical characteristics of rheumatic disease patients with COVID-19 over calendar time

	Time period					
		Early COVID-19	Early treatment	Early vaccination	Additional vaccination and Delta wave	Omicron wave
	Overall (n=1449)	1 March 2020–30 June 30 (n=261)	1 July 2020–31 January 2021 (n=492)	1 February 2021–30 June 2021 (n=123)	1 July 2021–16 December 2021 (n=172)	17 December 2021–31 January 2022 (n=401)
Age, years (mean±SD)	58.4 (17.5)	63.1 (16.6)	59.3 (17.0)	55.5 (16.9)	56.8 (17.7)	54.2 (17.6)
Female sex, n (%)	1090 (75.2)	196 (75.1)	370 (75.2)	90 (73.2)	111 (64.5)	323 (80.5)
Race, n (%)						
White	1035 (71.4)	154 (59.0)	353 (71.7)	93 (75.6)	136 (79.1)	299 (74.6)
Black	162 (11.2)	52 (19.9)	50 (10.2)	15 (12.2)	11 (6.4)	34 (8.5)
Asian, Hawaiian or Pacific Islander	52 (3.6)	9 (3.4)	19 (3.9)	6 (4.9)	7 (4.1)	11 (2.7)
Other or unknown	200 (13.8)	46 (17.6)	70 (14.2)	9 (7.3)	18 (10.5)	57 (14.2)
Hispanic ethnicity, n (%)	67 (4.6)	13 (5.0)	31 (6.3)	5 (4.1)	4 (2.3)	14 (3.5)
Comorbidities, n (%)						
Hypertension	635 (43.8)	154 (59.0)	220 (44.7)	49 (39.8)	63 (36.6)	149 (37.2)
Diabetes mellitus	234 (16.1)	61 (23.4)	92 (18.7)	20 (16.3)	20 (11.6)	41 (10.2)
Obesity	427 (29.5)	92 (35.2)	156 (31.7)	49 (39.8)	35 (20.3)	95 (23.7)
Cardiovascular disease	210 (14.5)	62 (23.8)	72 (14.6)	15 (12.2)	20 (11.6)	41 (10.2)
Obstructive lung disease	310 (21.4)	73 (28.0)	109 (22.2)	25 (20.3)	31 (18.0)	72 (18.0)
Interstitial lung disease	81 (6.0)	17 (6.5)	25 (5.1)	8 (6.5)	9 (5.2)	22 (5.5)
Vaccination status, n (%)						
Unvaccinated or prevaccine	938 (64.7)	261 (100)	492 (100)	102 (82.9)	40 (23.3)	43 (10.7)
Partially vaccinated	50 (3.5)	0 (0)	0 (0)	19 (15.4)	14 (8.1)	17 (4.2)
Two doses mRNA or one dose J&J	228 (15.7)	0 (0)	0 (0)	2 (1.6)	90 (52.3)	136 (33.9)
Additional doses	233 (16.1)	0 (0)	0 (0)	0 (0)	28 (16.3)	205 (51.1)
SARS-CoV2 diagnosis method, n (%)						
PCR	1126 (77.7)	246 (94.3)	426 (86.6)	100 (81.3)	129 (75.0)	225 (56.1)
Antigen/rapid test	123 (8.5)	0 (0)	0 (0)	0 (0)	1 (0.6)	122 (30.4)
Other or unknown	200 (13.8)	15 (5.7)	66 (13.4)	23 (18.7)	42 (24.4)	54 (13.5)

J&J, Johnson and Johnson/Janssen.

Table 2 shows the SARD characteristics for the COVID-19 cases, stratified by the five calendar periods. The most common type of SARD was rheumatoid arthritis (33.9%), followed by psoriatic arthritis and spondyloarthritis (14.7%) and systemic lupus erythematosus (13.1%). Most patients (74.1%) were in remission or had low disease activity at time of COVID-19 onset. Baseline glucocorticoids were used in 26.0% of cases. The most commonly used DMARD was methotrexate (22.0%), followed by antimalarials (21.7%) and TNF inhibitors (20.2%). Rituximab was used in 9.2% of cases.

Treatment and severe COVID-19 outcomes

The most common treatments for COVID-19 were remdesivir (14.2%), dexamethasone (13.2%) and neutralising monoclonal antibodies (12.6%). Few patients with SARD received tocilizumab (1.0%), baricitinib (0.2%) or convalescent plasma (0.5%) to treat COVID-19.

There were 399 (27.5%) who had the composite outcome of severe COVID-19 of hospitalisation or death (table 3). There were 391 (27.0%) hospitalisations and 60 (4.1%) deaths. The proportion of patients with SARD experiencing severe COVID-19 decreased over the calendar periods. The total and proportion of severe COVID-19 in each calendar period was 119 (45.6%), 144 (29.3%), 41 (33.3%), 36 (20.9%) and 59 (14.7%) in the early COVID-19, early treatment, early vaccination, additional vaccination and Delta wave, and Omicron wave periods, respectively (figure 2, p for trend <0.001). Compared with the reference of the early COVID-19 period, the multivariable ORs and 95%CIs for severe COVID-19 were 0.58 (0.41 to 0.81) in the early treatment period, 0.89 (0.54 to 1.46) in the early vaccination period, 0.39 (0.24 to 0.62) in the additional vaccination and Delta wave period, and 0.29 (0.19 to 0.43) in the Omicron wave period, adjusted for age, sex and race.

Figure 3 displays the vaccination status stratified by severe or not severe COVID-19. Among the 399 severe cases, 78.4% were unvaccinated. Among the 1050 cases that were not severe, 59.5% were unvaccinated.

Severe COVID-19 outcomes during the Omicron wave period

Online supplemental table 1 shows characteristics of the 401 patients with SARD in the initial Omicron wave, stratified by COVID-19 severity. Mean age of those with severe COVID-19 was 66.9 years (SD 19.1), 76.3% were female, 10.2% had interstitial lung disease, 37.3% had rheumatoid arthritis, 47.5% were on glucocorticoids, 18.6% were on methotrexate and 15.3% were on rituximab. Online supplemental table 2 shows the case

Table 2 Rheumatic disease characteristics of patients with SARD with COVID-19 over calendar time

	Time period					
		Early COVID-19	Early treatment	Early vaccination	Additional vaccination and Delta wave	Omicron wave
	Overall (n=1449)	1 March 2020–30 June 2020 (n=261)	1 July 2020–31 January 2021 (n=492)	1 February 2021–30 June 2021 (n=123)	1 July 2021–16 December 2021 (n=172)	17 December 2021–31 January 2022 (n=401)
Rheumatic disease diagnosis, n ()						
Rheumatoid arthritis	491 (33.9)	90 (34.5)	174 (35.4)	48 (39.0)	45 (26.2)	134 (33.4)
Psoriatic arthritis and spondyloarthritis	213 (14.7)	31 (11.9)	75 (15.2)	17 (13.8)	32 (18.6)	58 (14.5)
Systemic lupus erythematosus	190 (13.1)	39 (14.9)	64 (13.0)	16 (13.0)	21 (12.2)	50 (12.5)
Other inflammatory arthritis*	106 (7.3)	19 (7.3)	38 (7.7)	4 (3.3)	13 (7.6)	32 (8.0)
PMR and/or GCA	101 (7.0)	23 (8.8)	31 (6.3)	7 (5.7)	14 (8.1)	26 (6.5)
ANCA-associated vasculitis	68 (4.7)	11 (4.2)	21 (4.3)	7 (5.7)	12 (7.0)	17 (4.2)
Other vasculitis†	33 (2.3)	8 (3.1)	7 (1.4)	4 (3.3)	2 (1.2)	12 (3.0)
Sjogren's syndrome	36 (2.5)	2 (0.8)	13 (2.6)	3 (2.4)	5 (2.9)	13 (3.2)
Systemic sclerosis	35 (2.4)	6 (2.3)	11 (2.2)	5 (4.1)	3 (1.7)	10 (2.5)
Inflammatory myopathy	33 (2.3)	6 (2.3)	13 (2.6)	1 (0.8)	6 (3.5)	7 (1.8)
Other connective tissue diseases‡	37 (2.6)	9 (3.4)	8 (1.6)	3 (2.4)	3 (1.7)	14 (3.5)
Sarcoidosis	43 (3.0)	9 (3.4)	22 (4.5)	1 (0.8)	4 (2.3)	7 (1.8)
Multiple rheumatic diagnoses	35 (2.4)	6 (2.3)	9 (1.8)	4 (3.3)	4 (2.3)	12 (3.0)
Other diagnoses§	28 (1.9)	2 (0.8)	6 (1.2)	3 (2.4)	8 (4.7)	9 (2.2)
Disease activity, n (%)						
Remission or low activity	1074 (74.1)	181 (69.3)	365 (74.2)	91 (74.0)	131 (76.2)	306 (76.3)
Moderate or severe activity	259 (17.9)	50 (19.2)	100 (20.3)	23 (18.7)	27 (15.7)	59 (14.7)
Unknown	116 (8.0)	30 (11.5)	27 (5.5)	9 (7.3)	14 (8.1)	36 (9.0)
Rheumatic disease medications at time of infection, n (%)						
Glucocorticoids	377 (26.0)	80 (30.7)	125 (25.4)	29 (23.6)	42 (24.4)	101 (25.2)
None	1072 (74.0)	181 (69.3)	367 (74.6)	94 (76.4)	130 (75.6)	300 (74.8)
Low dose (1–10 mg)	317 (21.9)	70 (26.8)	106 (21.5)	26 (21.1)	36 (20.9)	79 (19.7)
Moderate/high dose (>10 mg)	53 (3.7)	10 (3.8)	18 (3.7)	3 (2.4)	4 (2.3)	18 (4.5)
Unknown dose	7 (0.5)	0 (0.0)	1 (0.2)	0 (0.0)	2 (1.2)	4 (1.0)
Conventional synthetic DMARDs and immunosuppressants						
Methotrexate	319 (22.0)	49 (18.8)	108 (22.0)	21 (17.1)	38 (22.1)	103 (25.7)
Antimalarials	314 (21.7)	58 (22.2)	102 (20.7)	20 (16.3)	36 (20.9)	98 (24.4)
Sulfasalazine	31 (2.1)	5 (1.9)	8 (1.6)	2 (1.6)	3 (1.7)	13 (3.2)
Leflunomide	54 (3.7)	12 (4.6)	23 (4.7)	1 (0.8)	5 (2.9)	13 (3.2)
Mycophenolate mofetil	101 (7.0)	13 (5.0)	27 (5.5)	13 (10.6)	17 (9.9)	31 (7.7)
Azathioprine	27 (1.9)	9 (3.4)	6 (1.2)	4 (3.3)	2 (1.2)	6 (1.5)
Calcineurin inhibitor	19 (1.3)	4 (1.5)	5 (1.0)	5 (4.1)	3 (1.7)	2 (0.5)
Cyclophosphamide	4 (0.3)	3 (1.2)	0 (0)	0 (0)	1 (0.6)	0 (0)
Biologic DMARDs						
TNF inhibitors	292 (20.2)	32 (12.3)	90 (18.3)	26 (21.1)	40 (23.3)	104 (25.9)
Rituximab	133 (9.2)	17 (6.5)	33 (6.7)	18 (14.6)	25 (14.5)	40 (10.0)
Belimumab	20 (1.4)	4 (1.5)	1 (0.2)	1 (0.8)	3 (1.7)	11 (2.7)
Abatacept	31 (2.1)	4 (1.5)	6 (1.2)	3 (2.4)	3 (1.7)	15 (3.7)
IL-6 inhibitors	45 (3.1)	3 (1.2)	18 (3.7)	3 (2.4)	5 (2.9)	16 (4.0)
IL-17, IL-12/23, and IL-23 inhibitors	36 (2.5)	8 (3.1)	16 (3.3)	2 (1.6)	3 (1.7)	7 (1.7)
IL-1 inhibitors	6 (0.4)	0 (0)	0 (0)	0 (0%)	3 (1.7)	3 (0.8)
Targeted synthetic DMARDs						
JAK inhibitors	55 (3.8)	8 (3.1)	21 (4.3)	4 (3.3)	7 (4.1)	15 (3.7)
Apremilast	5 (0.4)	0 (0)	2 (0.4)	0 (0)	0 (0)	3 (0.8)
IVIG	19 (1.3)	2 (0.8)	7 (1.4)	2 (1.6)	4 (2.3)	4 (1.0)

*Includes juvenile idiopathic arthritis, other unspecified inflammatory arthritis.
†Includes Takayasu's arteritis, Kawasaki disease, Behcet's disease, polyarteritis nodosa, other vasculitis.
‡Includes undifferentiated connective tissue disease, mixed connective tissue disease, antiphospholipid syndrome (without concurrent systemic lupus erythematosus).
§Includes relapsing polychondritis, IgG_4-related disease, sclerosing mediastinitis, periodic fever syndromes, adult-onset Still's disease.
ANCA, antineutrophil cytoplasmic antibody; DMARD, disease-modifying antirheumatic drug; GCA, giant cell arteritis; IL, interleukin; IVIG, intravenous immune globulin; JAK, Janus kinase; PMR, polymyalgia rheumatica; SARD, systemic autoimmune rheumatic disease; TNF, tumour necrosis factor.

Table 3 Outcomes and treatments of patients with SARD over time

	Time period					
		Early COVID-19	Early treatment	Early vaccination	Additional vaccination and Delta wave	Omicron wave
	Overall (n=1449)	1 March 2020–30 June 2020 (n=261)	1 July 2020–31 January 2021 (n=492)	1 February 2021–30 June 2021 (n=123)	1 July 2021–16 December 2021 (n=172)	17 December 2021–31 January 2022 (n=401)
Hospitalisation, n (%)	391 (27.0)	115 (44.1)	142 (28.9)	40 (32.5)	36 (20.9)	58 (14.5)
Mechanical ventilation, n (%)	57 (3.9)	29 (11.1)	12 (2.4)	6 (4.9)	3 (1.7)	7 (1.7)
Death, n (%)	60 (4.1)	23 (8.8)	12 (2.4)	9 (7.3)	8 (4.7)	8 (2.0)
Severe COVID-19*	399 (27.5)	119 (45.6)	144 (29.3)	41 (33.3)	36 (20.9)	59 (14.7)
OR		Reference	**0.49 (0.36, 0.67)**	**0.60 (0.38, 0.93)**	**0.32 (0.20, 0.49)**	**0.21 (0.14,0.30)**
Adjusted OR†		Reference	**0.58 (0.41, 0.81)**	0.89 (0.54, 1.46)	**0.39 (0.24, 0.62)**	**0.29 (0.19, 0.43)**
Treatments Received, n (%)						
Monoclonal antibodies‡	183 (12.6)	1 (0.4)	17 (3.5)	18 (14.6)	86 (50.0)	61 (15.2)
Remdesivir	206 (14.2)	18 (6.9)	90 (18.3)	29 (23.6)	21 (12.2)	48 (12.0)
Convalescent plasma	7 (0.5)	1 (0.4)	1 (0.2)	5 (4.1)	0 (0.0)	0 (0.0)
Dexamethasone	191 (13.2)	11 (4.2)	92 (18.7)	29 (23.6)	20 (11.6)	39 (9.7)
Tocilizumab	15 (1.0)	11 (4.2)	2 (0.4)	2 (0.4)	0 (0.0)	0 (0.0)
Baricitinib	3 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)	2 (0.5)

Bolding indicates p<0.05.

*Composite outcome of hospitalisation, mechanical ventilation or death.
†Adjusted for age, sex and race.
‡Includes bamlanivimab/etesevimab, casirivimab/imdevimab and sotrovimab. One patient received monoclonal antibodies in the Early treatment period through compassionate use prior to routine clinical availability.
SARD, systemic autoimmune rheumatic disease.

series of the eight patients with SARD who died during the initial Omicron wave.

Sensitivity analyses

The results of the sensitivity analyses with alternative calendar time periods and reference groups are shown in online supplemental tables 3–5. These showed significantly lower odds for severe COVID-19 in the initial Omicron wave compared with the reference group, similar to the main analysis. Results were also similar when removing patients who received monoclonal antibodies (online supplemental table 6). Monoclonal antibody use had an adjusted OR of 0.42 (95% CI 0.25 to 0.68) for severe COVID-19 compared with no use (online supplemental table 7).

DISCUSSION

In this large cohort study, we found that outcomes of COVID-19 in patients with SARDs have improved since the beginning of the pandemic. In particular, SARS-CoV-2 infections in the initial Omicron wave were associated with a 71% reduction in the risk of hospitalisation or death compared with the earliest time period. Despite these improvements, the absolute number of cases of severe COVID-19 was similar to that observed in other waves, suggesting that despite a reduced risk of severe disease, the initial Omicron wave had a substantial impact on patients with SARDs and the healthcare systems caring for them. The temporal improvement in outcomes is likely multifactorial, including differences in availability of testing, vaccination

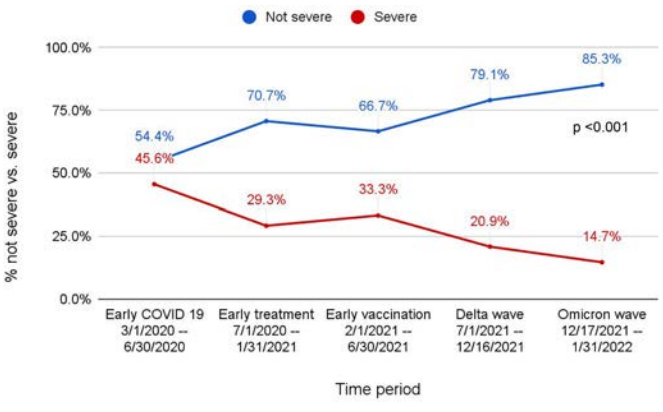

Figure 2 Proportion of cases with severe and non-severe COVID-19 in each time period. P value is for the trend across categories.

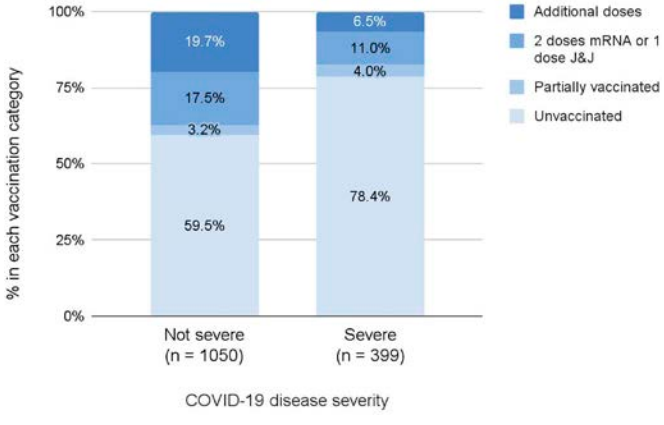

Figure 3 Vaccination status stratified by severe or not severe COVID-19. J&J, Johnson & Johnson-Janssen.

and other preventative strategies, hospital capacity, availability of effective treatments, depletion of susceptible individuals and virulence of SARS-CoV2 variants.

Previous studies conducted earlier in the pandemic found improvement in hospitalisation, mechanical ventilation, death and other outcomes for patients with SARDs over time, even during the first 6 months of the pandemic.[9 10] However, no other study to date has examined the temporal trends in COVID-19 outcomes among SARDs extending up to the initial Omicron wave. These findings are particularly relevant given the blunted vaccine response and associated higher risk of breakthrough infections that have been observed in some patients with SARDs.[24]

The introduction of effective vaccines represented a key turning point in the pandemic and despite waning efficacy with time and against novel variants, they continue to provide important protection against severe disease.[25] In our study of patients with SARDs, the majority of whom were on immunosuppressive treatments previously associated with blunted vaccine responses, we found that vaccination was associated with less severe COVID-19. These findings suggest that while some patients with SARD on immunosuppressives may be at higher risk for breakthrough infection, vaccination provided important benefits for many of these patients if infected with SARS-CoV-2. Additional studies are needed to further evaluate the efficacy of COVID-19 vaccinations among patients with SARDs during the most recent Omicron wave, a time characterised by substantial waning efficacy against breakthrough infection in the general population.[26 27]

Despite temporal improvements in the risk of severe COVID-19, some patients with SARDs during the initial Omicron wave experienced hospitalisation or death. Ours is one of the first to describe deaths from COVID-19 among SARDs that occurred during the initial Omicron wave. Several of these patients had serious comorbidities other than SARDs but were also on treatments associated with substantially blunted immune response to vaccine and infection (eg, B cell depletion),[12 28–30] so it is difficult to ascribe the contribution of underlying SARD and immunosuppression to these individual cases. These findings highlight the need for ongoing risk mitigating strategies for many patients with SARD on such treatments as well as those with other comorbidities that may be related to their SARD (eg, interstitial lung disease) or its treatment (eg, cardiovascular comorbidities). In addition to shielding practices such as masking, social distancing, and avoiding indoor congregation, the recent introduction of pre-exposure prophylaxis with tixagevimab/cilgavimab, a monoclonal antibody against SARS-CoV-2, represents an important strategy for protecting our highest risk patients. However, tixagevimab/cilgavimab was studied in high risk, unvaccinated patients, the vast majority of whom did not have SARDs or similar conditions.[31] Real-world effectiveness studies of tixagevimab/cilgavimab are now being reported and will be informative for guiding ongoing risk mitigating strategies for patients with SARDs.[31]

In addition to temporal improvements in the outcomes of COVID-19 among patients with SARDs during the ongoing pandemic, we also found other notable trends. First, there were shifts in the demographics of patients with COVID-19 during the course of the pandemic. For instance, there was a decrease in the proportion of patients with SARD with COVID-19 who identify as black or Hispanic and a decrease in the age of patients during the study period. These shifts are likely multifactorial, reflecting depletion of susceptible patients, changes in access to diagnostics and treatments, rates of vaccination and other factors. Second, a large portion of infections are now diagnosed at home using rapid antigen tests. This shift in diagnostics will make it increasingly difficult to capture more mild infections for the purpose of epidemiological studies like this one in both the general population and among SARDs. Leveraging electronic health record data, as we did here, will be an important way to capture and include patients like these in future studies.

Strengths of our study include the systematic identification of patients with SARDs in a large healthcare system that includes both tertiary care hospitals as well as community hospitals and their affiliated outpatient clinics. In contrast to studies relying only on administrative claims data, we were also able to capture COVID-19 diagnoses made using home rapid antigen testing because of the way these are captured in our electronic health record when reported to providers in our healthcare system. Additionally, we were able to confirm the SARD diagnosis and treatment, vaccination status at the time of infection, and details surrounding deaths and other outcomes with manual chart review.

Despite these strengths, our study has certain limitations. First, although we found temporal improvements in the outcomes of COVID-19 among patients with SARDs and an association between vaccination status and less severe disease, we cannot establish the causal effects of vaccinations, other risk mitigating strategies and COVID-19 treatments on these improvements. Second, this study was conducted in a single healthcare system in Massachusetts, USA. Our findings may not be generalisable to other areas of the USA or world because of differences in demographics as well as access to testing and treatment. Third, although we systematically identified cases in our healthcare system, patients who received COVID-19 diagnoses outside of our system may not have been captured, severe events occurring after diagnosis may have been missed, and patients with asymptomatic disease or mild courses may have been less likely to have testing or report their positive test. Thus, we were limited in our ability to calculate incidence and the true proportion of patients experiencing severe outcomes may be lower than we report with a larger denominator. However, those with SARDs, especially those on immunosuppression, are likely to contact their clinicians in the context of a COVID-19 infection to receive guidance regarding the management of their SARD treatments and to obtain treatments.

In conclusion, there have been substantial improvements in the outcomes associated with COVID-19 among patients with SARDs since the pandemic began in March 2020. In particular, the initial Omicron wave was associated with the largest number of cases but the lowest risk of severe disease. Despite these findings, some patients with SARDs continue to experience severe disease, especially those on immunosuppressives known to blunt the response to vaccine and infection, as well as those with other serious comorbidities. Additional studies are needed to refine risk mitigating strategies for patients at highest risk for severe outcomes.

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XW was involved in the analysis and interpretation of the data. CC, KV, EK, EPB, GQ, MD, TY-TH, MEW and DT were involved in the data acquisition, interpretation, and revision of the manuscript. JAS and ZSW are joint senior authors. All authors approved the final version of the article. JAS accepts full responsibility for the work and the conduct of the study, had access to the data, and controlled the decision to publish.

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

Patient consent for publication Not applicable.

Ethics approval This study was approved by the Mass General Brigham Institutional Review Board (2020P000833).

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Data availability statement Data are available on reasonable request. This study includes patient data from Mass General Brigham. The data that support the findings of this study may be made available upon reasonable request by contacting the corresponding author, JAS.

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CLINICAL SCIENCE

Immunogenicity and safety of a fourth COVID-19 vaccination in rituximab-treated patients: an open-label extension study

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ABSTRACT

Objectives Patients under rituximab therapy are at high risk for a severe COVID-19 disease course. Humoral immune responses to SARS-CoV-2 vaccination are vastly diminished in B-cell-depleted patients, even after a third vaccine dose. However, it remains unclear whether these patients benefit from a fourth vaccination and whether continued rituximab therapy affects antibody development.

Methods In this open-label extension trial, 37 rituximab-treated patients who received a third dose with either a vector or mRNA-based vaccine were vaccinated a fourth time with an mRNA-based vaccine (mRNA-1273 or BNT162b2). Key endpoints included the humoral and cellular immune response as well as safety after a fourth vaccination.

Results The number of patients who seroconverted increased from 12/36 (33%) to 21/36 (58%) following the fourth COVID-19 vaccination. In patients with detectable antibodies to the spike protein's receptor-binding domain (median: 8.0 binding antibody units (BAU)/mL (quartiles: 0.4; 13.8)), elevated levels were observed after the fourth vaccination (134.0 BAU/mL (quartiles: 25.5; 1026.0)). Seroconversion and antibody increase were strongly diminished in patients who received rituximab treatment between the third and the fourth vaccination. The cellular immune response declined 12 weeks after the third vaccination, but could only be slightly enhanced by a fourth vaccination. No unexpected safety signals were detected, one serious adverse event not related to vaccination occurred.

Conclusions A fourth vaccine dose is immunogenic in a fraction of rituximab-treated patients. Continuation of rituximab treatment reduced humoral immune response, suggesting that rituximab affects a second booster vaccination. It might therefore be considered to postpone rituximab treatment in clinically stable patients.

Trial registration number 2021-002348-57.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ B-cell-depleting therapy with rituximab can lead to severe disease courses after SARS-CoV-2 infection.
⇒ Humoral immune response after COVID-19 vaccination is severely impaired in rituximab-treated patients.
⇒ Third vaccination leads to an increased seroconversion rate in patients who did not respond to primary vaccination.

WHAT THIS STUDY ADDS

⇒ Fourth vaccination is immunogenic in the majority of rituximab-treated patients.
⇒ No unexpected safety signals could be detected on a fourth vaccination.
⇒ Continuation of rituximab treatment before booster vaccination severely impairs the humoral immune response.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Rituximab-treated patients should receive an additional booster vaccination.
⇒ In clinically stable patients, rituximab treatment should be evaluated and if possible postponed.

INTRODUCTION

COVID-19 vaccination is a critical component in the management of the COVID-19 pandemic. Despite the recent variants of concern (VOC) Omicron and Delta, the currently available vaccines are still effective in preventing severe disease courses and death, although at a reduced level compared with preceding variants.[1 2] Most importantly, due to mutations in the spike protein, VOC exhibit a higher degree of vaccine evasion, resulting in higher repertoire of antibodies required for effective virus neutralisation.[3–6] However, booster vaccinations improve protection against Delta and Omicron variants.[7 8] Patients under immunosuppressive therapy with rituximab, a B-cell-depleting antibody against the CD20 surface antigen, have impaired humoral responses after primary vaccination, depending on the number of detectable peripheral B- cells.[9–13] A booster dose given to these patients improved humoral responses, nonetheless overall seroconversion rate and antibody titers were significantly

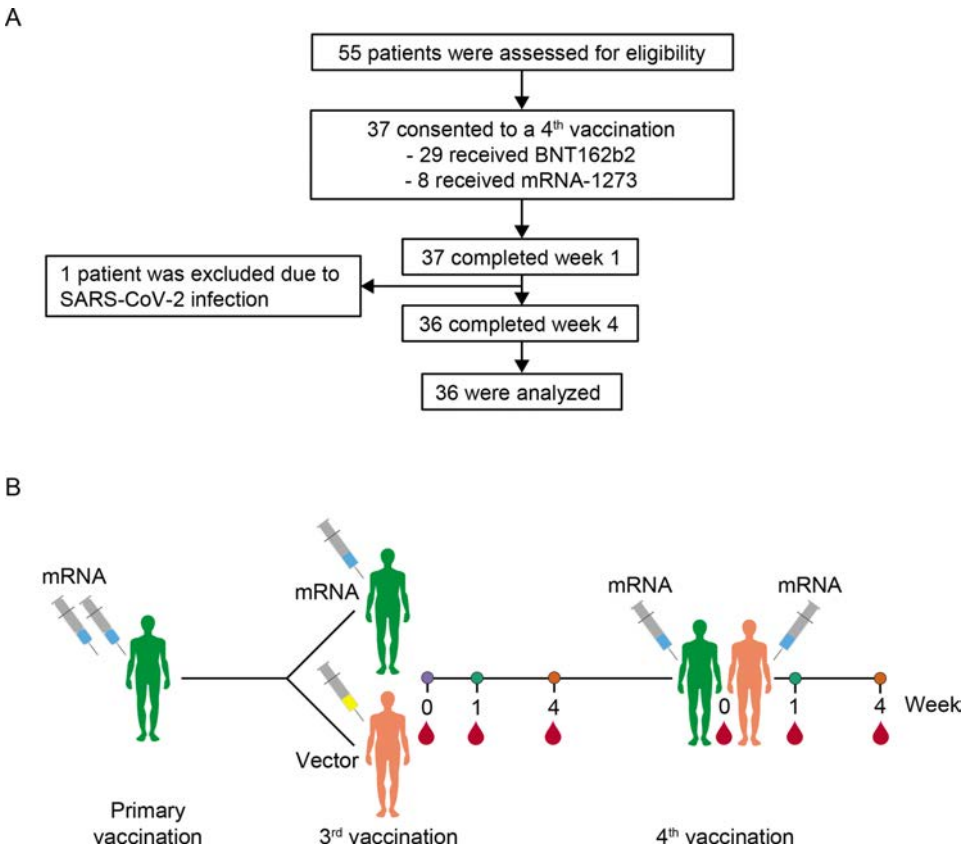

Figure 1 Study flow diagram. (A) Indicating screening, randomisation and follow-up of patients and (B) summary of the trial design.

lower than in healthy individuals.[14–19] Additionally, therapy with rituximab itself is associated with worse COVID-19 outcomes, such as the requirement of invasive mechanical ventilation or mortality.[20] Therefore, improving the level of protection against COVID-19 in this patient population is of utmost importance. The American College of Rheumatology guidelines suggest discussing optimal timing of dosing and vaccination prior to rituximab treatment.[21] The EULAR recommends that rituximab or any other B-cell-depleting therapy should be scheduled in a way to optimise vaccine immunogenicity.[22] However, due to a lack of high-level evidence, no specific recommendations are given. Currently, no data are available for the immunogenicity or safety of a fourth vaccination or how the continuation of rituximab therapy affects vaccine responses. We, therefore, investigated the immunogenicity and safety of a fourth vaccine dose in rituximab-treated patients and analysed the effect of continued rituximab treatment on vaccine immunogenicity.

METHODS

Trial design and participants

In this prospective open-label extension study, rituximab-treated patients received a fourth dose (second booster) with an mRNA-based vaccine. In the main study, patients who did not seroconvert after primary vaccination with an mRNA-based vaccine had received their third vaccination with either an mRNA (BNT162b2, Pfizer–BioNTech or mRNA-1273, Moderna) or a vector-based vaccine (ChAdOx1 nCoV-19, Oxford-AstraZeneca).[14] In the current trial, an mRNA-based vaccine was used as the fourth vaccination, in accordance with their primary vaccination (figure 1A). The most important exclusion criteria were previous COVID-19 infection and known allergies to vaccine components. Medical history regarding SARS-CoV-2 infections was verified before enrolment. Details can be found in the supplementary study protocol. The trial was registered on Eudra-CT (Number 2021-002348-57).

Interventions

Patients included in the main trial[14] were invited to a fourth vaccination 12 weeks after the third dose. At screening, concomitant medications, demographics and hypersensitivity reactions to previous SARS-CoV-2 vaccines were recorded. The vaccination was applied at the baseline visit. Immunogenicity and safety were assessed at week 1 and week 4 after vaccination. Serum samples obtained during screening visit, as well as visits 3 and 4 were stored below −70°C at the Biobank of the Medical University of Vienna, a centralised facility for the preparation and storage of biomaterial with certified quality management (certified according to International Organization for Standardization (ISO) 9001:2015).[23] Peripheral blood mononuclear cells (PBMCs) were isolated at screening and visit three by density gradient centrifugation and stored in the vapour phase of liquid nitrogen.

The vaccination compound was open label and selected according to the primary vaccination series. Vaccination with mRNA-1273 was carried out using the full dose (100 µg).

Assessment

Study outcomes included seroconversion rates, SARS-CoV-2 antibody levels at week 4 (overall and stratified for patients with different numbers of peripheral B-cells) and cellular immune responses at week 1. T-lymphocyte restimulation potential to SARS-CoV-2 antigens was assessed before and 1 week after the fourth vaccination. Laboratory assessors were blinded to patient

characteristics. Safety is presented as solicited adverse events over the first 7 days as reported by the patients using a paper-based diary. Adverse events and changes in the immunosuppressive treatment were assessed over a period of 28 days. Antibodies against platelet factor 4 (PF4) were routinely assessed at week 1 and 4 after fourth vaccination.

Assessment of CD19$^+$ peripheral B-cells

Flow cytometry (FACSCanto II, Becton Dickinson, San Jose, California, USA) was used to determine immunological phenotypes of lymphocyte subsets. Hereby, the whole blood staining was done previous to lysis (Becton Dickinson). A combination of the following monoclonal antibodies (all provided by Becton Dickinson) was applied: fluorescein isothiocyanate-labelled anti-CD3, phycoerythrin (PE)-labelled anti-CD16$^+$56$^+$, peridinin–chlorophyll–protein–cy5.5-labelled anti-CD4, PE–Cy7-labelled anti-CD19, allophycocyanin (APC)–Cy7-labelled anti-CD8, V450-labelled anti-human leucocyte antigen–DR, V500-labelled anti-CD45 and APC-labelled anti-CD14. CD19$^+$ B-cells are expressed as percentage among total lymphocytes.

Anti-SARS-CoV-2 antibody testing

Quantitative assessment of antibodies to the receptor-binding domain (RBD) of the viral spike (S) protein was performed by an Elecsys Anti-SARS-CoV-2 S immunoassay.[24 25] The detection range is between 0.4 and 2500.0 binding antibody units (BAU)/mL. A concentration greater than 0.8 BAU/mL was considered positive. Analysis was performed on a Cobas e801 (Roche Diagnostics, Rotkreuz, Switzerland) at the Department of Laboratory Medicine, Medical University of Vienna (certified acc. to ISO 9001:2015 and accredited acc. to ISO 15189:2012).

T-cell responses

For T-cell stimulation (see below), PepMix SARS-CoV-2 peptide pools were acquired from JPT (Berlin, Germany). The S peptides are split into two subpools S1 (aa 1-643) and S2 (aa 633-1273). Peptides were dissolved in dimethyl sulfoxide and diluted in AIM-V medium for use in enzyme-linked immunosorbent spot (ELISpot) assays as described previously.[14]

For ex vivo T-cell IFN-γ ELISpot assay, PBMCs from patients before and after the fourth vaccination were thawed and processed on the same day. A total of 1–2×10^5 cells per well were incubated with SARS-CoV-2 peptides (2 µg/mL; duplicates), AIM-V medium (negative control; 3–4 wells) or phytohemagglutinin (L4144, Sigma; 0.5 µg/mL; positive control) in 96-well plates coated with 1.5 µg anti-IFN-γ (1-D1K, Mabtech) for 24 hours. After washing, spots were developed with 0.1 µg biotin-conjugated anti-IFN-γ (7-B6-1, Mabtech), streptavidin-coupled alkaline phosphatase (Mabtech, 1:1000) and 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Sigma). Spots were counted using a Bio-Sys Bioreader 5000 Pro-S/BR177 and Bioreader software generation V.10. Data were calculated as spot-forming cells (SFCs) per 10^6 PBMCs after subtracting the spots from the negative control (mean spot numbers from three to four unstimulated wells).

Statistical analysis

All subjects vaccinated with a fourth dose who completed week four were included in the immunogenicity analysis. Seroconversion rates and increase in antibody concentrations were analysed and displayed in graphical form. Antibody levels were also examined towards peripheral B-cell status and whether rituximab therapy was continued between third and fourth dose. Cellular immunity is shown over three timepoints and in respect to the third dose applied. Given the fixed sample size, no formal sample size calculation was conducted and therefore trial outcomes and safety data are presented descriptively only. 'R' V.4.0.3 (R Development Core Team. Vienna, Austria) was used for the entire analysis. Following packages were used: 'ggplot2' and 'ggbeeswarm' for creating plots as well as 'tableone' to create baseline tables.

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

RESULTS

Patient characteristics

Overall, 55 patients who completed the main study were screened, of whom 37 patients consented to participate in the extension study to receive a fourth vaccine dose. Twenty nine were vaccinated with BNT162b2 and 8 with mRNA-1273, according to their primary vaccination, except for one patient who was switched to BNT162b2. Among these patients, 50% had received the ChAdOx1 nCoV-19 vaccine and 50% an mRNA vaccine as a booster. One patient experienced a SARS-CoV-2 infection and was therefore excluded from the immunogenicity analysis. Overall, 36 patients subsequently presented at follow-up visits and completed the trial 4 weeks after vaccination (figure 1). Patients continued their immunosuppressive therapy including rituximab following the EULAR guidelines at the treating physician's discretion.[22] Patient characteristics of all analysed patients are presented in table 1.

Humoral immune response

At screening, 12/36 patients (33%) had detectable anti-RBD antibodies; thus, the frequency of patients who seroconverted increased to 21/36 (58%) at week 4 after the fourth vaccination. Detectable anti-RBD antibodies were maintained between third and fourth vaccination. Accordingly, 9/24 (38%) of the patients who initially did not seroconvert after three vaccinations developed anti-RBD antibodies on receiving a fourth vaccination (figure 2A). Levels of antibodies were higher after an additional booster vaccination, increasing from median 0.4 BAU/mL (quartiles: 0.4; 8.1) at screening to 12.4 BAU/mL (quartiles: 0.4; 197.3) at week four in the total study population (figure 2B). In patients with detectable antibodies before vaccination (n=12), antibody levels increased from median 11.6 BAU/mL (quartiles: 8.1; 25.5) to 344.5 BAU/mL (quartiles: 119.0; 1387.8). Patients with no detectable antibodies at baseline, but who seroconverted on a fourth dose (n=9), had a median antibody concentration of 43.8 BAU/mL (quartiles: 22.8; 163.0) 4 weeks after the fourth dose, indicating further immunogenicity of a fourth vaccination. Anti-RBD antibody levels were lower in patients with CD19$^+$ peripheral B-cells<1% (n=26) than in those with B-cells≥1% (n=10). Furthermore, all patients with<1% peripheral B-cells and detectable anti-RBD antibodies at week four (n=11) already had detectable antibodies before the fourth dose, except for two. All patients with≥1% detectable peripheral B-cells (n=10) had antibodies at week 4, irrespective of their AB levels at screening (figure 2C), supporting the relevance of detectable peripheral B-cells for antibody production.

Overall, 15/36 (42%) of the patients received rituximab treatment between the third and the fourth vaccination. Patients who did not seroconvert on three vaccinations and continued rituximab treatment (n=9) did not develop anti-RBD antibodies

Table 1 Baseline patients characteristics.

n	36
Age, years	62.1 (14.0)
Sex: female	25 (69.4%)
Diagnosis	
Rheumatoid arthritis	14 (38.9%)
Connective tissue disease	12 (33.3%)
IgG4-related disease	1 (2.8%)
Multiple sclerosis	2 (5.6%)
Vasculitis	7 (19.4%)
Patients with detectable B-cells	14 (38.9%)
Months between last RTX and fourth dose	7.4 (5.8)
Concomitant medication	
Any csDMARD	18 (50.0)
Mycophenolate mofetil	4 (11.1)
Leflunomide	3 (8.3)
Hydroxychloroquine	1 (2.8)
Methotrexate	6 (16.7)
Azathioprine	5 (13.9)
Immunoglobulin therapy	3 (8.3)
Prednisone	10 (27.8)
Third vaccine dose	
ChAdOx1 nCoV-19	18 (50.0%)
BNT162b2	13 (36.1%)
mRNA-1273	5 (13.9%)
Patients with SARS-CoV-2-S AB at screening	12 (33.3%)
Level of SARS-CoV-2 -S AB at screening	0.4(0.4, 8.1)

Data are presented as n (%), mean±SD or median (quartiles), RTX, csDMARDs defined here as concomitant treatment with at least one of the following: methotrexate, mycophenolate mofetil, azathioprine, leflunomide, hydroxychloroquine—one patient had a combination of two csDMARDs, SARS-CoV-2-S AB: SARS-CoV-2 spike antibody.
csDMARD, conventional synthetic disease-modifying antirheumatic drug; RTX, rituximab.

(figure 3A). Patients with detectable antibody levels after three vaccinations who continued rituximab treatment (n=8) had lower antibody levels than those patients who postponed rituximab treatment (n=4) (median 173 BAU/mL (quartiles: 64; 300) vs 1880 BAU/mL (quartiles: 1311; 2449), respectively) (figure 3B), suggesting significant effects of rituximab treatment on antibody production in patients who received a fourth vaccination.

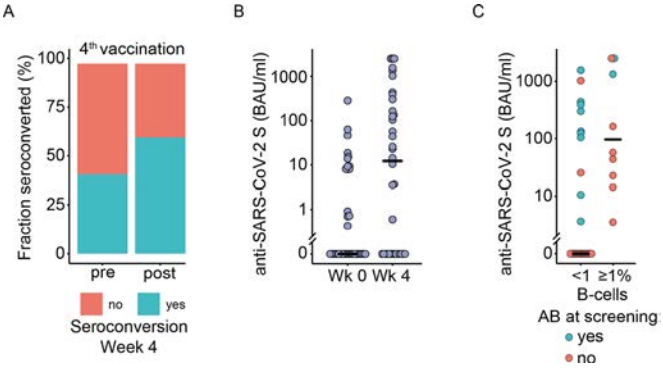

Figure 2 Humoral immune response to fourth COVID-19 vaccination in rituximab-treated patients. Antibodies to the receptor-binding domain (RBD) of the viral spike (S) protein were determined using an anti-SARS-CoV-2 immunoassay. (A) Fraction of seroconverted patients based on the presence of detectable anti-RBD antibodies (B) Anti-RBD antibody levels in patients at screening (n=36) and at week 4. (C) Anti-RBD antibodies grouped in patients according to the percentage of CD19+ peripheral B-cells. Median is shown, colour indicating detectable antibodies before a fourth dose. Wk: week.

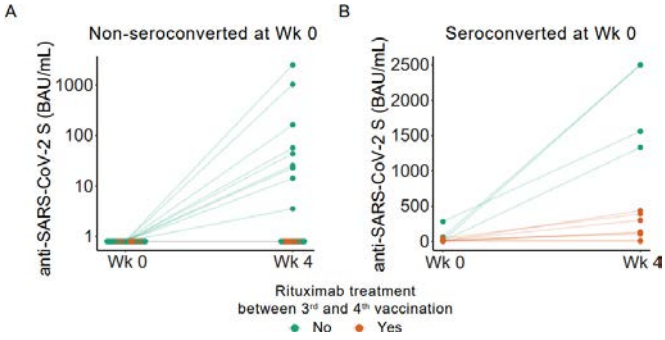

Figure 3 Humoral immune response in patients based on the time of last rituximab treatment. Antibodies to the receptor-binding domain of the viral spike (S) protein were determined using an anti-SARS-CoV-2 immunoassay in patients who (A) did not seroconvert on three vaccinations (n=24) and (B) seroconverted patients (n=12). Colours indicate whether rituximab was applied between third and fourth vaccination. Log scale was used in (A). Wk: week.

Cellular immune response

SARS-CoV-2-specific T-cell responses have been analysed over a period of 12 weeks before the fourth vaccination. The effect of a fourth vaccination was evaluated at week 1 (figure 4A). Overall, a decrease of the cellular immune response between week 1 (median 388 per 10^6 SFC (quartiles: 45; 861)) and week 12 (median 38 per 10^6 SFC (quartiles: 11; 110)) after third vaccination was observed. A fourth dose led to an only modest increase (median 56 per 10^6 SFC (quartiles: 10; 533)) (figure 4B). However, when analysing patients who received a

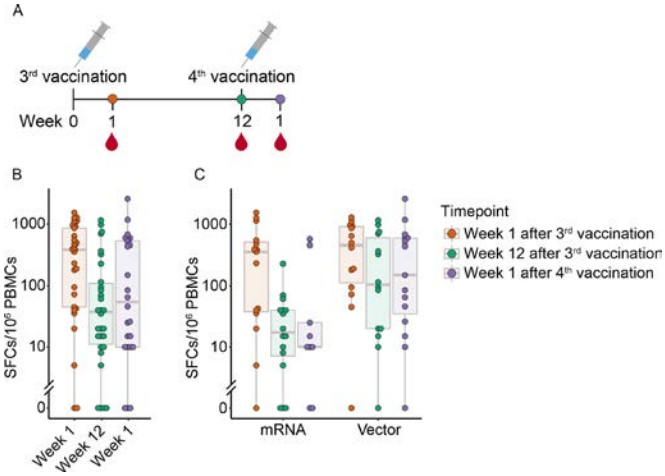

Figure 4 SARS-CoV-2-specific T-cell responses. (A) SARS-CoV-2-specific T-cell responses were determined by ELISpot assay from peripheral blood mononuclear cells (PBMCs) stimulated with spike subunit S1 and S2 peptide pools one and 12 weeks after the third vaccination as well as 1 week after the fourth vaccination. (B) average of SFCs/10^6 PBMCs from S1 and S2 peptide pools are shown for three time points. Y-axis indicates the number of spot forming cells (SFCs) per 10^6 PBMCs. data show the sum of average SFCs/10^6 PBMCs from S1 and S2 peptide pools. (C) composite ELISpot results divided into patients who received a third vaccination with either an mRNA or vector-based vaccine. The median and IQR are shown.

third vaccination with an mRNA or vector-based vaccine separately, we could observe a faster decline of SARS-CoV-2-specific cells in patients with a homologous mRNA-based vaccination regime (n=18) as compared with patients who received a vector-based regime as a third vaccination, suggesting that heterologous vaccination induces a more stable cellular immune response (figure 4C). However, this did not affect cellular immune responses after the fourth vaccination.

Reactogenicity

Adverse events were monitored using a paper-based patient diary throughout the first 7 days after vaccination and by an interview-based assessment at week 4. Prevalence of systemic reactogenicity was comparable between BNT162b2 and mRNA-1273 vaccinated patients, except for arthralgia and headache; arthralgia was reported by 4/7 (57%) as compared with 11/29 (38%) of patients vaccinated with mRNA-1273 and BNT162b2, respectively. Nausea only occurred in BNT162b2-vaccinated patients (5/29, 17%). Headache was prevalent in 5/7 (71%) of mRNA-1273, compared with 13/28 (45%) BNT162b2 vaccinated patients. Local pain and local pruritus were more often reported with mRNA-1273 than with BNT162b2 (6/7, 86% vs 14/29, 48% and 2/5, 40% vs 2/29 7%, respectively) (online supplemental figure 1). No thrombocytopenia or antibodies against PF4 were observed after an additional booster vaccination. One serious adverse event, hospitalisation due to lower back pain unrelated to vaccination, occurred during follow-up. No disease flares requiring a change of immunomodulating therapy were reported during the trial. None of the patients experienced an anaphylactoid reaction or neurological complication. One patient developed COVID-19 after the fourth vaccination (four doses of BNT162b2, no humoral vaccine response at the time of infection). The patient received sotrovimab as part of our routine clinical care after testing positive for SARS-CoV-2 and only suffered from mild COVID-19 associated symptoms. No hospitalisation was required.

DISCUSSION

In this open-label extension trial, we found an increase in seroconversion rates as well as in antibody levels after a fourth vaccination in rituximab-treated patients who mounted no or low antibody titers after their third vaccination. However, the antibody response was vastly diminished depending on the numbers of peripheral B-cells and the timing of the rituximab treatment in relation to the fourth vaccination. A modestly enhanced cellular immune response was observed.

Preliminary results of a fourth vaccination in healthy individuals from Israel indicate that a fourth dose of an mRNA dose restores antibody titers.[26] Patients under rituximab therapy have markedly reduced vaccine seroconversion rates and antibody concentration, mainly depending on the number of peripheral B-cells,[9 27] which is also consistent with results after the third vaccination.[12 14 16] Data on the fourth vaccination in immunosuppressed patients are sparse. Increased seroconversion rates and elevated antibody titre in kidney transplant patients with a possible improvement of the vaccination response after a temporary hold of immunosuppressive therapy were observed after four vaccinations.[28–30] In a case series of 18 patients with autoimmune diseases, 2 patients under mycophenolate mofetil therapy remained without humoral immune response after four vaccine doses.[31] In the present extension trial, we could reduce the rate of vaccine non-responders from almost 7 of 10 to about 4 of 10 rituximab-treated patients. Low antibody levels were observed in patients who received rituximab treatment between the third and fourth vaccination, suggesting that rituximab hampers seroconversion rates and exerts an adverse effect on the ability to booster SARS-CoV-2-specific humoral immune responses in these patients. This will be especially important for the use of possible variant vaccines to boost responses in the future, although first results of Omicron-specific vaccine doses report little advantage as compared with standard vaccination in animal models.[32 33] We have previously reported that cellular immune response can be mounted in B-cell-depleted patients.[9 10] Consecutive analysis revealed a drop in the cellular immune response 12 weeks after the third vaccination. We observed only moderate effects of a fourth vaccination on the cellular immune response. Subgroup analysis revealed a higher stability of a cellular immune response in those who received a heterologous vaccination in line with previously published data.[9 10] In the current trial, patients with different rheumatic diseases as well as two patients with multiple sclerosis (MS) have been included. Although a sufficient humoral immune response to COVID-19 vaccination has been reported in patients with MS,[34] we cannot exclude disease-specific effects. A fourth dose of the mRNA vaccine has shown a favourable safety profile. Reactogenicity of the vaccine dose over 7 days was in the expected range of our previous trial. Although no serious adverse events were observed in any group, reactogenicity was more pronounced in patients who received a fourth vaccination with mRNA-1273 as compared with BNT162b2, which is in line with previous trials in healthy individuals on a third vaccination.[35]

The major limitation of our study is the relatively small number of patients vaccinated; thus, further investigations are required to confirm our results. Furthermore, it still needs to be determined how antibodies (or their absence) are linked to protection against symptomatic infection with SARS-CoV-2 in these patients, especially with respect to novel VOCs. Recent data have shown, that memory T-cells with cross-reactive potential can exert protection against SARS-CoV-2 by rapid expansion.[36]

Comedication with conventional synthetic DMARDs and corticosteroids can lead to a reduced immunogenicity after COVID-19 vaccination.[37–39] Larger patient cohorts would be needed to decipher different effects of DMARD cotherapy in addition to rituximab. Our data show that a fourth vaccination dose in this high-risk population of patients is safe and can also increase seroconversion rates and antibody levels. Most importantly, as rituximab also seems to hamper the ability to boost previously seroconverted patients, the continuation of rituximab treatment should be carefully considered even in patients with a detectable vaccine response. Non-responders should be evaluated for therapy with monoclonal antibodies as prophylaxis or post exposure to improve COVID-19 outcome in this high-risk group of patients.

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Contributors All authors contributed to manuscript preparation. MB, DA, DS, DM, and SW contributed to the study design. DM, HR, LXA, ES and DS contributed to data analysis. TP, HH and KS performed antibody measurements. JHH, MM and PH contributed to cellular assays, MB, DA, JSS, DM and LXH contributed to the primary manuscript draft. ES, DM, MB, PM, AK, FK, TH and TD contributed to patient recruitment, RT determined leucocyte subsets. MB acts as guarantor and accepts full resposibility for the work.

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Competing interests PM reports speaker fees from AbbVie, Janssen and Novartis and research grants from AbbVie, BMS, Novartis, Janssen, MSD and UCB. MB reports about personal fees from Eli-Lilly, DA received grants and consulting fees from AbbVie, Amgen, Lilly, Merck, Novartis, Pfizer, Roche and Sandoz. JSS reports about grants, consulting and personal fees from AbbVie, Astra-Zeneca, Lilly, Novartis, Amgen, Astro, Bristol-Myers Squibb, Celgene, Celltrion, Chugai, Gilead, ILTOO, Janssen, Merck Sharp & Dohme, Novartis-Sandoz, Pfizer, Roche, Samsung and UCB. DM received support for meeting attendances from Pfizer. HH received grants from Glock Health, BlueSky Immunotherapies and Neutrolis. AK reports about speaker and consulting fees from AbbVie, Amgen, Bristol Myers Squibb, Eli Lilly, Gilead, Janssen, Merck Sharp and Dohme, Novartis and Pfizer. All other authors declare no competing interests.

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

Patient consent for publication Not applicable.

Ethical approval The trial was performed in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The trial was approved by the University of Vienna ethics committee in September 2021 (EK#: 1481/2021). All patients provided their written informed consent. All trial visits were conducted in a single centre (Vienna General Hospital).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Anonymous patient data are available under specific conditions. Proposals will be reviewed and approved by the sponsor, scientific committee and staff on the basis of scientific merit and absence of competing interests. Once the proposal has been approved, data can be transferred through a secure online platform after the signing of a data access agreement and a confidentiality agreement.

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EPIDEMIOLOGICAL SCIENCE

Breakthrough infections with the SARS-CoV-2 omicron (B.1.1.529) variant in patients with immune-mediated inflammatory diseases

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ABSTRACT

Objectives To compare the cumulative incidence and disease severity of reported SARS-CoV-2 omicron breakthrough infections between patients with immune-mediated inflammatory diseases (IMID) on immunosuppressants and controls, and to investigate determinants for breakthrough infections.

Methods Data were used from an ongoing national prospective multicentre cohort study on SARS-CoV-2 vaccination responses in patients with IMID in the Netherlands (Target-to-B! (T2B!) study). Patients wih IMID on immunosuppressants and controls (patients with IMID not on immunosuppressants and healthy controls) who completed primary immunisation were included. The observation period was between 1 January 2022 and 1 April 2022, during which the SARS-CoV-2 omicron (BA.1 and BA.2 subvariant) was dominant. A SARS-CoV-2 breakthrough infection was defined as a reported positive PCR and/or antigen test at least 14 days after primary immunisation. A multivariate logistic regression model was used to investigate determinants.

Results 1593 patients with IMID on immunosuppressants and 579 controls were included. The cumulative incidence of breakthrough infections was 472/1593 (29.6%; 95% CI 27% to 32%) in patients with IMID on immunosuppressants and 181/579 (31.3%; 95% CI 28% to 35%) in controls (p=0.42). Three (0.5%) participants had severe disease. Seroconversion after primary immunisation (relative risk, RR 0.71; 95% CI 0.52 to 0.96), additional vaccinations (RR 0.61; 95% CI 0.49 to 0.76) and a prior SARS-CoV-2 infection (RR 0.60; 95% CI 0.48 to 0.75) were associated with decreased risk of breakthrough infection.

Conclusions The cumulative incidence of reported SARS-CoV-2 omicron breakthrough infections was high, but similar between patients with IMID on immunosuppressants and controls, and disease severity was mostly mild. Additional vaccinations and prior SARS-CoV-2 infections may reduce the incidence of breakthrough infections.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Some immunosuppressants used in patients with immune-mediated inflammatory diseases (IMIDs) impair humoral or cellular immune responses after SARS-CoV-2 vaccination.
⇒ These patients may, therefore, be at increased risk of (severe) SARS-CoV-2 breakthrough infections.

INTRODUCTION

The emergence of the SARS-CoV-2 variant omicron has led to an unprecedented number of SARS-CoV-2 cases worldwide. Multiple mutations in the receptor binding domain (RBD) of the spike (S) protein of this variant increased transmissibility and infectivity, and reduced effectiveness of

WHAT THIS STUDY ADDS

⇒ SARS-CoV-2 omicron breakthrough infections in patients with IMID on immunosuppressants are frequent but mostly mild and incidence and severity is similar to controls.

⇒ Humoral responses after primary immunisation, additional vaccinations and hybrid immunity, resulting from prior SARS-CoV-2 infections, were associated with a lower risk of SARS-CoV-2 omicron breakthrough omicron infections in both patients with IMID on immunosuppressants and controls.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings suggest that additional vaccinations and development of hybrid immunity both contribute in reducing the risk of SARS-CoV-2 omicron breakthrough infections in patients with IMID, even despite the use of immunosuppressants. Severe SARS-CoV-2 breakthrough infections are rare for the omicron variant.

⇒ In case of new SARS-CoV-2 infection waves, it can be speculated that offering additional and/or updated vaccinations is an effective strategy to reduce risks, also for patients with IMID.

standard SARS-CoV-2 vaccination regimens.[1–3] In the general population, disease severity after infection with the SARS-CoV-2 omicron variant were shown to be generally mild and less severe compared with the delta variant.[4–7] Booster vaccinations help to protect against symptomatic infection by increasing SARS-CoV-2 omicron neutralising antibodies and by broadening the antibody repertoire.[8–12] However, in patients with immune-mediated inflammatory diseases (IMIDs) treated with specific immunosuppressants, cellular and humoral efficacy of (booster) vaccinations may be impaired.[13–17] Therefore, these patients may be at increased risk for more severe SARS-CoV-2 breakthrough infections. We previously reported that there was no difference in incidence of SARS-CoV-2 delta variant breakthrough infections and disease severity between patients with IMID on immunosuppressants compared with controls, with the exception of anti-CD20 treatment in patients with additional risk factors (ie, older age and comorbidities).[6] The primary objective of this study is to compare cumulative incidence and disease severity of reported SARS-CoV-2 omicron breakthrough infections between patients with IMID on immunosuppressants, and controls (patients with IMID not on immunosuppressants and healthy controls). The secondary objective is to explore determinants associated with the risk of SARS-CoV-2 omicron breakthrough infections, including use of immunosuppressants, humoral responses after primary immunisation, administration of additional vaccines and prior SARS-CoV-2 infections.

METHODS

Study design

This is a study on SARS-CoV-2 omicron breakthrough infections from an ongoing prospective multiple-arm multicentre cohort study, the T2B! study (Trial ID NL8900; Dutch Trial Register). The primary objective of the T2B! study was to assess humoral and cellular immune responses after SARS-CoV-2 vaccination in patients with various IMIDs treated with predefined types of immunosuppressants. Monitoring SARS-CoV-2 breakthrough infections is a predefined secondary outcome in the study. Full study protocol, data on patient characteristics, humoral and cellular responses and SARS-CoV-2 infections other than omicron has been published elsewhere.[6 15–18]

Participants

Patients with IMID on immunosuppressants during primary immunisation and a combined control group of patients with IMID without systemic immunosuppressants and healthy controls who had been included as part of the overall study between 2 February 2021 and 1 October 2021 were included. Participants were included if primary immunisation with either with two doses of BNT162b2 (Pfizer/BioNtech), CX-024414 (Moderna) or ChAdOx1 nCoV-19 (AstraZeneca), or one dose of Ad.26.COV2.S (Janssen/Johnson & Johnson) was completed. Participants with a SARS-CoV-2 infection prior to or within 90 days after first vaccination who had received only one dose of any of the above vaccines were also included. See online supplemental methods for the full inclusion and exclusion criteria.

Vaccination campaign Netherlands

See online supplemental methods for information about the vaccination campaign in the Netherlands. In short, in September 2021 an additional ('third') vaccination was offered to several vulnerable groups, including patients with IMID treated with 'strongly antibody-impairing immunosuppressants' (see below) and from December 2021 onwards additional ('booster') vaccinations were offered to all individuals in the Netherlands.

Procedures

Electronic questionnaires were sent to participants every 2 months after first vaccination. An extra questionnaire was sent on 13 April 2022 to those who had not completed follow-up questionnaires. Demographics and data on SARS-CoV-2 (breakthrough) infections were retrieved from these questionnaires. Medical files were used to register IMID and start, and stop dates of all immunosuppressants. Testing for a SARS-CoV-2 infection was participant driven and performed independently of this study. When a participant indicated a positive PCR or antigen test they were contacted by a researcher at least 2 weeks after the positive test to verify and determine disease severity. If hospital admission was reported, clinical discharge letters were retrieved to assess disease severity.

From the ongoing T2B! cohort study, serum samples collected at baseline (before vaccination) and at 28 days after first and second vaccination (when applicable). Anti-RBD and anti-NP antibodies were measured at Sanquin as described before (see online supplemental methods).

Outcomes

The primary outcome was the cumulative incidence of reported breakthrough infections with the SARS-CoV-2 omicron variant in patients with IMID on immunosuppressants and controls. Patients with IMID not on immunosuppressants and healthy controls were combined in one control group because we did not observe differences between these groups in humoral responses after SARS-CoV-2 vaccination nor in the incidence of the delta variant breakthrough infections.[6 17] A SARS-CoV-2 omicron breakthrough infection was defined as a reported PCR or antigen confirmed infection at least 14 days after primary immunisation occurring between 1 January 2022 and 1 April 2022 when the SARS-CoV-2 omicron variant (BA.1 and BA.2 subvariant) was dominant in the Netherlands.[19]

Disease severity and determinants for breakthrough infections were secondary outcomes. Disease severity was based on

the WHO classification and was defined as either asymptomatic (WHO 1), mild symptomatic (WHO 2–3), hospitalised moderate disease (WHO 4–5), hospitalised severe disease (WHO 6–9) or dead (WHO 10).[20] Definitions of immunosuppressants as monotherapy or as part of combination therapy and definition of active treatment are described in online supplemental methods. A SARS-CoV-2 infection prior to SARS-CoV-2 omicron breakthrough infection was defined as having one or more positive PCR or antigen tests prior to 1 January 2022, presence of anti-RBD antibodies in any serum sample obtained prior to vaccination or the presence of anti-NP antibodies prior to 1 January 2022. Seroconversion after primary immunisation was defined as an anti-RBD IgG response of >4.0 AU/mL measured at 28 days after primary immunisation.[21]

Analysis

Sample size calculation for the primary outcomes of the T2B! study have been described previously.[17] As primary analysis, we calculated the 95% CIs for the cumulative incidence of reported SARS-CoV-2 omicron breakthrough infections in patients with IMID on immunosuppressants and controls. A post hoc sensitivity analysis was done to compare characteristics of participants included for analyses compared with participants who were lost to follow-up. Differences in disease severity of reported SARS-CoV-2 omicron breakthrough infections between patients with IMID on immunosuppressants and controls were compared using the WHO COVID-19 Clinical Progression Scale.[20]

As a secondary analysis, we investigated possible determinants of SARS-CoV-2 omicron breakthrough infections. Previously, we showed that seroconversion after primary immunisation and hybrid immunity (ie, immunity after both infection and vaccination) were the most important determinants of breakthrough infections with the delta variant.[6] To this end, we compared the cumulative incidences of SARS-CoV-2 omicron breakthrough infections between participants with and without seroconversion after primary immunisation. In addition, we defined three medication groups: (1) treatment with anti-CD20 (combination) therapy, S1P modulators or MMF (combination) therapy as 'strongly antibody-impairing immunosuppressants' as we previously showed strongly reduced seroconversion rates with these treatments, (2) other immunosuppressants or (3) no immunosuppressants.[17] We compared the cumulative incidences of SARS-CoV-2 omicron breakthrough infections between these three medication groups. To investigate the role of additional vaccinations, that is, vaccinations after primary immunisation, we compared the cumulative incidence of SARS-CoV-2 omicron breakthrough infections in participants with and without additional vaccinations, separately for patients with IMID on immunosuppressants and controls. Participants vaccinated against SARS-CoV-2 less than 14 days prior to a SARS-CoV-2 omicron breakthrough infection (N:32) were analysed as not having received an additional vaccination. Also, we compared the proportion of participants with a SARS-CoV-2 omicron breakthrough infection who had received 0, 1 or 2 additional vaccinations separately for the three medication groups. To assess the impact of hybrid immunity, the incidence of SARS-CoV-2 omicron breakthrough infections was compared between participants with and without a prior SARS-CoV-2 infection at the start of the SARS-CoV-2 omicron wave on 1 January 2022, separately for patients with IMID on immunosuppressants and controls.

A time-to-event curve was constructed from the start of the omicron wave (ie, 1 January 2022) up to the time of SARS-CoV-2 omicron breakthrough infection or 1 April 2022 stratified for the different determinants except for seroconversion (due to low number of observations in subgroups) and medication group (due to no observed difference; see online supplemental figure 1 for curves). As the proportional hazard assumption was not met for all determinants, we used a multivariate logistic regression model (reported with relative risk and 95% CIs) to investigate risk associations for the potential determinants. The following determinants were studied: medication group (strongly antibody-impairing immunosuppressants/other immunosuppressants/no immunosuppressants), prior SARS-CoV-2 infection at the start of the omicron wave (yes/no), additional vaccination (yes/no) and seroconversion after primary immunisation (yes/no). Age and sex were added as confounders to the multivariate model. Interaction terms between determinants were explored, but were not significant. Differences between cumulative incidences were analysed using a χ^2 test. Analysis was done using R V.4.2.0.

RESULTS

A total of 1593 patients with IMID on immunosuppressants and 579 controls, consisting of 398 patients with IMID not on immunosuppressants and 181 healthy controls were included. Figure 1 shows the flow chart of this study. Table 1 shows baseline characteristics of all participants. The mean age of patients with IMID on immunosuppressants was 51 years (SD 14) and controls 52 years (SD 12), and most participants were female (62% and 67%, respectively). A total of 336/1593 (21.1%) patients with IMID were treated with strongly antibody-impairing immunosuppressants (anti-CD20 (combination) therapy, S1P modulators or MMF (combination) therapy). Online supplemental table 1 shows characteristics of participants included for analyses compared with those who were lost to follow-up. Participants included for analyses were older (51 years (SD 13) vs 41 years (SD 14), p<0.01) and more frequently female (36% vs 46%, p<0.01) compared with those lost to follow-up. Online supplemental table 2 shows characteristics separate for patients with IMID on immunosuppressants, patients with IMID not on immunosuppressants and healthy controls. Online supplemental table 3 shows characteristics separately for the different strongly antibody-impairing immunosuppressants.

Cumulative incidence of reported SARS-CoV-2 omicron breakthrough infections

SARS-CoV-2 omicron breakthrough infections were reported by 472/1593 (29.6%; 95% CI 27% to 32%) patients with IMID on immunosuppressants and by 181/579 (31.3%; 95% CI 28% to 35%) controls (p=0.42; controls: 126/398 (32%) patients with IMID not on immunosuppressants and 55/181 (30.4%) healthy controls). Figure 2 shows the incidence rate of SARS-CoV-2 omicron breakthrough infections per week during the observation period. No difference in trends of incidence rates was observed between patients with IMID on immunosuppressants and controls.

Determinants of SARS-CoV-2 omicron breakthrough infection

A total of 1746/1961 (89.0%) of all participants reached seroconversion after primary immunisation. Patients with IMID on strongly antibody-impairing immunosuppressants reached seroconversion in 150/314 (47.8%), while 1100/1143 (96.2%) in patients with IMID on other immunosuppressants and 496/504 (98.4%) in controls reached seroconversion. SARS-CoV-2 omicron breakthrough infections were detected in 81/215 (37.7%) of participants without seroconversion after primary immunisation compared with 508/1746 (29.1%) of participants

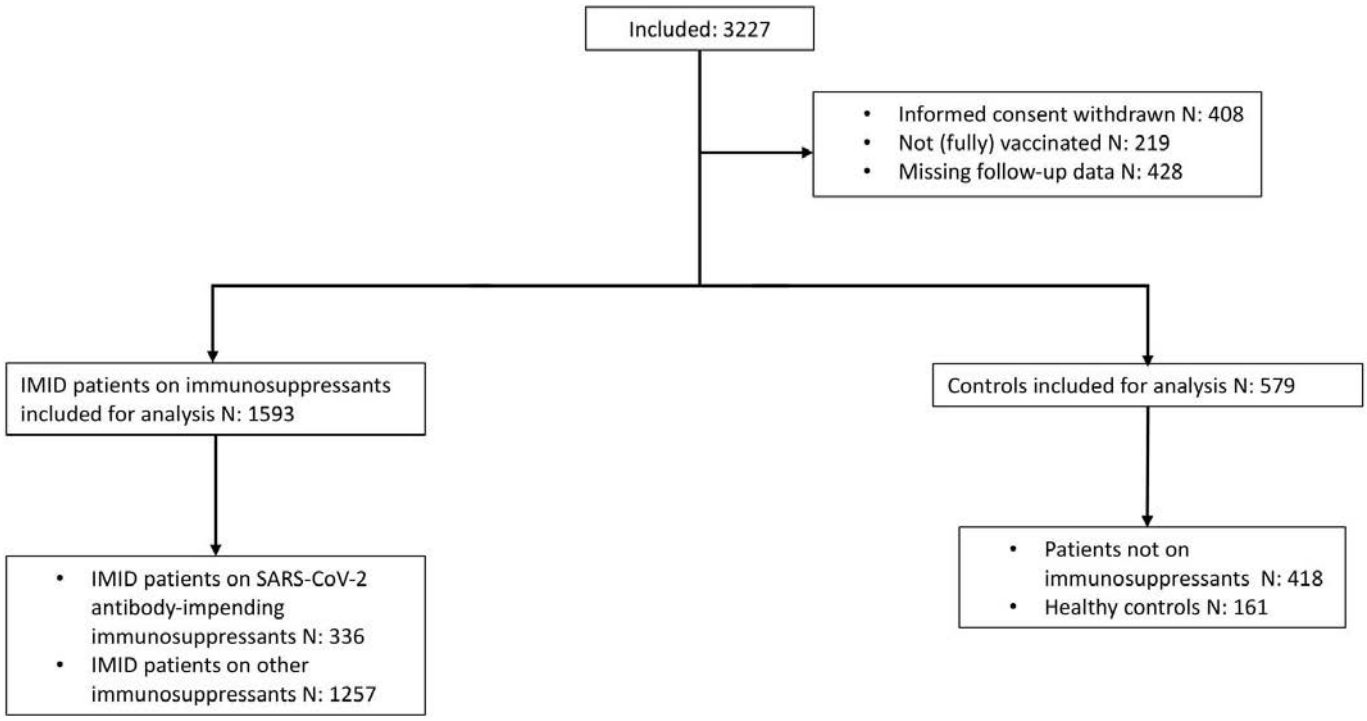

Figure 1 Shows baseline characteristics of flow chart. Figure showing the flow chart of the study. IMID, immune-mediated inflammatory disease.

with seroconversion (p=0.01). SARS-CoV-2 omicron breakthrough infections were detected in 122/336 (36.3%) of patients with IMID on strongly antibody-impairing immunosuppressants as opposed to 350/1257 (27.8%) of patients with IMID on other immunosuppressants (p<0.01). SARS-CoV-2 omicron breakthrough infections were observed more frequently in patients with IMID on S1P modulators compared with other immunosuppressants (table 1).

In 1403/1593 (88.1%) of patients with IMID on immunosuppressants and 490/579 (84.6%) of controls, additional vaccinations were administered. In patients with IMID on immunosuppressants, 387/472 (82.0%) with a SARS-CoV-2 omicron breakthrough infection had received any additional vaccination compared with 1016/1121 (90.6%) without a SARS-CoV-2 omicron breakthrough infection (p<0.01). In controls, 134/181 (74.0%) with a SARS-CoV-2 omicron breakthrough infection had received any additional vaccination compared with 356/398 (89.4%) without a SARS-CoV-2 omicron breakthrough infection (p<0.01). Figure 3 displays the proportion of SARS-CoV-2 omicron breakthrough according to the number of additional vaccines received for the different medication groups. Only in patients with IMID treated with strongly antibody-impairing immunosuppressants, we observed a lower proportion of breakthrough infections in those who had received two additional vaccinations as compared with one additional vaccination.

A total of 344/1593 (21.6%) patients with IMID on immunosuppressants and 158/579 (27.3%) controls had one or more prior SARS-CoV-2 infections. In patients with IMID on immunosuppressants, 78/472 (16.5%) with a SARS-CoV-2 omicron breakthrough infection had a prior SARS-CoV-2 infection compared with 266/1121 (23.7%) without a SARS-CoV-2 omicron breakthrough infection (p<0.01; table 1). In controls, 38/181 (21.1%) with a SARS-CoV-2 omicron breakthrough infection had a prior SARS-CoV-2 infection compared with 120/398 (30.2%) without a SARS-CoV-2 omicron breakthrough infection (p=0.03; table 1).

Figure 4 shows the combined effects of additional vaccination and prior SARS-CoV-2 infections on the cumulative incidence of SARS-CoV-2 omicron breakthrough infections. The cumulative incidence of SARS-CoV-2 omicron breakthrough infections ranged from 72/381 (18.8%) for participants with additional vaccination(s) and prior SARS-CoV-2 infection to 88/158 (55.7%) for participants without additional vaccination and prior SARS-CoV-2 infection. Figure 5 shows the results when combining the potential determinants into a logistic regression model. Reaching seroconversion after primary immunisation, any additional vaccination and a prior SARS-CoV-2 infection were associated with decreased risks for SARS-CoV-2 omicron breakthrough infections while the type of immunosuppressants was not a risk factor.

Disease severity of reported SARS-CoV-2 omicron breakthrough infections

SARS-CoV-2 omicron breakthrough infections were asymptomatic in 6/472 (1.3%) of patients with IMID on immunosuppressants compared with 5/181 (2.8%) in controls, mild symptomatic in 464/472 (98.3%) compared with 175/181 (96.7%) in controls, while hospitalisation was required in 2/472 (0.4%) compared with 1/181 (0.6%) in controls. Four out of 472 (0.8%) patients with IMID on immunosuppressants had been treated with recombinant anti-SARS-CoV-2 monoclonal antibodies during January–March 2022 and were not admitted to the hospital. Of the three hospitalised participants, none required oxygen therapy. The first hospitalised patient with IMID on immunosuppressants was treated with anti-CD20 therapy, did not reach seroconversion after primary immunisation and had received an additional vaccination. The second patient with IMID was treated with corticosteroids, reached seroconversion after primary immunisation and had not received an additional vaccination. The third participant did not use any immunosuppressants, reached seroconversion and had received

Table 1 Baseline characteristics

	Patients with immune-mediated inflammatory disorders on immunsuppressants				Controls			
	(n=1593)				(n=579)			
	With SARS-CoV-2 omicron breakthrough infection (n=472)		Without SARS-CoV-2 omicron breakthrough infection (n=1121)		With SARS-CoV-2 omicron breakthrough infection (n=181)		Without SARS-CoV-2 omicron breakthrough infection (n=398)	
Group—no (%)								
Patients with IMID	472	(100)	1121	(100)	126	(70)	292	(73)
Healthy controls	–		–		55	(30)	106	(27)
Patient characteristics								
Age, years—mean (SD)	46	(13)	53	(13)	48	(13)	53	(11)
Female sex—no (%)	317	(67)	675	(60)	128	(71)	261	(66)
Comorbidities—no (%)								
Cardiovascular disease	37	(8)	113	(10)	6	(4)	30	(9)
Chronic pulmonary disease	19	(4)	93	(8)	3	(2)	17	(5)
Diabetes	13	(3)	56	(5)	3	(2)	13	(4)
Obesity	195	(42)	553	(50)	75	(42)	182	(46)
Missing	0	0	0	0	26	(14)	51	(13)
IMID type—no (%)								
Rheumatological diseases	157	(33)	425	(38)	22	(12)	44	(11)
Rheumatoid arthritis	53	(11)	181	(16)	8	(4)	13	(3)
Spondylarthritis	29	(6)	71	(6)	7	(4)	12	(3)
Systemic lupus erythematosus	53	(11)	100	(9)	3	(2)	11	(3)
Other rheumatological*	22	(5)	73	(7)	4	(2)	8	(2)
Neurological†	140	(30)	307	(27)	42	(23)	120	(23)
Gastroenterological‡	127	(27)	246	(22)	22	(12)	68	(17)
Dermatological§	48	(10)	143	(13)	39	(22)	58	(15)
Immunosuppressants—no (%)¶								
Other immunosuppressants	192	(41)	502	(45)	–		–	
MTX	58	(12)	225	(20)	–		–	
TNF-inhibitors	100	(21)	180	(16)	–		–	
Anti-CD20	64	(14)	129	(12)	–		–	
MMF	27	(6)	56	(50)	–		–	
S1P modulator	31	(7)	29	(26)	–		–	
Prior SARS-CoV-2 infection—no (%)								
Any infection prior omicron wave	78	(17)	266	(24)	38	(21)	120	(30)
Two infections prior to omicron wave	1	(0.2)	4	(0.4)	1	(0.6)	3	(0.8)
Additional vaccination prior to SARS-CoV-2 omicron—no (%)								
Any additional vaccination	387	(82)	1016	(91)	134	(74)	356	(89)
Two additional vaccinations	62	(13)	170	(15)	0	0	4	(1)
Available humoral response data after primary vaccination—no (%)	n=431		n=1026		n=158		n=346	
Seroconversion	354	(82)	896	(87)	154	(97)	342	(99)

Table showing baseline characteristics of participants divided into patients with immune-mediated inflammatory disorders on immunosuppressants and controls (patients with immune-mediated inflammatory diseases not on immunosuppressants and healthy controls), with and without a SARS-CoV-2 omicron breakthrough infection.
*Including vasculitis (small-vessel, medium-vessel and large-vessel vasculitis and other forms of vasculitis except giant cell arteritis), other rheumatological (giant-cell arteritis, polymyalgia rheumatica and others).
†Multiple sclerosis and neuromyelitis optica spectrum disorder, Inflammatory neuropathies and myopathies (chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and inflammatory myositis), myasthenia gravis.
‡Crohn's disease, ulcerative colitis, autoimmune hepatitis, other inflammatory bowel disorders (autoimmune hepatitis, autoimmune sclerosing cholangitis).
§Atopic dermatitis, psoriasis, pemphigus, other dermatological (vitiligo, pemphigus, psoriasis and others); e: anti-CD20 therapy, sphingosine-1-phosphate receptor (S1P) modulators and MMF.
¶Therapies are either monotherapy or combination therapy, calculated percentage of total patients with IMID treated with a type of immunosuppressant.
IMID, immune-mediated inflammatory disease; MMF, mycophenolate mofetil; MTX, methotrexate; S1P, sphingosine-1-phosphate receptor; TNF-inhibitor, tumour necrosis factor inhibitor.

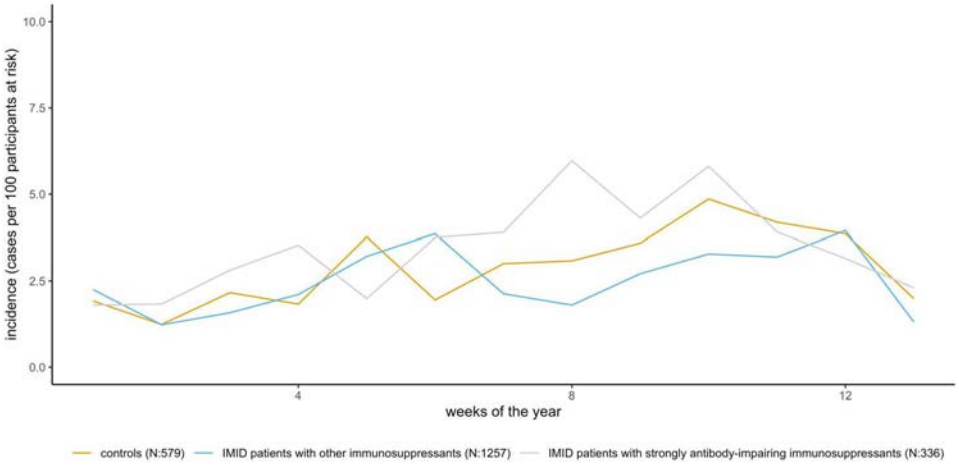

Figure 2 Incidence rates for SARS-CoV-2 omicron breakthrough infections. Figure showing the incidence rates for SARS-CoV-2 omicron breakthrough infections per week of the year for patients with immune-mediated inflammatory disorder (IMID) treated with strongly antibody-impairing immunosuppressants (ie, anti-CD20 (combination) therapy, S1P modulators or MMF (combination) therapy), patients with IMID treated with other immunosuppressants and controls (patients with IMID without immunosuppressants and healthy controls). MMF, mycophenolate mofetil.

an additional vaccination. None of the hospitalised participants had a prior SARS-CoV-2 infection.

DISCUSSION

A cumulative incidence of reported SARS-CoV-2 omicron breakthrough infections of 30% was found that did not differ between patients with IMID on immunosuppressants and controls. Overall disease severity of SARS-CoV-2 infections was mild as hospitalisation was seen in only a few cases and disease severity did not differ between patients with IMID on immunosuppressants and controls. As part of exploratory analyses, we established that the risk of SARS-CoV-2 omicron breakthrough infections was lower in participants with seroconversion after primary immunisation, with additional vaccinations, and with prior SARS-CoV-2 infections.

We found that the incidence of SARS-CoV-2 breakthrough infections with the omicron variant was considerably higher than with the delta variant of SARS-CoV-2, as observed by others and by us.[6 22 23] Disease severity of reported SARS-CoV-2 omicron breakthrough infections was generally mild in line with other studies in healthy controls[22 23] and similar to what we observed earlier for delta breakthrough infections, irrespective of the use of immunosuppressants for patients with IMID.[6 22 24] Others have reported increased disease severity of delta variant breakthrough infections when compared with omicron infections in healthy controls.[4 7] Comparing disease severity between variant strains is challenging, because of the many determinants involved, including differences in risk behaviour and evolving immunological protection induced by repeated vaccinations and/or infections with SARS-CoV-2 leading to an increased proportion of individuals having hybrid immunity which has been shown to be superior to other forms of immunity.[25–28]

Our study focused on possible determinants mitigating the risks of SARS-CoV-2 omicron breakthrough infections in patients with IMID on immunosuppressants. First, we confirm that a poor humoral response after primary immunisation is a risk factor. This is in line with previously found data for delta variant breakthrough infections and observations in

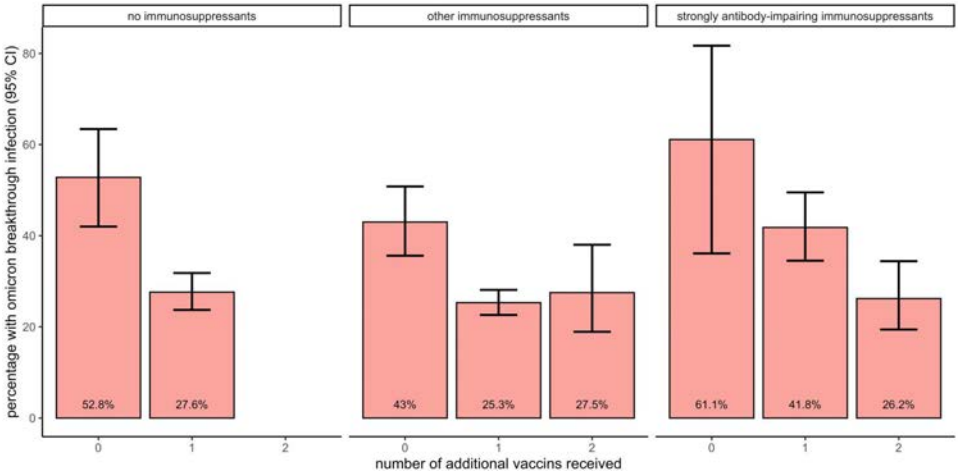

Figure 3 Proportion of SARS-CoV-2 omicron breakthrough infections and number of additional vaccinations received. Figure showing the proportion with 95% CI of SARS-CoV-2 omicron breakthrough infections for patients with immune-mediated inflammatory disorder (IMID) treated with strongly antibody-impairing immunosuppressants (ie, anti-CD20 (combination) therapy, S1P modulators or MMF (combination) therapy), patients with IMID treated with other immunosuppressants and controls (patients with IMID without immunosuppressants and healthy controls) stratified for the number of additional vaccines received. MMF, mycophenolate mofetil.

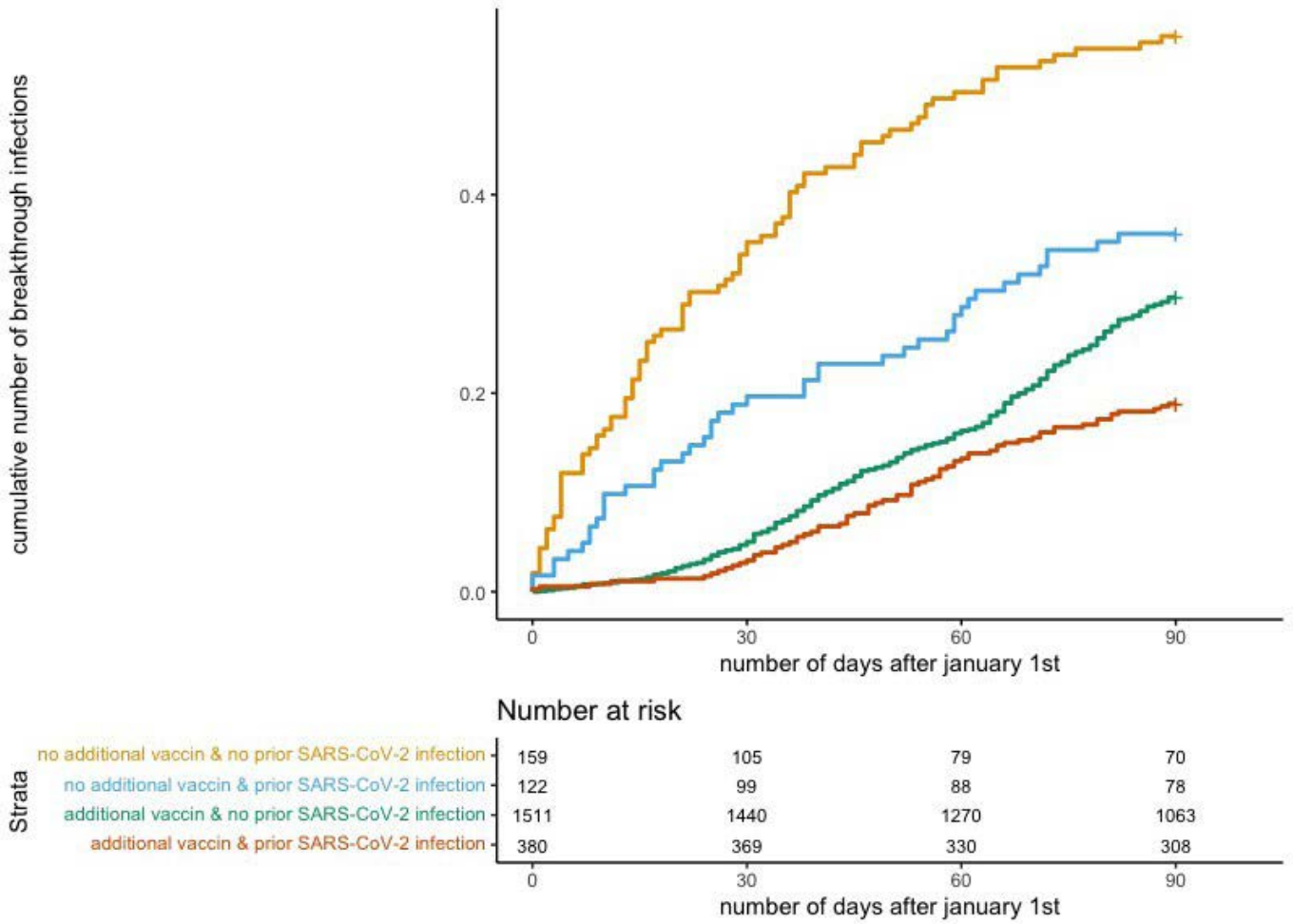

Figure 4 Cumulative event curves for SARS-CoV-2 omicron breakthrough infections. Figure showing the cumulative incidence for SARS-CoV-2 Omicron breakthrough infections stratified for having received an additional vaccination and prior SARS-CoV-2 infection.

other SARS-CoV-2 vaccination trials.[6] Of note, the humoral response after primary immunisation in this analysis should not be interpreted as a direct reflection of humoral immunity at the moment of the omicron breakthrough infections (eg, antibody titres or antibody affinity), but more as an indirect risk factor reflecting an overall decreased (humoral) response after

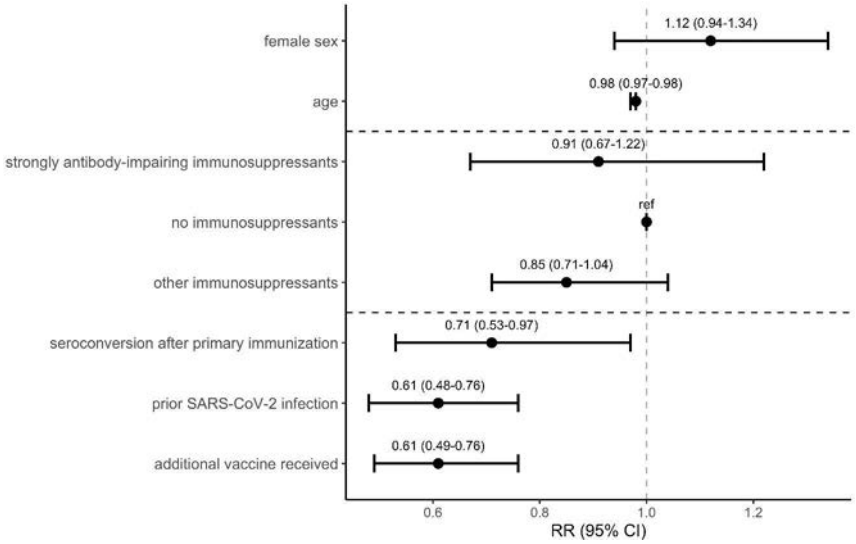

Figure 5 Risk estimates of determinants for SARS-CoV-2 omicron breakthrough infections. Figure showing the estimated relative risks (RR; shown with 95% CI) for SARS-CoV-2 Omicron breakthrough infections for the different determinants. *N: 209 participants excluded because of missing serological data after primary vaccination.

(repeated) vaccination. In many individuals with demonstrated poor humoral responses after primary immunisation, a 'third' or additional vaccination did not increase humoral response rates up to levels seen in the general population.[17] Ongoing decreased immunological responses, despite repeated vaccinations, are a likely cause for the observed increased incidence of breakthrough infections in patients with IMID on strongly antibody-impairing immunosuppressants, like anti-CD20 (combination) therapy, S1P modulators or MMF (combination) therapy, that have previously been shown to greatly impair humoral and (variably) cellular vaccination responses.[16 17 29–31] Second, for the first time we demonstrate in patients with IMID on immunosuppressants that additional vaccinations are associated with decreased risk of SARS-CoV-2 omicron breakthrough infections. This is in line with recent studies in healthy individuals showing that additional vaccinations were either highly effective against infection or disease severity with various SARS-CoV-2 variants.[8–12] Moreover, in patients with IMID treated with strongly antibody-impairing immunosuppressants, two additional vaccinations seem to be better compared with a single additional vaccination whereas this added benefit could not be observed in other groups. Third, similar to our previous results on the delta variant, we found that prior SARS-CoV-2 infections are associated with a decreased risk of new, in this case, omicron SARS-CoV-2 breakthrough infections.[6] Also in other studies, hybrid immunity, as opposed to vaccine responses only, was associated with increased protection against a SARS-CoV-2 breakthrough infections due to an increased breadth of humoral and cellular immune responses.[25 26 28]

Together, these observations suggest that for the majority of patients with IMID on immunosuppressants, immunological protection against severe disease can be achieved through vaccination and previous SARS-CoV-2 infection (or both) and that short-term as well as long-term protective immunological mechanisms are in play despite immunosuppressive treatment. No seroconversion after primary immunisation remains a risk factor, but this is only relevant for a relatively small subgroup of patients with IMID on immunosuppressants. To better understand risk profiles for individual patients with IMID, vaccinations and prior infections should be taken into account besides other known risk factors, like older age and comorbidities as suggested by our previous study in delta breakthrough infections.[6]

A limitation of our study is that we relied on a participant driven test approach to identify SARS-CoV-2 infections and did not employ a test-negative design as has been used in (phase 4) studies on vaccine efficacy. Given the mild disease course in the majority of SARS-CoV-2 omicron breakthrough infections, it is likely that the true rate of infections was higher due to undetected asymptomatic infections. We, therefore, limit our conclusions to reported infections and not all infections as antigen testing was used frequently and studies show a broad variety of sensitivity in symptomatic SARS-CoV-2 cases.[32] However, as this underestimation of the incidence of SARS-CoV-2 infections would occur throughout the cohort and would not have led to a difference between the groups. Also, we were unable to correct for risk behaviour in our analyses. Participants were aware of their SARS-CoV-2 antibody titre after vaccination and could have adapted their behaviour accordingly. In particular patients with IMID with immunosuppressants might be stricter in adhering to the infection preventive measures which could have led to an underestimation of the incidence of SARS-CoV-2 breakthrough infection in this group. Also, we did not analyse the actual humoral immune response after additional vaccination(s) or prior to breakthrough infection. Finally, although our cohort is a broad disease-overarching reflection of IMID, this inherently leads to an under-representation of various other known risk factors for increased incidence or severity of breakthrough infections. Most importantly, our cohort is composed of relatively young participants and consequently the burden of comorbidities, such as diabetes, is low. Age and comorbidities have been identified as important risk factors in many other studies and our results should therefore be interpreted with caution when dealing with older patients with IMID and/or patients with IMID with comorbidities or other known risk factors relevant for (breakthrough) infections.[33] An important strength of this study is the use of a well-characterised ongoing large cohort of participants that has been prospectively studied clinically and serologically from before the start of primary immunisation.

In conclusion, we found that the cumulative incidence of reported SARS-CoV-2 omicron breakthrough infections is relatively high compared with the delta variant, but similar between patients with IMID on immunosuppressants and controls, and that disease severity of SARS-CoV-2 infections was almost exclusively mild. Seroconversion after primary immunisation, additional vaccinations, and prior SARS-CoV-2 infections were associated with decreased risks of SARS-CoV-2 omicron breakthrough infections. Our findings suggest that offering additional vaccinations can be an effective strategy to reduce risks of (future) breakthrough infections also in patients with IMID.

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Competing interests FE and TWK report (governmental) grants from ZonMw to studyimmune response after SARS-Cov-2 vaccination in autoimmune diseases. FE also reports grants from Prinses Beatrix Spierfonds, CSL Behring,Kedrion, Terumo BCT, Grifols, Takeda Pharmaceutical Company, andGBS-CIDP Foundation; consulting fees from UCB Pharma and CSlBehring; and honoraria from Grifols. AJvdK reports grants from CSLBehring and participation on an advisory board for Argen-X. ML reports agrant from Galapagos not related to this study, and honoraria from BristolMyers Squibb, Pfizer, Takeda, and Tillotts. PIS is involved in clinical trialswith many pharmaceutical industries that manufacture drugs used for thetreatment of, for example, psoriasis and atopic dermatitis, for whichfinancial compensation is paid to the department or hospital, and is achief investigator of the TREAT NL registry taskforce and SECURE-ADregistry. MWB is a secretary for the Dutch Experimental DermatologyBoard; head of the pigmentary disorders group within the DutchDermatology Board; and reports honoraria from Pfizer, Sanofi, Novartis,and Fondation René Touraine. JK has speaking relationships with MerckSerono, Biogen Idec, TEVA, Sanofi, Genzyme, Roche, and Novartis;received financial support to his institution for researchactivities from Merck Serono, Bayer Shcering Pharma, Biogen Idec,GlaxoSmithKline (GSK), Roche, Teva, Sanofi, Genzyme, and Novartis. BHreports unpaid positions as a medical adviser for several patient groups, aboard position for ERN-SKIN, and associate editor for The British Journalof Dermatology; reports grants from AbbVIe, Akari Therapeutics, Celgene, and Novartis; consulting fees from UCB Pharma, Novartis, and Janssen; and honoraria from AbbVie. JJGMV reports consulting fees from Argenx,Alexion, and NMD Pharma, and is a co-inventor on patent applicationsbased on MuSK-related research. DJH reportsgrants from AbbVie, AstraZeneca, Janssen, LEO Pharma, and UCB;honoraria from AbbVie, Galderma, Janssen, Lilly, Pfizer, Sanofi, and UCB;and a paid position on an advisory board for BIOMAP IMI. PAvDparticipated on an advisory board for Octapharma. PvP reports grantsfrom Alexion Pharma and GSK, and participation on advisory boards forGSK and Vifor Pharma. GRAMD'H reports consulting fees from AbbVie,Agomab, AstraZeneca, AM Pharma, AMT, Arena Pharmaceuticals, BristolMyers Squibb, Boehringer Ingelheim, Celltrion, Eli Lilly, ExeliomBiosciences, Exo Biologics, Galapagos, Index Pharmaceuticals, Kaleido,Roche, Gilead, GSK, Gossamerbio, Pfizer, Immunic, Johnson andJohnson, Origo, Polpharma, Procise Diagnostics, PrometheusLaboratories, Prometheus Biosciences, Progenity, and Protagonist;honoraria from AbbVie, Arena, Galapagos, Gilead, Pfizer, Bristol MyersSquibb, and Takeda; and participation on advisory boards for AbbVie,Seres Health, Galapagos, and AstraZeneca. RBT reports honoraria fromSobi and Norgine, and participation on an advisory board for Norgine.SHG is a board member of the Dutch Society of Clinical Neurophysiology(unpaid), reports grants from Prinses Beatrix Spierfonds, and receivedspeaker fees from Shire/Takeda. KAHZ reports paid data safetymonitoring board positions for Torrent and Foresee. All other authorsdeclare no competing interests.

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 applicable.

Ethics approval This study involves human participants and was approved by the medical ethical committee of the Amsterdam UMC, location AMC. Reference number: 2020.194. Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available on reasonable request. Aggregated data and code for reproducing the results of this analysis can be shared on reasonable request.

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News from EULAR

THE LATEST RECOMMENDATIONS FROM EUROPEAN ALLIANCE OF ASSOCIATIONS FOR RHEUMATOLOGY (EULAR)

- EULAR points to consider for minimal reporting requirements in synovial tissue research in rheumatology (https://ard.bmj.com/content/early/2022/03/02/annrheumdis-2021-221875).
- The 2021 EULAR/American College of Rheumatology points to consider for diagnosis, management and monitoring of the interleukin-1-mediated autoinflammatory diseases: cryopyrin-associated periodic syndromes, tumour necrosis factor receptor-associated periodic syndrome, mevalonate kinase deficiency and deficiency of the interleukin-1 receptor antagonist (https://ard.bmj.com/content/81/7/907).
- Effects of diet on the outcomes of rheumatic and musculoskeletal diseases (RMDs): systematic review and meta-analyses informing the 2021 EULAR recommendations for lifestyle improvements in people with RMDs (https://rmdopen.bmj.com/content/8/2/e002167).
- EULAR/PRES recommendations for vaccination of paediatric patients with autoimmune inflammatory rheumatic diseases: update 2021 (https://ard.bmj.com/content/early/2022/06/20/annrheumdis-2022-222574).
- EULAR points to consider for including the perspective of young patients with inflammatory arthritis in patient-reported outcome measures (https://rmdopen.bmj.com/content/8/2/e002576).
- Procedures for the content, conduct and format of EULAR/PRES paediatric musculoskeletal ultrasound courses (https://rmdopen.bmj.com/content/8/2/e002455.info).
- Gender equity in academic rheumatology, current status and potential for improvement: a cross-sectional study to inform an EULAR task force (https://rmdopen.bmj.com/content/8/2/e002518).

Find more EULAR recommendations at EULAR|Recommendations and initiatives.

EULAR HAS LAUNCHED A NEW PAGE ON ITS WEBSITE: 'NEWS FROM SOCIETIES'

This new section aims to share the latest guidelines and news from scientific societies about important topics related to rheumatology, such as new guidelines from British Society for Rheumatology for treating rheumatic conditions during conception, pregnancy and breastfeeding.

EULAR 2023 CONGRESS IN MILAN, ITALY

Registration for EULAR 2023 Congress and Abstract submission is now open!

Important note: the abstract submission deadline is 15 January 2023.

"The EULAR 2023 Congress will take place in one of the most beautiful and vibrant cities in Europe – Milan! We look forward to returning to a city that acknowledges our organisation and supports its three pillars and communities, aiming to reduce the impact of RMDs on those afflicted and improve their social position and quality of life. We look forward to sharing our new strategy for 2024–2028, recent developments in Rheumatology, scientific sessions, abstracts, and more. I am looking forward to meeting you all on-site, in Milan, on 31 May – 3 June 2023. " - Annamaria Iagnocco, EULAR President

More details are available at EULAR 2023—Annual European Congress of Rheumatology.

EULAR'S CAMPAIGN FOR WORLD ARTHRITIS DAY (WAD) 2022

WAD is a global awareness day, celebrated on 12 October, aimed at increasing knowledge of the existence and impact of RMDs among all audiences.

EULAR's WAD 2022 campaign focused on understanding medical professionals' and patients' issues in rheumatological care and addressing them nationally and internationally. Besides other activities, EULAR also created a video showing medical professionals and a patient representative from Denmark talk about how their collaboration is based on the principle of togetherness, ensuring all three pillars (rheumatologists, healthcare professionals and patients) work together to provide the best possible therapy option - Rheumatology Workforce—The Danish Example, YouTube.

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The next application deadline for the EULAR Research Vouchers Programme is 31 December 2022. The programme aims to help investigators cover ad hoc expenses or services supporting their ongoing rheumatic and musculoskeletal diseases (RMD) research projects.

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► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/ard-2022-223330).

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Serum urate outcomes of treat-to-target urate lowering treatment: results of a nationwide cohort study from 1997 to the COVID-19 pandemic using data from the Clinical Practice Research Datalink

Despite long-standing recommendations,[1–3] most gout patients prescribed urate lowering treatment (ULT) do not achieve serum urate (SU) target.[4] The time between treat-to-target (T2T) recommendations and achievement of SU treatment target, and how the latter was impacted by the COVID-19 pandemic has not been evaluated. We used UK-wide nationally representative primary-care data from the Clinical Practice Research Datalink (CPRD) GOLD to evaluate temporal trends in achievement of T2T–SU levels within 12 months of first ULT prescription in successive years from 1997 to 2020. CPRD contains anonymised healthcare records from >18 million individuals, originating during their routine care in the National Health Service, a healthcare system with universal coverage.[5]

This study spanned from 01 January 1997 to 31 December 2021. Prevalent gout cases age≥18 years, first prescribed ULT in the study period was followed from the first prescription to earliest of prescription end, death, transfer out, last data collection, 12 months after ULT prescription or 31 December 2021. Participants were required to have≥1 year ULT prescription-free registration prior to study entry to prevent prevalent ULT users appearing as new users. Gout and ULT prescription status were defined using Read and product codes.[6]

Prevalence (95% CI) of achieving SU<360 and <300 µmol/L within 12 months of ULT initiation were calculated. The latest SU within 12 months of ULT initiation was used to define achievement of target thresholds. Cox proportional HR with 95% CI were used to estimate the likelihood of achieving SU target for patients starting ULT in each year compared with those starting in the year 2006 as the first British Society for Rheumatology gout guidelines were published in 2007. Analyses were adjusted for age, time between first primary care consultation for gout and first ULT prescription, sex and region. Sensitivity analysis included additional adjustment for pre-ULT SU. Data were analysed using Stata-MP V.16.

Data for 119 903 gout patients (77.19% men) were included (online supplemental figure S1). Their mean (standard deviation) age and time from first gout consultation to ULT prescription were 63.09 (15.06) and 2.54 (5.14) years. Overall, 99.32%, 0.50% and 0.18% were prescribed allopurinol, febuxostat and uricosurics, respectively. Overall, 34 137 (28.47%) and 18 926 (15.78%) achieved SU<360 and <300 µmol/L, respectively. Among the 73 657 participants prescribed ULT in 2007 or later, 23 446 (31.83%) and 12 630 (17.15%) achieved SU<360 and <300 µmol/L, after mean (SD) 1.05 (1.73) and 1.44 (2.95) years. The median (IQR) allopurinol dose at treatment start was 100 (100–300) mg/day (n=107 214). Participants who achieved and did not achieve SU<300 µmol/L by 1 year were prescribed allopurinol at median (IQR) dose of 300 (200–300) and 200 (100–300) mg/day (p<0.0001, Wilcoxon rank-sum test). Similarly, participants who achieved and did not achieve SU<360 µmol/L at 1 year were prescribed allopurinol at median (IQR) dose of 300 (100–300) and 200 (100–300) mg/day (p<0.0001, Wilcoxon rank-sum test). Increasing proportion of gout patients commenced on ULT in calendar years 1997–2018 achieved SU target (figure 1). The age and SU at the start of ULT increased modestly over time (online supplemental table S1). Overall, 5228 (15.31%) and 2979 (15.74%) participants who achieved SU<360 and <300 µmol/L by 12-month consulted at their General Practice surgery for gout flare subsequently, defined as Read code specific for gout flare or any consultation for gout and prescription colchicine, corticosteroids or NSAIDs on the same or next date.

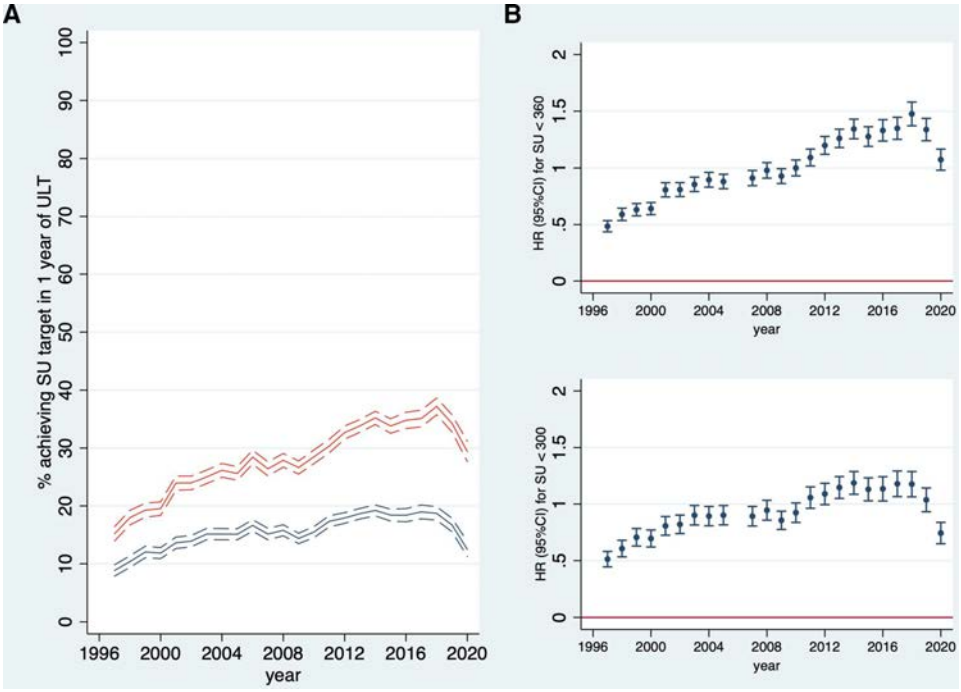

Figure 1 (A) The proportion (solid line) and 95% CI (dotted line) of gout cases commenced on urate lowering treatment (ULT) in each calendar year that achieved serum urate (SU) treatment target<360 (red) and <300 (blue) µmol/L within 1 year. (B) Adjusted HRs (95% CI) for achieving SU outcomes<360 (top) and <300 (bottom) µmol/L within 1 year in gout cases commenced on ULT in successive years with the year 2006 reference.

There was a 5-year lag between EULAR and British Society for Rheumatology recommendations to treat gout to target before significant improvement in achievement of recommended SU treatment target was apparent. Compared with those prescribed ULT in 2006, participants commenced on ULT in the year 2020 were significantly less likely to achieve SU<300 µmol/L (figure 1, online supplemental table S2).

This study evaluated T2T–ULT in consecutive annual new-prescription cohorts spanning 25 years. There was a sharp reduction in achievement of SU targets among those commenced on ULT in the year 2019 and 2020 potentially due to the impact of the COVID-19 pandemic. This was comparable to 37.2% reduction in healthcare utilisation during the pandemic reported in a systematic review, with 29.6% and 31.4% reduction in therapeutics and diagnostics, respectively.[7] T2T–ULT prevents recurrent gout flares and our findings point to a potential epidemic of uncontrolled gout. The modest improvement in SU outcomes pre pandemic was lost during the COVID-19 pandemic. As the pandemic resolves, additional efforts, for example, engagement with primary-care providers will be required to increase the use of T2T–ULT.

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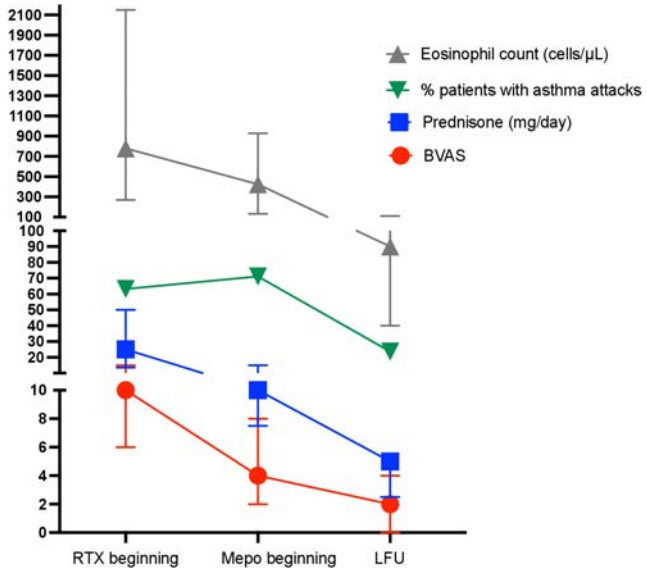
Sequential rituximab and mepolizumab in eosinophilic granulomatosis with polyangiitis (EGPA): a European multicentre observational study

Eosinophilic granulomatosis with polyangiitis (EGPA) is an anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis characterised by eosinophilic (eg, respiratory involvement, cardiomyopathy, gastroenteritis) and vasculitic manifestations (eg, neuropathy, glomerulonephritis).[1]

Rituximab is an established treatment in granulomatosis with polyangiitis and microscopic polyangiitis, and growing evidence indicates that it seems effective also in EGPA, mainly to induce and maintain remission of vasculitic involvement.[2 3] However, its efficacy on respiratory manifestations seems limited. Conversely, the anti-IL5 mepolizumab, recently licensed for relapsing-refractory EGPA, is effective on respiratory manifestations, although it may also partially control systemic ones.[3–5]

Based on the idea that combining treatments with complementary mechanisms of action might induce and maintain remission of both disease components,[6 7] we investigated the efficacy and safety of a regimen based on sequential rituximab and mepolizumab for the control of EGPA.

This multicentre, European, retrospective study included patients meeting the American College of Rheumatology classification criteria for EGPA or the eligibility criteria proposed in the MIRRA trial.[1] Only patients who received therapy with rituximab (any dosage), and subsequent treatment with mepolizumab (100–300 mg/4 weeks) within 12 months from last rituximab



	Rituximab beginning (n=38)	Mepolizumab beginning (n=38)	Last follow-up (n=38)	P-value
Time elapsed, months (median, IQR)	-	5 (3-11) from last Rituximab dose	26 (13-33) from Mepolizumab beginning	
Efficacy				
Remission (n,%)	2 (5.3%)	6 (15.8%)	11 (28.9%)	0.003
Active disease (n,%)	36 (94.7%)	32 (84.2%)	27 (71.1%)	
BVAS (median, IQR)	10 (6-15)	4 (2-8)	2 (0-4)	<0.001
Eosinophil count, cells/µL (median, IQR)	780 (270-2150)	424 (133-929)	90 (40-110)	<0.001
Asthma attacks in the last months (median, IQR)	1 (0-3)	1 (0-2)	0 (0-0)	<0.001
Patients with 1+ asthma attacks	24 (63.2%)	27 (71.1%)	9 (23.7%)	<0.001
ANCA positivity (n,%)	12/17 (70.6)	5/16 (31.3%)	2/17 (11.8%)	0.001
Concomitant treatments				
Glucocorticoids	37 (97.4%)	37 (97.4%)	31 (81.6%)	0.034
Prednisolone dosage, mg/day (median, IQR)	25.0 (13.5-50.0)	10.0 (7.5-15.0)	5.0 (2.5-5.0)	<0.001
Immunosuppressants	22 (57.9%) AZA (n=8); MTX (n=8); MMF (n=3); CSA (n=3)	12 (31.6%) AZA (n=5); MTX (n=3); MMF (n=3); CSA (n=1)	8 (21.1%) MMF (n=4); AZA (n=2); MTX (n=2)	<0.001

Figure 1 Efficacy of sequential rituximab and mepolizumab. P values for the paired comparison between data at last follow-up and at the start of rituximab. ANCA, anti-neutrophil cytoplasmic antibody; AZA, azathioprine; BVAS, Birmingham Vasculitis Activity Score; CSA, ciclosporin; MMF, mycophenolate mofetil; MTX, methotrexate.

administration, without other induction/maintenance therapies in the meanwhile, were included.

Treatment efficacy was assessed considering disease activity (by the Birmingham Vasculitis Activity Score, BVAS), eosinophil count and glucocorticoid dose.[1] Asthma attacks and adverse events (AEs) were also assessed.

The study received ethical approval (University of Florence IRB; ref.16821_OSS); as this is a retrospective study, patient representatives were not involved in designing the study.

We included 38 patients (53% female), whose median age at diagnosis was 52 years (IQR 42–61). Eighteen (47%) were ANCA positive, mostly with an anti-myeloperoxidase specificity (17/18). Rituximab (1 g every 2 weeks (q2w) in 26/38; 375 mg/m^2/week for 4 weeks in 11/38; 500 mg q2w in 1/38) was mostly initiated for the control of active disease (36/38, median BVAS of 10 (IQR 6–15), median eosinophil count of 780 (270–2150) cells/µL), particularly of systemic (±respiratory) manifestations (33/38; 87%) (figure 1). Sixty-three per cent of patients had experienced one or more asthma attacks in the preceding month. At rituximab initiation, 97% of patients were receiving glucocorticoids (median prednisone dose of 25 mg/day (13.5–50)), and 58% immunosuppressants.

Mepolizumab (100 mg every 4 weeks (q4w) in 36/38) was started after a median of 5 months (3–11) from last rituximab dose, usually for the presence of active manifestations (32/38, 84%; median BVAS of 4 (2–8)), mostly respiratory (28/32). All except one patient were still receiving glucocorticoids (97%; median dose of 10 mg/day (7.5–15), mostly for respiratory manifestations), and 32% immunosuppressants.

After a median of 26 months (13–33) from mepolizumab initiation, the median BVAS significantly decreased to 2 (0–4), as well as the median eosinophil count (90 cells/µL (40–110)), and the use of glucocorticoids and immunosuppressants (median prednisone dose of 5 mg/day (2.5–5); 21% of patients on immunosuppressants). Only 24% of patients reported asthma attacks in the previous month. Notably, following sequential rituximab and mepolizumab treatment, ANCA negativisation occurred in a relevant proportion of patients. Indeed, at the start of rituximab, 17 out of the 18 ANCA+ patients at EGPA diagnosis had available data on ANCA status, and 12 of them still tested positive

(70.6%). At the start of mepolizumab, 5 out of 16 patients with available data were positive (31.3%). At last available follow-up, only 2 out of 17 patients with available results tested ANCA+, the remaining displaying ANCA negativisation (p=0.001 as compared with the time of rituximab beginning).

Both rituximab and mepolizumab were well tolerated. Six patients had non-serious AEs on rituximab, while five patients had AEs on mepolizumab, including one serious (COVID-19 pneumonia).

Taken together, our findings confirmed previous literature evidence on the efficacy of rituximab for the control of systemic EGPA manifestations,[2] while proving limited efficacy on respiratory symptoms. Conversely, the introduction of mepolizumab allowed reducing asthma attacks, while also contributing to the sustained remission of systemic features and glucocorticoid sparing.

Notably, we confirmed[5] that in real clinical practice, mepolizumab was mostly used at the dosage for eosinophilic asthma (100 mg/4 weeks), rather than at the dosage approved for EGPA (300 mg/4 weeks).[4]

The tolerability of the sequential rituximab-mepolizumab treatment was good.

Our study has some limitations, mostly related to this retrospective nature. First, data on quality of life and on specific scores of ear-nose-throat involvement could not be retrieved, as they are not routinely collected in medical charts. Second, given the wide time window elapsed between last rituximab administration and start of mepolizumab (up to 12 months), disease flares due to an 'end-of-dose' effect cannot be fully excluded. Third, the small sample size did not allow to conduct separate analysis according to the ANCA status.

Despite these limitations, our findings suggest that a regimen based on sequential rituximab and mepolizumab might be effective to induce and maintain remission of both systemic and respiratory EGPA manifestations.

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Correspondence on 'Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): a multicentre cohort'

We read with great interest the article by Pouletty *et al* reporting 16 paediatric patients presenting with Kawa-COVID-19, an inflammatory syndrome similar to Kawasaki disease (KD) associated with SARS-CoV-2 infection.[1] All 16 patients met criteria for complete or incomplete KD. Severe cases in children involving systemic inflammation and multiorgan involvement related to COVID-19 are increasingly being reported. These cases, named multisystem inflammatory syndrome in children (MIS-C) in the USA and pediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 in the UK, share features of both KD and macrophage activation syndrome.[2–6] In contrast to children, few adults with KD-like cases have been reported.[7 8] Herein, we describe an adult who presented with KD-like illness similar to children in the Kawa-COVID-19 cohort 4 weeks following a documented SARS-CoV-2 infection.

A 38-year-old Hispanic woman developed fever, dyspnoea, cough, anosmia, myalgias and polyarthralgias of the hands, wrists, elbows and knees 4 weeks prior to admission. At that time, nasopharyngeal SARS-CoV-2 PCR was positive. Her symptoms completely resolved within 2 weeks. Five days prior to admission, she developed fevers up to 39.4°C, dyspnoea and polyarthralgias. Additionally, she described occipital headaches, conjunctival injection, lip peeling, odynophagia, vomiting and a maculopapular rash on her chest and arms (figure 1A). The conjunctival injection and rash resolved within a week, but arthralgias, dyspnoea and fevers persisted.

On admission, vitals showed temperature 39.1°C, pulse 114 beats/min, blood pressure 114/67 mm Hg and 97% oxygen saturation. Physical examination revealed clear conjunctiva, erythematous tongue, lip peeling, clear lung fields and a normal cardiac examination with exception of tachycardia (figure 1B).

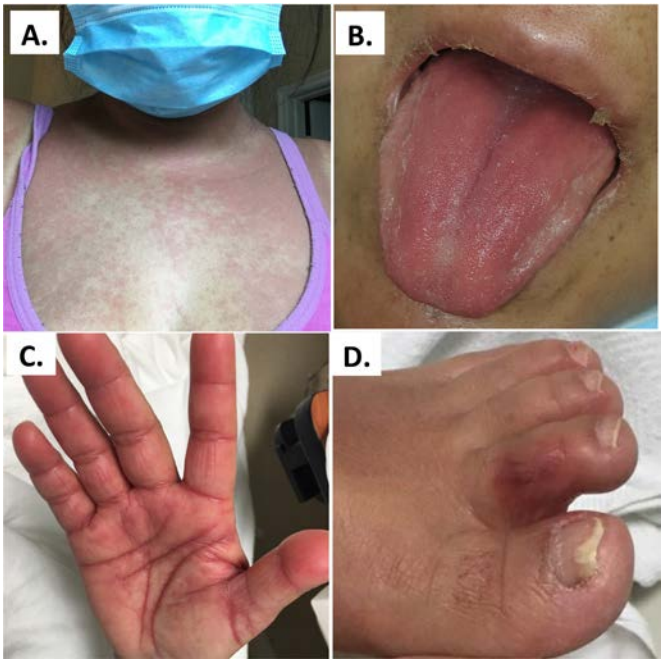

Figure 1 (A) Maculopapular rash on chest. (B) Dry oral mucosa with peeling lips. (C) Palmar erythema. (D) Toe discoloration.

Musculoskeletal examination demonstrated synovitis of the proximal interphalangeal joints. On hospital day 4–5, she developed palmar erythema and discoloration of two toes (figure 1C,D).

Admission laboratories showed alanine aminotransferase (126 units/L (7–52)), alkaline phosphatase (337 U/L (24–104)), B-natriuretic peptide (404 pg/mL (<100)), sedimentation rate (34 mm/hour (<20)), C-reactive protein (21.7 mg/dL (<10)), d-dimer (0.77 μg/mL fibrinogen equivalent units (0.27–0.48)), an absolute lymphocyte count of 560 per μL (1.18–3.74) and albumin of 3.3 g/dL (3.7–5.3). Serum creatinine, troponin, creatine kinase, lactate dehydrogenase, haptoglobin and ferritin were normal. Repeat nasopharyngeal SARS-CoV-2 PCR and serum IgG and IgA to the spike protein of SARS-CoV-2 were positive. Infectious workup was negative, including blood and urine cultures as well as testing for HIV-1/2, parvovirus, arbovirus, gonorrhoea, chlamydia and murine typhus. Echocardiogram showed trace pericardial effusion, elevated pulmonary artery pressure (46–51 mm Hg), and normal left ventricular ejection fraction but no coronary artery abnormalities. CT chest with angiography was negative for pulmonary emboli but showed right upper lobe ground glass opacities, septal and bronchial wall thickening, and bilateral pleural effusions.

This patient met diagnostic criteria for both KD and MIS-C, with the exception of age.[3 9] Accordingly, she was treated with intravenous immunoglobulin (IVIG) 80 g on hospital day 1 and 81 mg of aspirin daily. On hospital day 3, due to persistent fevers, she was given a second dose of IVIG (100 g). Prednisone 10 mg daily was given for the inflammatory arthritis. She defervesced and her symptoms improved by hospital day 6. She was discharged on hospital day 7 with daily aspirin 81 mg for 6 weeks and prednisone taper from 10 mg over 5 weeks. Two weeks post hospital discharge, she reported desquamation of the hands and feet, and her only remaining symptoms were bilateral ankle arthralgia and mild headaches.

This case describes an adult with a KD-like presentation following a SARS-CoV-2 infection similar to the Kawa-COVID-19 cohort described by Pouletty *et al*. KD in adults is rare and often associated with HIV infection.[10] KD-like presentations in the setting of COVID-19 have been reported in two adults, but the time of initial SARS-CoV-2 infection in relation to KD-like presentation was unknown.[7 8] Our case demonstrates a timeline of a symptomatic COVID-19 infection followed by complete symptom resolution prior to the onset of a KD-like illness. This case emphasises the need for adult as well as paediatric rheumatologists to be aware of the potential for KD-like illness from COVID-19 infection. Further study of this and similar cases is important to aid clinicians in recognising SARS-CoV-2-related inflammatory syndromes in adults.

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Response to: 'Correspondence on 'Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): a multicentre cohort' by Ventura *et al*'

We thank Ventura *et al* for their correspondence[1] on our study on paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 (MIS-TS) mimicking Kawasaki disease (KD) (Kawa-COVID-19).[2] They report a 38-year-old woman with a KD-like presentation following SARS-CoV-2 infection and highlight the need for physicians to be aware of this syndrome also in adults. We fully agree that, despite being initially described and more frequent in children, a similar presentation may occur following SARS-CoV-2 infection in adults.[3] To assess similarities and differences between paediatric and adult cases, we collected data of nine adult cases of Kawa-COVID-19 in three hospitals of the Great Paris region (six were previously reported in another case series[3]) and compared them with our paediatric Kawa-COVID-19 cohort.[2] The main characteristics of adult and paediatric patients are described in table 1.

Median (range) age of the adults was 25 (19–33) years and 55% were male. None of the adults had criteria for complete KD while 10/16 children had complete KD criteria. Adults and children shared similar characteristics including fever, gastrointestinal and neurological signs, hyponatremia, hypoalbuminaemia, lymphopaenia and biological inflammatory syndrome. Of note, differences in the presentation between adult and paediatric Kawa-COVID-19 were also observed. Respiratory features were reported in the majority of adults. Mucocutaneous manifestations were less frequent, while myocarditis, acute kidney injury and vasoplegic shock were more common in adult MIS-TS. Adults seemed in a more severe condition: six (66%) of them required intensive care unit admission, three (33%) were placed on mechanical ventilation and six (66%) required vasopressor therapy. Inflammation parameters were also more elevated in adults with significantly higher ferritin level (2124 (833–6205) g/L) and C reactive protein (CRP) (363 (278–439) mg/L). Regarding specific treatments, children received more frequently a second intravenous immunoglobulin (Ig) infusion than adults (p=0.057). All patients were in remission 3[3 4] days after treatment initiation. No patient died.

The different descriptions of this new entity (ie, multisystem inflammatory syndrome in children in the USA,[4] paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 in the UK[5] and Kawasaki-like disease or Kawa-COVID-19) reflect the uncertainty about the pathophysiology and specificities associated with SARS-CoV-2. The temporal link observed between the occurrence of COVID-19 and MIS-TS, together with positive SARS-CoV-2 serology results strongly suggest a postinfectious mechanism, which seems to occur later in age and to include more frequently myocarditis, gastrointestinal signs and inflammatory syndrome than classical KD.

In MIS-TS, the adult presentation is very similar to children, except for frequent respiratory features and uncommon mucocutaneous symptoms, without complete KD criteria. These dissimilarities should not prevent physicians to consider MIS-TS in adult patients, especially because the main difference between children and adults seems to be a higher severity of the adults' condition, with consistent myocarditis, and a higher prevalence of acute kidney injury and circulatory failure. The adult cohort seems to present higher severe prognostic factors that we identified in our initial study, with respectively higher ferritin levels (median above 1400 µg/L) and older age. Moreover, median CRP levels were higher in adults (363 mg/L) compared with children (207 mg/L): the threshold of 300 mg/mL was reported as a feature of severity by a British Delphi study.[6]

This discrepancy might be explained by a recruitment bias of our adult cases, but also by under-recognition of mild forms in

Table 1 Comparison between paediatric Kawa-COVID-19 cohort and adult Kawa-COVID cohort

Clinical and biological results	Adult Kawa-COVID (N=9)	Paediatric Kawa-COVID-19 cohort (N=16)	P value
Median age (IQR)	25 (19–33)	10 (4.7–12.5)	
Male gender	5 (55%)	8 (50%)	1.0
Comorbidities; n (%)	4	6 (37%)	1.0
Overweight	4	4	0.63
Clinical features: n (%)			
Fever	9 (100%)	16 (100%)	1.0
Skin rash	1 (11%)	13 (81%)	0.035
Hands and feet erythema/ oedema	2 (22%)	11 (68%)	0.14
Conjunctivitis	4 (44%)	15 (94%)	0.062
Dry cracked lips	1 (9%)	14 (87%)	0.024
Cervical lymphadenopathy	3 (33%)	6 (37%)	0.84
Gastrointestinal signs	8 (88%)	13 (81%)	1.0
Neurological signs	6 (66%)	9 (56%)	0.69
Respiratory symptoms	8 (88%)	2 (12%)	0.0003
KDSS	4 (44%)	7 (14%)	0.67
Complete Kawasaki disease: n (%)	0	10 (71%)	<0.0001
Biological results: median (IQR)			
CRP (mg/L)	363 (278–439)	207 (162–236)	0.0004
Platelets (g/L)	240 (128–243)	188 (164–244)	0.64
Lymphocytes (g/L)	0.6 (0.33–0.87)	1.15 (0.8–1.7)	0.023
Natremia (mmol/L)	132 (129–134)	130 (127–134)	0.91
Creatinine (µmol/L)	140 (83–439)	59 (44–124)	*
Albumin (g/L)	24 (20–25)	21 (19–23)	0.29
SGOT (UI/L)	120 (75–166)	48 (33–86)	0.012
SGPT (UI/L)	103 (69–139)	35 (33–86)	0.042
Ferritinaemia (g/L)	2124 (833–6205)	1067 (272–1709)	0.049
Troponin (ng/L)	1164 (765–2666)	58 (36–165)	0.006
BNP (pg/ml)	24 540 (2585–35 000)	4319 (2747–6493)	0.17
Echocardiography abnormalities: n (%)	9 (100%)	11 (69%)	
Myocarditis	9 (100%)	7 (44%)	0.008
Coronary dilations	1 (11%)	3 (19%)	0.63
Pericarditis	1 (11%)	4 (25%)	0.32
Treatment: n (%)			
Intravenous Ig	6 (66%)	15 (93%)	0.12
Single infusion	6 (66%)	9 (56%)	0.69
Second infusion	0	6 (37%)	0.057
Steroids	3 (33%)	3 (18%)	0.63
No anti-inflammatory treatment	3 (33%)	1 (6%)	0.12

*No statistical analysis was possible due to different standards between children and adults.
BNP, brain natriuretic peptide; CRP, C reactive protein; Ig, immunoglobulin; KDSS, Kawasaki disease shock syndrome; SGOT, Serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

adults, which may be confounded with ongoing SARS-CoV-2 infection. Moreover, this could lead to delayed diagnosis, and therefore delayed treatment. This latency might partially explain an increased severity in adults. Given the potential life-threatening injury and the current active pandemic of SARS-CoV-2, clinicians should be alert and look for signs of MIS-TS, including myocarditis features in adults. Diagnosing these forms as early as possible may optimise clinical management and outcome. Ig infusions and corticosteroids with proven benefits in KD[7] may have a potential effect in this novel entity. Further studies are warranted to determine the risk factors associated with MIS-TS, its relevant pathogenesis, the benefit of IVIg and/or corticosteroids, and long-term outcome.

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Correspondence on 'Glucocorticoid-induced relapse of COVID-19 in a patient with sarcoidosis'

Patients with interstitial lung disease have been considered at high risk of complications of COVID-19 because of their underlying lung disease and use of immunosuppressive agents.[1] However, data on COVID-19 in patients with sarcoidosis are scarce.[2–4] Several reasons for an increased risk of severe forms of COVID-19 among sarcoidosis patients have been hypothesised: the involvement of the lung in almost 90% of patients with COVID-19, some of whom have reduced pulmonary function and comorbidities such as diabetes or hypertension, which are largely associated with the use of glucocorticosteroids for treating sarcoidosis; and the use of immunosuppressive agents in a subset of these patients.[5] Recently, Gyorfi *et al*[6] described the case of a patient with sarcoidosis who experienced a symptomatic SARS-CoV-2 infection with spontaneous clinical improvement, and a virological relapse after steroids treatment. This case illustrated the fact that immunosuppression with glucocorticoids may induce relapse of COVID-19 in patients with sarcoidosis. However, we lack data on the outcomes of patients with sarcoidosis affected by COVID-19. We retrospectively collected data for all patients with sarcoidosis and SARS-CoV-2 infection seen among 15 French centres between 1 March and 20 May 2020. The inclusion criteria were a sarcoidosis diagnosis based on the American Thoracic Society/European Respiratory Society/World Association for Sarcoidosis and other granulomatous diseases criteria[7] and SARS-CoV-2 infection based on at least one of the following: nasopharyngeal or tracheal swab reverse transcription (RT)-PCR positive for SARS-CoV-2; SARS-CoV-2-positive serology; or typical clinical and radiological findings.

Thirty-six patients were included. Among them, 34 patients had a confirmed SARS-CoV-2 infection: 3 were not tested with SARS-CoV-2 RT-PCR but had positive serology; 31 were tested with SARS-CoV-2 RT-PCR, which was positive in 29 patients. The two remaining patients had typical clinical and radiological findings despite negative RT-PCR results and were admitted to the hospital, with one patient being admitted to the intensive care unit (ICU). These two patients were not tested with serology. The demographic data and clinical features of the patients are detailed in table 1. Among the patients, 33% had lung fibrosis. The presenting symptoms of COVID-19 were classical and included fever in most of the patients (67%), despite the use of glucocorticosteroids. Twenty-five (69%) patients were receiving long-term treatment with glucocorticosteroids at the time of COVID-19 diagnosis. The steroids were stopped in only one patient at the time of COVID-19 diagnosis (this patient initially received a daily dose of 7.5 mg). The daily dose of steroids was increased in five patients because of COVID-19 diagnosis. Corticosteroids were introduced in two patients who did not previously receive this treatment for sarcoidosis. In comparison with steroids, methotrexate was more frequently stopped at the time of COVID-19 diagnosis, in 4/8 (50%) patients. Among the six patients under tumour necrosis factor (TNF)-alpha antagonist treatment, the treatment was temporarily suspended in all. All patients were admitted to ICUs when needed.

Five patients died during the SARS-CoV-2 infection: four patients died from acute respiratory failure due to SARS-CoV-2 infection. Of note, two patients had chronic renal failure, one

Table 1 Demographic and clinical characteristics and outcomes of 36 patients with sarcoidosis and COVID-19

Characteristics	All (n=36) n (%)	Admitted in ICU (n=13) n (%)	Not admitted in ICU (n=23) n (%)
Age at sarcoidosis diagnosis (years), median (range)	38.5 (21–74)	39 (30–61)	38 (21–74)
Age at COVID-19 diagnosis (years), median (range)	54.5 (26–100)	55 (36–100)	51 (26–90)
Sex (female/male)	10/26	03/10/20	7/16
BMI, kg/m^2, median (range)	26.4 (12.6–38.4)	25.0 (12.6–38.4)	26.1 (19.5–35.2)
Active smoker	4 (11)	2 (15)	2 (9)
Past smoker	9 (25)	4 (31)	5 (22)
Overweight	13 (36)	3 (23)	10 (43)
Obesity	7 (19)	3 (23)	4 (17)
Chronic comorbidities			
COPD	2 (6)	1 (8)	1 (4)
Diabetes	12 (33)	8 (62)	4 (17)
With insulin	5 (14)	4 (31)	1 (4)
Hypertension	14 (39)	6 (46)	8 (35)
Malignant tumour	2 (6)	1 (8)	1 (4)
Chronic kidney disease	5 (14)	3 (23)	2 (9)
Dialysis	1 (3)	1 (8)	0
Cardiovascular disease	3 (8)	2 (15)	1 (4)
ACE inhibitor or ARB	3 (8)	0	3 (13)
History of thrombosis	1 (3)	1 (8)	0
Organ transplantation	2 (6)	0	2 (9)
Sarcoidosis involvement			
Pulmonary	35 (97)	13 (100)	22 (96)
Intrathoracic lymph nodes	32 (89)	11 (85)	21 (91)
ILD	26 (72)	10 (77)	16 (70)
Lung fibrosis	12 (33)	5 (38)	7 (30)
Skin	6 (17)	2 (15)	4 (17)
Peripheral lymph nodes	5 (14)	2 (15)	3 (13)
Liver	7 (19)	5 (38)	2 (9)
Heart	4 (11)	2 (15)	2 (9)
CNS	7 (19)	3 (23)	4 (17)
PNS	3 (8)	2 (15)	1 (4)
Kidney	3 (8)	2 (15)	1 (4)
Löfgren syndrome	1 (3)	0	1 (4)
Treatments at the time of COVID-19			
Corticosteroids	25 (69)	11 (85)	14 (61)
Daily dose, median (range)	7.8 (5–50)	7.5 (5–50)	8 (5–35)
Hydroxychloroquine	3 (8)	0	3 (13)
Methotrexate*	8 (22)	4 (31)	4 (17)
MMF*	3 (8)	1 (8)	2 (9)
Azathioprine*	3 (8)	1 (8)	2 (9)
TNF-alpha antagonist*	6 (17)	1 (8)	5 (22)
Signs and symptoms at diagnosis			
Fever	24 (67)	12 (92)	12 (52)
Cough	29 (81)	11 (85)	18 (78)
Shortness of breath	24 (67)	11 (85)	13 (57)
Anosmia	9 (25)	0	9 (39)
Dysgeusia	7 (19)	0	7 (30)
Diarrhoea, nausea or vomiting	10 (28)	2 (15)	8 (35)
RT-PCR positive	31/33 (94)	12/13 (92)	19/20 (95)
Serology positive	3/3 (100)	0/0 (0)	3/3 (100)
Chest CT findings: extension of GGO and/or consolidation			
Chest CT performed	27 (75)	11 (85)	16 (70)
None	0 (0)	0	0
Minor	5 (19)	0	5 (22)
Moderate	9 (33)	5 (45)	4 (17)

Continued

Table 1 Continued

Characteristics	All (n=36) n (%)	Admitted in ICU (n=13) n (%)	Not admitted in ICU (n=23) n (%)
Severe	11 (41)	5 (45)	6 (23)
Missing data	1 (4)	1 (9)	0 (0)
Treatments for COVID-19			
Antiviral	4 (11)	4 (31)	0
Hydroxychloroquine	5 (14)	3 (23)	2 (9)
Steroids	7 (19)	4 (31)	3 (13)
Management			
Admission to hospital	28 (78)	13 (100)	15 (65)
Admission in ICU	13 (36)	13 (100)	0
Mechanical ventilation	4 (11)	4 (31)	0
Outcomes			
Deaths	5 (14)	4 (31)	1 (4)
Thrombosis	3 (8)	2 (15)	1 (4)
Acute kidney injury	3 (8)	2 (15)	1 (4)
Bacterial infection	5 (14)	3 (23)	2 (9)

*All patients with methotrexate, MMF, azathioprine and/or TNF-alpha antagonists also received corticosteroids.
ARB, angiotensin receptor blockade; BMI, body mass index; CNS, central nervous system; COPD, chronic obstructive pulmonary disease; GGO, ground glass opacity; ICU, intensive care unit; ILD, interstitial lung disease; MMF, mycophenolate mofetil; PNS, peripheral nervous system; RT, reverse transcription; TNF, tumour necrosis factor.

was undergoing haemodialysis and the last patient had an acute kidney injury complicating the chronic renal disease and required renal replacement therapy during the COVID-19 course. One patient also had an active thromboembolic disease. The fifth patient, who was receiving a TNF-alpha antagonist treatment for sarcoidosis, died from acute and uncontrollable hypercapnia in the context of chronic obstructive pulmonary disease and obesity. Thirteen patients (36%) were admitted in ICU (table 1). The admission in ICU was always possible, when decided by the treating physician.

Our findings support previous data obtained on COVID-19 in autoimmune diseases.[8 9] Although it is estimated that 15%–20% of people infected with COVID-19 develop severe pneumonia and that 5%–10% require critical care in the general population, we found a higher percentage of patients requiring intensive care support (36%) among the sarcoidosis population, probably because the study population was recruited from hospital-based centres. The percentage of patients with lung fibrosis was similar in patients admitted in ICU and those who were not.

The role of immunosuppressive agents in the course and severity of COVID-19 is still debated.[10] Our results share similarities with those of previous studies about COVID-19 in patients with immune-mediated inflammatory diseases.[11–13] In a previous study, the use of oral glucocorticosteroids and methotrexate was higher among patients for whom hospitalisation was warranted.[11] We found that TNF-alpha antagonist treatment was not associated with more severe forms of the disease, even if this should be considered with caution in this small sample. This is in accordance with the results of previous studies that supported the safety of chronic use of TNF-alpha antagonist treatment.[9 11]

With this study of 36 patients with sarcoidosis and COVID-19 from a French multicenter registry, we provide a better understanding of the implications of COVID-19 in the sarcoidosis population.

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FJ and RL contributed equally.
HN and FC-A contributed equally.

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HN and FC-A are joint senior authors.

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Response to: 'Correspondence on 'Glucocorticoid-induced relapse of COVID-19 in a patient with sarcoidosis" by Jeny *et al*

We thank Cohen Aubart *et al* for their correspondence.[1] Their study on 36 patients with sarcoidosis and COVID-19 from 15 French centres provides interesting insights on the outcome of COVID-19 in this group of patients. These data provide evidence that a higher percentage of patients with sarcoidosis with COVID-19 might require intensive care support than in the general population. In contrast, sarcoidosis does not seem to affect the severity of COVID-19, and patients with sarcoidosis-associated interstitial lung disease (ILD) are not admitted more often to the intensive care unit (ICU) than patients without ILD.

The data by Cohen Aubart *et al*[1] show that 85% of the patients with sarcoidosis with COVID-19 admitted to the ICU received a long-term glucocorticoid therapy, in comparison with 61% of the patients who did not require admission to the ICU. The two groups received glucocorticoids in similar doses (median daily dose 7.5 and 8 mg of prednisolone, respectively). Although the numbers are too low for statistical analyses, these data may support a more severe course of COVID-19 in patients treated with glucocorticoids. This conclusion is also supported by other publications on the outcome of COVID-19 in patients with other immune-mediated inflammatory diseases treated with glucocorticoids.[2 3] Moreover, our case report provides first evidence that initiation of glucocorticoid treatment might induce relapse of COVID-19.[4]

The study of Cohen Aubart *et al*[1] also provides insights how the SARS-CoV-2 pandemic affected treatment decisions in rheumatology. Between 1 March and 30 May 2020, physicians decided to continue glucocorticoid therapy, whereas most tumour necrosis factor α (TNF-α) inhibitors were discontinued in patients with sarcoidosis. Recent data show, however, that patients with immune-mediated inflammatory diseases receiving treatment with cytokine inhibitors, in particular TNF-α blockers, have low prevalence of COVID-19 and tend to have a milder course of the SARS-CoV-2 infection.[3 5]

Taken together, the available data indicate that glucocorticoids may have a negative impact on the outcome of COVID-19, whereas cytokine-targeting therapies such as TNF-α blockers may not. In the context of sarcoidosis, this may argue for a temporary suspension of glucocorticoid therapy, but continuation of TNF-α inhibition.

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Correspondence to 'Prevalence of hospital PCR-confirmed COVID-19 cases in patients with chronic inflammatory and autoimmune rheumatic diseases'

We read with great interest the article by Pablos *et al*.[1] However, we consider some methods and findings in the study that need to be further clarified.

First, detection bias may exist in this study because patients with AI/IMD visit hospitals for medical examinations regularly. Therefore, patients under follow-up in rheumatology department exactly showed higher prevalence of hospital PCR+-COVID-19 than the reference population.

Second, to the best of our knowledge, Sjögren's syndrome is a slow-developing syndrome.[2] However, we can find out that in this study, patients with Sjögren's syndrome showed remarkably higher rates of COVID-19 than those in the other AI/IMD groups, despite the fact that Sjögren's syndrome is mild than others. Therefore, we suggest that the authors need to explain this result in the discussion.

Third, in general, more serious cases of autoimmune diseases, that is, systemic lupus erythematosus (SLE), will have tendency to become more susceptible to COVID-19. However, it is not the case in this research article. The authors explained the reason was due to frequent use of antimalarial drugs, that is, chloroquine/hydroxychloroquine in patients with SLE. However, despite the side effects of chloroquine/hydroxychloroquine, it has been proved to have no therapeutic effect in patients with COVID-19.[3] So, the explanation may not be true in SLE. In addition, spectrum of autoantibodies in different autoimmune diseases may co-relate with the susceptibility for COVID-19.

If the reasons mentioned in the correspondence could be clarified and discussed further, the study will become a great pioneer to identify the risk factors of COVID-19 in the future and to combat the new and threatening virus.

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Response to: 'Correspondence to 'Prevalence of hospital PCR-confirmed COVID-19 cases in patients with chronic inflammatory and autoimmune rheumatic diseases" by Wu *et al*

We thank Wu *et al* for their interest in our study reporting on the prevalence of COVID-19 in patients with rheumatic diseases (RMD).[1] Wu *et al* suggest that potential detection bias due to more frequent regular visits to hospitals in patients with RMD may explain the observed greater prevalence of hospital COVID-19 cases in these patients. However, this is highly unlikely since the identification of hospital PCR+ cases was performed in April 2020, still in the peak phase of COVID-19 pandemic in Spain, when the regular scheduled follow-up visits had been cancelled and restricted to urgent medical care. In addition, PCR test shortage resulted in the need to restrict testing to 'COVID-19-likely' symptomatic patients at emergency departments, making unlikely preferential testing of RMD or other specific patients. Nevertheless, we cannot exclude other biases such as greater attendance to emergency departments of patients with RMD due to other factors, such as greater concerns on infection risk related to immunosuppressive therapy. However, this is also unlikely to be the case since in a further analysis of these patients, we found very high and similar rates of hospitalisation (>70%), which we can use as an estimate of severity in both RMD and reference cases, suggesting that the greater prevalence of COVID-19 in RMD is not explained by more attendance to emergency departments of milder cases.[2]

An important consideration to interpret our study is that we report crude prevalences not adjusted for age and sex. Since both factors influence COVID-19 severity, they should similarly influence the prevalence of hospital cases that are representative of the more severe symptomatic patients attending reference hospitals and not of mild community cases.[3] These factors may partially explain some of the observed differences in prevalences between different RMD groups, that is, the lower prevalence in patients with systemic lupus erythematosus (SLE), since these were younger and more often women than reference cases. Despite these limitations, our study suggests differences in the prevalence of COVID-19 among different RMD diagnostic groups, that is, non-SLE autoimmune diseases vs inflammatory arthritis, which do not seem to be explained just by age and sex bias.

With respect to the apparent greater prevalence of COVID-19 observed in Sjögren's syndrome in our patients, we should note that the number of cases with this condition in our study was small, not allowing for a firm conclusion on this aspect. Only grouped data on types of diseases (ie, SLE vs non-SLE autoimmune diseases) could be analysed. Comparing prevalence of hospital cases and outcomes in each RMD will require higher sample sizes.

It is not possible at this time to hypothesise a parallelism between the potential severity of RMDs and the prevalence of hospital COVID-19. In fact, our data do not support this concept. In our further analysis of the severity factors of COVID-19 in patients with RMD, age, sex or having any autoimmune or immunomediated disease, but neither chronic arthritis, nor therapies or comorbidities, were independent factors for severe outcomes.[2] Regarding the effect of antimalarials, we acknowledge that newer evidence suggests their unlikely therapeutic role. However, we believe that a potential prophylactic effect in patients with SLE patients is an still unanswered question, despite the results of the small study referenced by Wu *et al*.[4]

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No increased rate of SARS-CoV-2 infection for patients with inflammatory rheumatic diseases compared with the general population in the city of Hamburg (Germany)

We highly appreciated the work on the paper by Gianfrancesco *et al*.[1] While this large international registry provides information, for example, about the course of the disease in regard to the intensity of immunosuppression or complications, they do not allow any conclusions about the actual incidence rate of infections in patients with rheumatic diseases compared with the overall population. In addition to the data by Gianfrancesco *et al* we here like to share our data and experience of the Hamburg COVID-19 registry.

Until 9 June 2020, a total of 5120 proven severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections were reported in Hamburg.[2] This corresponds to 0.28% of the total population of the city of Hamburg (1.814 million inhabitants), with a reported mortality rate of 4.4% (in total 226 patients).

With the beginning of the COVID-19 pandemic we initiated a SARS-CoV-2 registry, where all reported COVID cases were documented anonymously by all rheumatologists of the city of Hamburg. In total, 11 771 patients were prescribed any disease-modifying antirheumatic drug (DMARD) during this period. Of these, a total of 30 (0.25%) patients had a clinically tested SARS-CoV-2 infection (clinical symptoms and SARS-CoV-2 PCR and/or IgG positive). Three out of 30 patients with rheumatic diseases (10%) were treated with severe disease in intensive care unit, in contrast to 4.4% of patients with COVID of the general population. So far, no deaths were reported in our cohort (mortality rate 0%) (see table 1).

In analogy to the COVID-19 Global Rheumatology Alliance registry, our cohort found no evidence that individual rheumatological diseases lead to a higher risk for or a severe course of infection. Additionally, so far, no accumulation of infection among one of the therapy groups (conventional synthetic DMARD, biological DMARD or targeted synthetic DMARD) was apparent.[1]

Table 1 Number of SARS-Cov-2 infections, ICU admissions and death in the general population compared to DMARD treated patients as well as calculated incidences

	General population	DMARD-treated patients with rheumatic diseases
Total, n	1.814.000	10.771
SARS-CoV-2 infected, n (%)	5120 (0.28)	30 (0.25)
ICU, n (%)	227 (4.4)	3 (10)
Death, n (%)	226 (4.4)	0 (0)

DMARD, disease-modifying antirheumatic drug; ICU, intensive care unit; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

To the best of our knowledge, this is the largest population-based study to date in this particular risk group. We consider the risk of unreported cases for the group of the general population comparable with that for the patients with rheumatic diseases.

In summary, patients with rheumatic diseases and under DMARD therapy do not seem to have a higher risk of a SARS-CoV-2 infection.

Additionally, in this cohort patients with rheumatic diseases did not have a higher rate of a severe course of SARS-CoV-2 infection.

Peer Aries,[1] **Christof Iking-Konert** [2]

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Correspondence on 'Long-term outcome of a randomised controlled trial comparing tacrolimus with mycophenolate mofetil as induction therapy for active lupus nephritis'

I read with great interest the recently published long-term outcome of a randomised controlled trial comparing tacrolimus with mycophenolate mofetil as induction therapy for active lupus nephritis.[1] Emerging evidence showed the efficacy of calcineurin inhibitors (CNIs) in patients with lupus nephritis, including ciclosporin A, tacrolimus and voclosporin.[1–5] However, I am concerned about the risks of thrombotic microangiopathy (TMA) and posterior reversible encephalopathy syndrome (PRES) when CNIs were given for patients with systemic lupus erythematosus (SLE) with concomitant antiphospholipid syndrome (APS). The anticardiolipin or lupus anticoagulant was present in 48 out of 150 patients (28%) in the study by Mok *et al*,[3] which was not reported in other studies.[2 4 5] There were about 17.6% patients with TMA in a cohort of 341 patients with stable lupus nephritis, who had the poorest renal outcome.[6] The use of CNIs in patients with SLE/APS should be prudent because both CNI and APS are risk factors for the occurrence of TMA,[7] especially for whom with the presence of histological features of TMA. PRES is contributed by endothelial cell dysfunction. SLE/APS, renal impairment and CNIs are known risk factors for PRES.[8] There were 4 out of 177 cases reported PRES in the voclosporin group regardless of the dosage, whereas none in the placebo group from the Aurinia Urinary Protein Reduction Active - Lupus With Voclosporin (AURA-LV) study.[4] I also noted one case developed epilepsy in the group of multitarget therapy, but none in the group of cyclophosphamide from the study by Liu *et al*.[2] The use of CNIs in patients with SLE/APS may confer a higher risk of PRES. In summary, the risks of TMA and PRES should not be neglected when CNIs are used for patient with SLE/APS. The authors should be encouraged to report the outcomes of CNIs use in patients with SLE and APS, which would shed light on the management of these patients.

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Response to: 'Correspondence on 'Long-term outcome of a randomised controlled trial comparing tacrolimus with mycophenolate mofetil as induction therapy for active lupus nephritis" by Xu

I would like to thank Dr Xu[1] for his interest in our lupus nephritis (LN) randomised controlled trial.[2] Although 28% of the recruited patients were ever positive for the antiphospholipid (aPL) antibodies at study entry, only four (2.7%) of them had history of thromboembolism and two times positivity of the aPL antibodies that qualified the consensus criteria for the antiphospholipid antibody syndrome (APS).[3] Four more patients developed the APS on follow-up, giving rise to an overall prevalence of 5.3%, which is consistent with the figure reported in our entire cohort of systemic lupus erythematosus.[4] Only one patient with APS at entry developed new onset hypertension after induction therapy with tacrolimus. The posterior reversible encephalopathy syndrome (PRES) was not observed in any of the tacrolimus-treated patients.

Thrombotic microangiopathy (TMA) in kidney biopsy is a well-recognised poor prognostic feature of LN. Factors associated with TMA include the APS, thrombotic thrombocytopenic purpura and chronic use of the calcineurin inhibitors (CNIs).[5] Renal insufficiency, pre-existing hypertension, high lupus activity and the use of heavy immunosuppression that include high-dose glucocorticoids, cyclophosphamide, mycophenolate mofetil, CNIs and rituximab have been linked to the PRES, which occurred in <2% of Asian patients with SLE.[6–9] While the contribution of each of these factors cannot be easily differentiated, an inflammatory mechanism is increasingly suggested for the endothelial dysfunction in the PRES.[7] Although there is no evidence to indicate that the CNIs are contraindicated in APS patients, blood pressure, renal function, electrolytes and neurological symptoms should be closely monitored in users of this class of drugs. The APS or the presence of the aPL antibodies were not in the exclusion criteria of the voclosporin study mentioned by Dr Xu.[10] In view of the paucity of data in the literature, the prognostic value of TMA and its interaction with other risk factors in LN should be further explored in Chinese patients.

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Correspondence on 'EULAR recommendations for the management of antiphospholipid syndrome in adults'

2019 antiphospholipid syndrome (APS) guideline[1] has been launched and has given recommendations to the rheumatologists on how to manage APS in different situations. The guideline has been presented in detail but there are still some questions remain to be discussed. For example, we are still confused in some circumstances, in secondary APS, such as systemic lupus erythematous, glucocorticoids (GC) is widely used. But according to the guideline in primary APS, GC treatment is recommended only in first trimester of pregnancy or in catastrophic APS (CAPS). But what about in second and third trimester of pregnancy? What dosage of GC is recommended? It needs more open discussion.

In terms of CAPS, the definition has a strict request on biopsy. Yet, in the clinic practice, there are little chance to do the biopsy. Besides, thrombosis happened in 1 week is also a criterion hard to meet; sometimes, it could happen in more than 1 week. Is it possible that in the future, the definition of CAPS will be modified? Although we could give the diagnose of probable CAPS in these cases, patients are still in danger and have a great risk of severe complications, whose treatment should be similar to CAPS. For instance, GC and plasma exchange need to be used. Furthermore, the guideline did not recommend the choice of disease-modifying antirheumatic drugs in severe cases, is there a preference? More studies are needed to answer this question as well.

Another circumstance is that patients with high-risk aPL profiles and new-onset thrombosis, besides heparin, is there a high-priority for GC and plasma exchange or intravenous immunoglobulins ? Moreover, in obstetric APS, another argument is that if a woman with positive medium–high titres of antiphospholipid antibodies, but with just once or twice spontaneous miscarriages (less than 10 weeks' gestation), meaning not meeting the APS classification criteria, what should be done, to use aspirin or not? For these older pregnant women, it sounds like a disaster to have another miscarriage without any intervention. Maybe these questions need more evidence and more clear explanation from experts.

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Response to: 'Correspondence on 'EULAR recommendations for the management of antiphospholipid syndrome in adults' by Zhou *et al*

We thank Zhou *et al*[1] for their interest in the 2019 European League Against Rheumatism (EULAR) recommendations for the management of antiphospholipid syndrome (APS) in adults.[2]

Although glucocorticoids (GC) are widely used in systemic lupus erythematous-associated APS, there is some uncertainty about their use in primary APS, and more specifically, about their use and dosage in the second and third trimester of pregnancy. In the EULAR recommendations for APS management, the use of GC in patients with primary APS is recommended only in catastrophic APS (CAPS), and may be considered in refractory cases of obstetric APS at low-doses (≤10 mg prednisolone daily) and for only the first trimester.[2] The latter statement is based on expert opinion due to the limited evidence. The only retrospective cohort study that addressed this question is described in the accompanying article with the results of the systematic literature review (SLR) informing the EULAR recommendations.[3] This study compared the pregnancy outcomes between women treated with a combination with low dose aspirin and heparin, with or without the addition of prednisolone 10 mg/day which was discontinued at week 14 of gestation.[4]

In their second point, Zhou *et al* note some issues related to the feasibility of a diagnosis of CAPS in clinical practice. The 2019 EULAR recommendations include statements about the management of APS patients based on the currently available classification criteria for APS and for CAPS. The statement for the management of CAPS refers to patients with definite CAPS based on the McMaster RARE-Best practices guidelines for CAPS management.[5] Consideration of revising the classification criteria for CAPS was beyond the scope of these recommendations.

Zhou *et al* have also commented that EULAR recommendations 'did not recommend the choice of disease-modifying antirheumatic drugs (DMARDS) in severe cases, and whether there is a preference'. According to the current evidence, the use of DMARDS was discussed only for patients with refractory CAPS. The following statement was included in the table of recommendations which was graded as 4/D since it was based only on case reports: 'In patients with refractory CAPS, B cell depletion (eg, rituximab) or complement inhibition (eg, eculizumab) therapies may be considered'.[2] Ongoing studies have shown that inflammatory and thrombotic mechanisms coexist in APS and the role of immunoregulatory agents in APS, especially in refractory APS, is under investigation.[6]

Another question raised was whether 'there is a high-priority for GC and plasma exchange or intravenous immunoglobulins in patients with high-risk antiphospholipid antibody (aPL) profiles and new-onset thrombosis, besides heparin'. Currently, there is no evidence to support the use of GC and plasma exchange or intravenous immunoglobulins after a thrombotic event in patients with high-risk aPL profile, except the case of definite CAPS.

Finally, the use of aspirin in women with positive medium-high aPL titres but with just one or two spontaneous miscarriages (<10th week), not meeting the APS classification criteria, was addressed by the corresponding recommendation in Table 1 and the text (section 8.C, page 6).[2] Additionally, in the publication of the results of the SLR informing the EULAR recommendations,[3] there is a specific section about the 'Treatment of women with a history of two recurrent spontaneous abortions <10th week of gestation'. The task force agreed that treatment with low dose aspirin alone or in combination with heparin might be considered based on an individual's risk, but this statement was mainly based on expert opinion due to limited evidence since the majority of studies combined several types of pregnancy losses without specifying on 'non-criteria' APS. The presence of only one spontaneous miscarriage <10th week of gestation was not included in our search since there is no evidence that this sole manifestation might support the suspicion of obstetric APS.

We agree that more evidence is needed to adequately address these questions in the future, hopefully in the update of the EULAR recommendations for the management of APS in adults.

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Correspondence on 'Safety and tolerability of nintedanib in patients with systemic sclerosis-associated interstitial lung disease: data from the SENSCIS trial'

The Safety and Efficacy of Nintedanib in Systemic Sclerosis (SENSCIS) trial,[1] published in May 2019 in *New England Journal of Medicine*, analysed the efficacy and safety of nintedanib in the treatment of systemic sclerosis-related interstitial lung disease (SSc-ILD) over 52 weeks. A reanalysis of the safety and tolerability data was recently published in *Annals of the Rheumatic Diseases*.[2] In both articles, we could not find information on the incidence of serious infections and serious respiratory tract infections.

Further safety results of the SENSCIS trial, using a wider time frame than the original publications (i.e., up to 100 weeks of follow-up), are accessible at ClinicalTrials.gov website since December 2019.[3] In a closer look at the table reporting serious adverse events (you must do the math), there were 34 infections in nintedanib versus 14 in placebo group (each group had 288 patients). [1]Notwithstanding the fact that this is not the primary outcome of the study (what may affect the interpretation of the p values), the risk of serious infections is significantly higher in nintedanib group (risk ratio, 2.43, 95% confidence interval [CI], 1.33 to 4.43, p=0.003; risk difference, 6.9%, 95% CI, 2.1 to 11.8%). Bacterial or viral respiratory tract infections represented apparently 18/34 (53%) and 7/14 (50%) of serious infections in nintedanib and placebo groups, respectively. Eleven cases of serious infectious pneumonia were reported with nintedanib comparing with two in placebo arm,[3] representing a risk ratio of 5.50 (95% CI, 1.23 to 24.59, p=0.012; risk difference, 3.1%, 95% CI, 0.4 to 5.9%). Two fatalities in nintedanib arm were attributed to pneumonia after adjudication of the causes of deaths.[1]

It is possible that the net clinical beneficial effects of nintedanib are restricted to patients with the usual interstitial pneumonia (UIP)-like pattern on high-resolution CT (HRCT). In systemic sclerosis, the overwhelming majority of patients presents the non-specific interstitial pneumonia pattern on HRCT. In the INBUILD trial,[4] which included patients with progressive ILD of different aetiologies, randomisation of patients was stratified by the pattern on HRCT, and mortality and serious adverse events (SAEs) seem to have behaved differently in the subgroups. In non-UIP-like pattern subgroup, the incidence of fatality was 4/126 (3.2%) with nintedanib and 1/125 (0.8%) with placebo, while in UIP subgroup it was 7/206 (3.4%) with nintedanib and 16/206 (7.8%) with placebo (at 52 weeks; test of heterogeneity of odds ratios (ORs), p=0.036). SAEs occurred in 44/126 (34.9%) patients with nintedanib versus 33/125 (26.4%) with placebo in the non-UIP subgroup; in UIP subgroup, SAEs occurred in 63/206 (30.6%) patients with nintedanib versus 77/206 (37.4%) with placebo (heterogeneity of ORs, p=0.041). Therefore, there may be a shift in the safety pattern, with nintedanib causing a higher incidence of complications in non-UIP-like ILD and reducing clinically serious events in UIP-like ILD.

Despite the observed effect of nintedanib in reducing the loss of forced vital capacity in SSc-ILD, changes in pulmonary function tests are still surrogate endpoints. Further studies are necessary to prove the safety and the capacity of nintedanib in improving clinical outcomes that represent the burden of disease to patients with SSc-ILD.

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Response to: 'Correspondence on 'Safety and tolerability of nintedanib in patients with systemic sclerosis-associated interstitial lung disease: data from the SENSCIS trial'' by Bredemeier

Following the publication of data on the safety and tolerability of nintedanib in the SENSCIS trial,[1 2] and INBUILD trial,[3] Dr Bredemeier has raised the question of the risk of serious respiratory infections with nintedanib treatment in patients with systemic sclerosis-associated interstitial lung disease (SSc-ILD) and other interstitial lung diseases (ILDs).[4] We have made a thorough investigation into this question and concluded that the evidence from clinical trials does not suggest an increased risk of infections in patients treated with nintedanib. Further, the mechanistic effects of nintedanib, an inhibitor of tyrosine kinases, do not suggest a plausible mechanism by which nintedanib would affect the risk of infection.[5]

We acknowledge that in the SENSCIS trial, there were numerical imbalances between the nintedanib and placebo groups in the percentages of patients with overall serious infections (6.6% vs 3.5%) or serious lower respiratory tract infections (3.5% vs 1.7%). This difference was driven largely by serious adverse events of pneumonia (2.8% (n=8) vs 0.3% (n=1)), when pneumonia was defined using the single preferred term 'pneumonia' from the Medical Dictionary for Regulatory Activities. A detailed review of the cases of serious pneumonia in patients treated with nintedanib revealed that none was considered related to nintedanib by the investigator. Most of the cases (6 of 8) occurred after nintedanib had been discontinued and an explanation for the infection was apparent in the majority of cases (predominantly the use of immunosuppressive drugs such as mycophenolate or cyclophosphamide). In the INPULSIS trials in patients with idiopathic pulmonary fibrosis (IPF),[6] there was no evidence of an increased risk of overall serious infections, serious lower respiratory tract infections or serious pneumonia with nintedanib versus placebo (8.5% vs 8.5%, 5.6% vs 5.4% and 3.6% vs 3.8%, respectively). Similarly, in the INBUILD trial in patients with progressive fibrosing ILDs other than IPF, the risk of these infections was similar between the nintedanib and placebo groups (8.7% vs 8.2%, 5.7% vs 6.3% and 3.6% vs 3.3%, respectively).

Dr Bredemeier queries whether nintedanib has a worse safety profile in patients with fibrosing ILDs who do not have a usual interstitial pneumonia (UIP) pattern on high-resolution computed tomography (HRCT). When looking at data from the whole INBUILD trial, in patients who had other fibrotic patterns on HRCT, the frequencies of serious adverse events and fatal adverse events were similar between the nintedanib and placebo groups (42.9% vs 44.8% and 4.8% vs 7.2%, respectively). The heterogeneity of the patient population, with various comorbidities and comedications, needs to be borne in mind when interpreting the safety data from the INBUILD trial, but descriptive analyses suggest that nintedanib had a consistent safety profile between subgroups by fibrotic pattern on HRCT. Importantly, both in patients with a UIP-like fibrotic pattern on HRCT and in patients with other fibrotic patterns on HRCT, nintedanib was associated with a significant reduction in the rate of decline in forced vital capacity (mL/year) over 52 weeks compared with placebo (by 61% and 49%, respectively).[3]

In conclusion, our analyses indicate that in patients with SSc-ILD and with fibrosing ILDs with a progressive phenotype, nintedanib reduces the rate of progression of ILD, and the totality of the data does not suggest an increased risk of serious infections.

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Correspondence on 'Tumour necrosis factor inhibitors slow radiographic progression in patients with ankylosing spondylitis: 18-year real-world evidence'

We recently read with great interest the article by Koo *et al*.[1] In this retrospective study, the authors evaluated the effect of tumour necrosis factor (TNF) inhibitors on radiographic progression in patients with ankylosing spondylitis (AS) using real-world patient data with long-term follow-up information. They found that the TNF inhibitors could significantly impede the radiographic progression of AS patients. We commend the authors for performing this important study as the results could be very helpful in guiding practice in clinics. However, we noticed that the authors did not clarify the influence of TNF inhibitors on osteoporosis or vertebral fracture risk on imagings in the patients.

Osteoporosis is a commonly recognised problem in AS population, which can lead to serious consequences for the patients. For instance, patients with AS may have severe fractures and resulting neurological dysfunctions even after minor trauma due to osteoporosis. Similarly, AS patients may be vulnerable to have complications (such as instrumentation loosing and displacement) after surgery because of poor bone quality. Given the potentially dismal outcomes, it is highly imperative to aggressively treat the osteoporosis in AS patients. Currently, the effect of TNF inhibitors on osteoporosis in AS remains inconclusive. Previous studies have shown that osteoporosis or bone loss in AS are mainly caused by the inflammatory activities mediated by TNF-alpha.[2] Considering that TNF inhibitors could effectively decrease the inflammatory response in AS, it is easy to speculate that TNF inhibitors may likely improve the osteoporosis of AS patients. In support of this, prior observations have suggested beneficial effects of TNF inhibitors on bone mineral density (BMD) at lumbar spine of AS patients,[3 4] with infliximab, etanercept and adalimumab most commonly studied. However, most of these studies are observational cohort studies without long-term follow-up and control groups. In addition, impact of TNF inhibitors on hip BMD of patients is still unclear.[4] A further comprehensive literature search revealed a lack of data with regard to the effect of other TNF inhibitors (including certolizumab and golimumab) on osteoporosis in AS. Moreover, studies have also demonstrated that TNF inhibitors fail to prevent vertebral fracture progression in AS patients.[4–7] Therefore, according to these data available, we are still not able to determine the effect of TNF inhibitors on bone metabolism in AS. A recent systematic review and meta-analysis showed similar findings concerning this issue.[8] Specifically, this study disclosed no strong evidence for TNF inhibitors in increasing BMD at hip and spine of AS patients.[8] Given this situation, we recommend that more prospective and well-designed studies with appropriate sample size are needed to define the impact of TNF inhibitors on osteoporosis in AS. Noticeably, these studies should define the relationship between specific TNF inhibitors as well as their optimal regimen of administration (dosage, rhythm and duration) and osteoporosis in AS, considering that different drugs and administration methods may possibly have different therapeutic effects.

Published data have indicated that the osteoporosis in AS may also be attributed to other factors, such as age, menstrual status and pro-inflammatory interleukin-17 signalling.[9] Considering these aspects, whether additional drugs should be prescribed in AS patients for osteoporosis amelioration deserves investigation. Supporting this idea, a recent study evaluated the effect of a 2-year pharmacotherapy (combination of bisphosphonates and calcium/vitamin D supplements) on osteoporosis in AS patients and revealed a positive effect of this therapy on lumbar spine BMD of patients,[10] similar to preceding findings.[8] These preliminary data suggest an effective strategy to ameliorate osteoporosis in AS, although further confirmation is necessary. Importantly, this approach can also be further justified by previous reports showing bisphosphonates (including neridronate and pamidronate) as alone therapy or combination therapy with TNF inhibitors display beneficial effects on disease activity of patients with AS.[11–13] Similarly, interleukin-17 inhibitors are also shown to be able to relieve symptoms and radiographic progression of AS patients.[4] Evaluation of these drugs including those targeting other non-TNF mediated pathways (such as interleukin-6, JAK-STAT, CD20 and CTLA-4) on improvement in BMD of patients should be performed at present, as this result may impact the choice of osteoporosis treatment in AS.

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Response to 'Correspondence on "Tumour necrosis factor inhibitors slow radiographic progression in patients with ankylosing spondylitis: 18-year real-world evidence" by Zhang *et al*

We thank Zhang *et al*[1] for their interest in our study, titled "Tumour necrosis factor inhibitors slow radiographic progression in patients with ankylosing spondylitis: 18-year real-world evidence." We investigated long-term observational data using advanced statistical methods to determine whether changes in radiographic progression are affected by tumour necrosis factor inhibitors (TNFi) in patients with ankylosing spondylitis (AS).[2] However, our study did not consider the risk of osteoporosis or vertebral fractures while evaluating the effects of TNFi.

There are several studies on bone mineral density (BMD) in patients with AS. Compared with the general population, patients with AS have a lower BMD.[3–5] Furthermore, it is interesting that an increase in the modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS) correlated with the decreased lumbar BMD.[4] These results are possibly related to decreased physical activity and functional capacity owing to pain and stiffness.[6] Importantly, AS is a chronic inflammatory disease, and inflammation plays a key role in bone loss. Therefore, it is conceivable that anti-inflammatory drugs can affect not only mSASSS but also BMD.

Tumour necrosis factor-α is an important cytokine for the regulation of bone homoeostasis in arthritis. Therefore, there are various experimental results showing that TNFi can affect osteoblast differentiation and osteoclastogenesis.[7] Studies have also assessed how TNFi affect osteoporosis in patients with rheumatic disease.[8 9] TNFi differ from existing osteoporosis medications (such as bisphosphonate or denosumab) with respect to the mechanism that affects bone remodelling in rheumatic diseases such as ankylosing spondylitis. However, the effect of TNFi on BMD in AS is unclear. In addition, there is insufficient evidence for determining the effects of TNFi on BMD because of time consuming and confounding by indication . Studying the role of TNFi in osteoporosis and fractures will provide important experimental and clinical evidence for pathogenesis of AS.

However, showing the effect of TNFi on osteoporosis was difficult using our data. First, patients with AS in our electronic medical records (EMR) were young (mean age, 33.1±9.8 years) and mostly men (90%). Moreover, there is no reason to routinely measure BMD in young patients in real-world clinical practice. Therefore, there is limited or no information on BMD in the EMR, and hence, it was impossible to study the relationship between TNFi and osteoporosis. Second, dual-energy X-ray absorptiometry and quantitative computed tomography along with several laboratory indicators have been used for measuring BMD using data from the EMR. However, incorporating these various methods into statistical models was difficult.

Notably, we showed that TNFi can affect the radiographic progression of AS with either direct or indirect effects via inflammation. Although further research is needed, these effects of TNFi may be considered similarly in the study for BMD.

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Correspondence to 'Normal human enthesis harbours conventional CD4+ and CD8+ T cells with regulatory features and inducible IL-17A and TNF expression'

We read with great interest the work by Watad *et al*,[1] which the authors demonstrated the characterisation of enthesis-resident T cells and their corresponding cytokine responses upon stimulation. This commendable work mimicked enthesitis-involved inflammatory pathogenesis of spondyloarthritis, for instance, psoriatic arthritis (PsA). Particularly, the authors proposed that entheseal T cells may secrete interleukin (IL)-17 and much more tumour necrosis factor α (TNF-α) in response to anti-CD3 and anti-CD28 (as suggested in figure 3 by Watad *et al*[1]); furthermore, phosphodiesterase 4 (PDE4) inhibitors suppressed the expression of the above mentioned inflammatory cytokines (as suggested in figure 5 by Watad *et al*[1]). As PDE4 inhibitors have been used to treat autoimmune diseases and advanced malignancies,[2] we are highly interested in whether infliximab, a neutralised antibody for TNF-α and a widely prescribed biologic disease-modifying antirheumatic drugs for a number of autoimmune diseases, would as well modulate the immunity of entheseal or synovial T cells in patients with PsA in clinical settings.

We compared the RNA-sequencing profiles of synovial biopsies from patients with PsA naive to anti-TNF-α agents before and 10 weeks after infliximab treatment registered in the National Center for Biotechnology Information-Gene Expression Omnibus database. Overall, we identified 39 significantly expressed pathways using p value and Z-score visualisation, with 26 pathways up-regulated at a Z-score of above 1, and 13 pathways down-regulated at a Z-score of less than −1 (figure 1). Among the 26 upregulated pathways after infliximab treatment, well-documented immunomodulatory signalling pathways, including adrenomedullin signalling pathway,[3] transforming growth factor-β (TGF-β) signalling and aryl hydrocarbon receptor signalling, were noted; furthermore, B cell-involved pathways, including B cell receptor signalling and systemic lupus erythematosus-associated B cell signalling pathway, were as well activated after TNF-α blockage. Among the 13 downregulated pathways after infliximab treatment, both Tec kinase signalling and signalling by Rho family GTPases were significantly inhibited at a Z-score of less than −2. These findings are consistent with previous studies reporting that Tec kinases regulate signalling pathways downstream of T cell receptor (TCR) activation, followed by T cell development, cytokine production and T-helper cell differentiation.[4] On the other hand, these findings are in line with the fact that Rho GTPases initiate signalling following TCR activation, which allow them to modulate pathways responsible for T cell development, differentiation and activation.[5] Moreover, as IL-23 signalling, a pathway upstream of Th17 induction,[6] was also downregulated after infliximab treatment, it was suggested that reciprocal regulation between TNF-α and IL-17 took place in synovial T cells during anti-TNF-α therapy.[7]

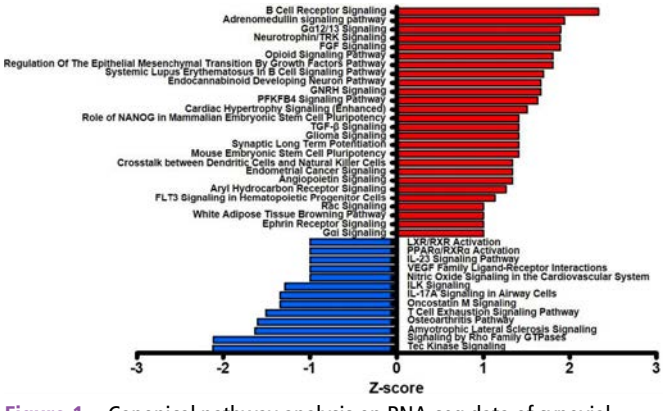

Figure 1 Canonical pathway analysis on RNA-seq data of synovial biopsy from patients with psoriatic arthritis receiving infliximab treatment after a follow-up of 10 weeks. Upregulated pathways are labelled in red. Downregulated pathways are labelled in blue. FGF, fibroblast growth factor; FLT3, FMS-like tyrosine kinase 3; GNRH, gonadotropin-releasing hormone; IL, interleukin; ILK, integrin-linked kinase; LXR, liver X receptor; NANOG, homeobox transcription factor Nanog; PFKB4, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4; PPARα, peroxisome proliferator-activated receptor α; RXR, retinoid X receptor; TRK, tropomyosin receptor kinase; VEGF, vascular endothelial growth factor.

In conclusion, our data supported that the activity of entheseal and synovial T cells was suppressed in patients with PsA treated with TNF-α inhibitors, potently accompanying with an overall downregulation in pathways underlying the pathogenesis of PsA.

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Response to: 'Correspondence to 'Normal human enthesis harbours conventional CD4+ and CD8+ T cells with regulatory features and inducible IL-17A and TNF expression" by Wang and Ma

We thank Wang and Ma[1] for their comments on our description of T-cells and their cytokines profile at the normal human spinal enthesis.[2] Wang and Ma[1] report on synovial T-cells in psoriatic arthritis (PsA) obtained from synovial biopsies, and amongst other things, describes that infliximab therapy leads to a reduction in interleukin (IL)-23 related pathway transcripts indicating a potential pathogenic interplay between tumour necrosis factor (TNF)-α and IL-23/IL-17 axis at the synovium.[1]

The enthesis and synovium form what is known as the synovio-entheseal structure complex.[3] A major unresolved issue in the immunopathology of PsA, is the link between synovial and entheseal immune cells. Animal models suggest that disease either TNF or IL-23 originating enthesitis may drive synovitis,[4 5] but it is unclear if this is the case humans. It is possible that the findings of Wang and Ma[1] could be extended to the enthesis and bone but formal studies are needed since the precise link between these immune compartments is unclear. The authors' results suggest a 'dampening' of the IL-23/IL-17 axis following infliximab therapy by acting on TGF-β and aryl hydrocarbon receptor signalling, that are involved in the regulation of the 23/17 axis. These findings are of interest towards the further understanding of the link between the enthesis and synovium in PsA and spondyloarthritis.

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Correspondence on 'Long-term efficacy and safety of canakinumab in patients with colchicine-resistant familial Mediterranean fever: results from the randomised phase III CLUSTER trial'

We read with great interest the article "Long-term efficacy and safety of canakinumab in patients with colchicine-resistant familial Mediterranean fever: results from the randomised phase III CLUSTER trial" by Özen *et al*.[1] This article makes a remarkable contribution to treatment in patients with colchicine-resistant familial Mediterranean fever (FMF), which shows a great remission rate with minimal side effects. However, there are some aspects that need to be clarified and discussed.

First, it would be better to report the dominant attack-type of the patients because we think musculoskeletal type attacks, especially mild ones, could be skipped and not remembered by the patients when they are admitted to outpatient control. These attacks are also associated with increased damage.[2] Second, there is an inconsistency regarding patient number in canakinumab dosage group between figure 1 and text in the results section. It was written as "44 patients received <2700 mg canakinumab and 16 received ≥2700 mg". However, in the figure, it was stated as 42 and 15, respectively. Baseline median C reactive protein (CRP) levels were higher than normal, and we wonder whether the increased CRP levels are persistent. It was shown that persistent inflammation was related to the increased risk of amyloidosis, kidney dysfunction and proteinuria,[3] but the latter was not discussed in the manuscript. We would like to also know the effect of canakinumab on the proteinuria of those patients.

We appreciate the work of Özen *et al* to highlight the treatment of patients with colchicine-resistant FMF. We believe that this comprehensive study will help the clinician to manage these high-risk group of patients.

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Response to: 'Correspondence on 'Long-term efficacy and safety of canakinumab in patients with colchicine-resistant familial Mediterranean fever: results from the randomised phase III CLUSTER trial" by Satis *et al*

We thank Satis *et al*[1] for their interest in our article,[2] and will try to address their queries. Our colleagues suggest that flares with musculoskeletal symptoms as predominant signs may not be remembered by the patients when they report them. We reported that in Epoch 4 of the CLUSTER study, >90% of the patients treated with canakinumab experienced no flares or one flare throughout the 72-week period, while a median of 17.5 flares per year was reported before baseline. As detailed in the methods section and according to the study protocol, during the trial patients were considered to experience a flare when they present with a physician global assessment (which includes the assessment of musculoskeletal symptoms) ≥2 and C-reactive protein (CRP) ≥30 mg/L. On the other hand, if the phenomenon that Satis *et al*[1] mentioned occurred when patients reported the number of flares before the trial, it could have potentially led to an underestimation of the number of flares experienced in the previous year, thus making the difference with the rate of flares during the study even higher. Overall, we believe that it is unlikely that this phenomenon would affect significantly the results and conclusions in our manuscript. However, as we mentioned in the discussion of the limitations of the study, we acknowledge that a more standardised definition of flare would help to better define the target of familial Mediterranean fever (FMF) treatment.

Satis *et al*[1] suggest that there is an inconsistency between the reported number of patients receiving <2700 or ≥2700 mg as cumulative doses of canakinumab in the text and elsewhere. As explained in the patient disposition section, from the 60 patients who entered Epoch 4 of the CLUSTER study, three discontinued the study and 57 completed it. We correctly mentioned in the text that overall, 44 patients received <2700 mg canakinumab and 16 received ≥2700 mg. Figure 1 of our referred paper[2] indicates the patients in the lower boxes (ie, those who completed the study) who received each cumulative dose, and as Satis *et al*[2] mentioned, when we add the numbers in the boxes, these were 42 and 15. This is also correct as it refers only to the 57 patients who completed the study. What the figure does not mention explicitly is the cumulative dose received by the three patients who discontinued the study, it was ≥2700 mg for the patient receiving 150 mg every 4 weeks who discontinued the study due to pregnancy, and <2700 mg for the other two patients.

Satis *et al*[1] ask why baseline CRP levels were high. As mentioned in the article, patients had to have active disease with an ongoing flare when they entered the study (ie, baseline flare), and this is the reason for which their CRP levels were high. This is also mentioned specifically in the figure legend. Average CRP levels decreased quickly during Epoch 2 in patients treated with canakinumab, as previously reported.[3]

We would also like to point out that none of the patients had amyloidosis nor renal failure during the study. All patients entered the study with normal renal function, and the effect of canakinumab on proteinuria was not systematically analysed in this trial. Renal function was studied by creatinine clearance, as reported. However, only two adult patients with colchicine-resistant FMF presented with isolated events of newly occurring proteinuria during the whole study, as measured by protein urine dipstick. One of these patients presented with proteinuria at the last visit of the study and one with intermittent low levels of proteinuria at four different visits during Epochs 2, 3 and 4.

We hope that this additional information helps to further clarify some aspects of our study, and thank again Satis *et al*[1] for their correspondence.

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Correspondence on 'Rheumatoid arthritis-associated DNA methylation sites in peripheral blood mononuclear cells'

We read with interest the paper by Zhu *et al*[1] on DNA methylation, mRNA expression and their role in the pathology of rheumatoid arthritis (RA). We found that their findings highlighted the importance of *PARP9* gene DNA methylation in RA. However, we found that the study had some limitations that necessitate a cautious interpretation of the findings and merit further attention.

First, 43 subjects (RA:healthy controls=25:18) were included during the discovery stage, 52 (RA:healthy controls=25:27) for DNA methylation and 70 (RA:healthy controls=35:35) for mRNA expression. However, we found that the ratio of patients to healthy controls in the first two phases was not suitable. The number of healthy controls should be greater than that of patients (patients vs healthy controls=1:*R*, where *R* is equal to or less than 4) in case–control studies.

Second, patients with RA were separated into three sample sets, and the consistency of the results needs to be interpreted with caution. The main reasons for this are as follows: (1) patients with RA may have different disease status (eg, disease activity and age) across the three stages; (2) the three assays had different levels of sensitivity (ie, microarray analysis, bisulfate sequencing and real-time quantitative-PCR). However, the authors did not take these matters into account or adjust for them accordingly.

Third, we do not know why the authors chose the *PARP9* gene instead of other genes (eg, *IFI44L* and *MX1*) for their in vitro study. Is the *PARP9* gene the most representative gene available with respect to diagnostic value?

Fourth, Jurkat cells, as the authors said, are an immortal line of human T lymphocytes. They are frequently used as a cell model in studies of immune-related diseases.[2] The authors used Jurkat cells to investigate the functional effects of the *PARP9* gene. Are the Jurkat cells specific or RA-related? If they are not specific, we can say that these effects are also suitable for other autoimmune diseases including systemic lupus erythematosus.

Lastly, the authors used T cells from patients with active RA to explore the correlation between methylation and mRNA, which may mean that their results are applicable to many situations. Because the disease activities and inflammation levels of active RA are more intense than those of stable patients, it may be more appropriate to use T cells from newly detected and newly diagnosed cases.

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Response to 'Correspondence on 'Rheumatoid arthritis-associated DNA methylation sites in peripheral blood mononuclear cells" by Wang and Niu

We appreciate the interest and comments shown by Wang *et al* about our study in their letter.[1 2] We would like to clarify and discuss some issues Wang *et al* indicated.

First, matching design is generally used in case–control studies to eliminate the interference of potential confounding factors. Ideally, the subjects are perfectly matched in case–control (1:R) groups. In our research design stage, we also planned the ratio (1:1) of case–control that is commonly used in genetic and/or epigenetic studies. However, several steps of quality control were adopted in DNA/RNA preparation and microarray experiment, and some subjects were excluded, which led to the mismatched numbers, even though the sample ratio was originally designed as 1:1. The mismatched sample probably introduced some confounding effects, but such effects would be excluded to the greatest extent through our multiomics integration, replication in independent samples and in-depth functional validation. Actually, it is frequently observed that the numbers of cases and controls were mismatched in the genome-wide profiling study.[3]

Second, it is a common strategy that the identified DNA methylation sites and messenger RNAs (mRNAs) from microarray assays are validated using different technical methods (herein, bisulfate sequencing for DNA methylation and reverse transcription PCR for mRNA). Our research strategy is to integrate multiomics expression profiling (methylome and transcriptome) by using high-throughput microarray analysis and then to technically and biologically validate the significant findings in additional study samples with larger sample size. Although the three assays had different levels of sensitivity, we believe that the consistent results from different methodology and biological validation at DNA methylation level and mRNA expression level would be reasonable to warrant their significance for rheumatoid arthritis (RA), especially for the significance of poly (ADP-ribose) polymerase family member 9 (PARP9).

Third, the PARP9 gene was the most interesting gene after exploratory analysis and a series of confirmatory analyses.[1] PARP9 was affirmed to be causative among the regulatory chains of DNA methylation–mRNA–RA and highlighted in interaction networks constructed by the differentially methylated genes/differentially expressed genes. Among five validated methylation sites, three (cg00959259, cg08122652 and cg22930808) were located in the PARP9 gene. The significant correlations between methylation levels in PARP9 and gene expression were verified in peripheral blood mononuclear cells and Jurkat T cells, as well as in primary T cells. The above evidence taken together could justify the priority of PARP9.

Fourth, the study also used Jurkat cells as cell models to investigate the functional effect of the PARP9 gene. Based on current evidence, we cannot conclude that the effect is specific. As shown in the Discussion section, we have discussed that some of our findings were also reported in other autoimmune diseases,[4–6] suggesting that the identified sites in our study may serve as common sites shared by other autoimmune diseases. Further research would be needed to explore whether the RA-related methylation sites identified in the present study are unique to RA or common to other autoimmune diseases including systemic lupus erythematous.

Last, as shown in the Results section, a significant correlation between the methylation level (cg00959259) and PARP9 gene expression (r=0.752, p=0.019) was detected in the active RA cases. This experiment is to investigate whether the detected methylation sites have regulation effects on mRNA expression of PARP9 in the patients with RA with active disease status. Such patient selection strategy is consistent with those in the discovery and replication stages. The original purpose of this study is to find the abnormal methylation sites between active RA cases and healthy controls. Therefore, all the patients with RA in our study were recruited according to their active disease status but not limited to newly detected and newly diagnosed cases.

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Role of interaction between B cells and epithelial cells in pSS

We read with great interest the recent publication by Rivière *et al*, concerning the activation of B cells by salivary gland epithelial cells (SGECs) from patients with primary Sjögren's syndrome (pSS).[1] The authors elegantly show that coculture of $CD19^+$ peripheral blood B cells with SGECs isolated from labial salivary glands (LSGs) of patients with pSS increases expression of the B cell activation marker CD38, and the memory B cell marker CD27. This effect was the most noticeable with further stimulation of toll-like receptor 3 by means of Poly(I:C) medium supplementation. Treatment of cocultures with agents ablating specific B cell signalling pathways (ibrutinib, blocking BTK signalling), and non-B cell specific pathways (LY294002 blocking the PI3K pathway and leflunomide, inhibiting lymphocyte proliferation) reduced this effect somewhat.

We would like to take the opportunity to comment on these interesting findings. First, their main conclusion is that SGECs (by presumption ductal cells) from patients with pSS support the activation and survival of B cells. We would like to add that we have previously demonstrated in situ the presence of an epithelium-associated subset of B cells, expressing FcRL4, in both minor (labial) and major (parotid) SGs of patients with pSS.[2] This $FcRL4^+$ B cell subset is highly proliferative, and located both periductally and intraductally.[2] Follow-up studies by Verstappen *et al* using RNAseq revealed that parotid $FcRL4^+$ B cells in SGs express genes indicative of both chronic (T-bet, CD11c) and general (TACI, CXCR3, NF-kB signalling genes, IL-6) activation.[3] We further provided some evidence that these chronically activated (intra-)epithelial B cells are involved in epithelial cell proliferation, resulting in the formation of lymphoepithelial lesions (LELs).[2–4] The findings of Rivière *et al* support our notion of an intimate relationship between epithelium and B cells in SGs of patients with pSS, which might be very relevant for the pathogenesis of the disease. We would be extremely curious to see if in the studies of Rivière *et al*, FcRL4 is also differentially expressed in B cells sorted from LSGs of patients with pSS.

Second, Rivière *et al* employed cocultures of bulk (blood-derived) $CD19^+$ B cells with SGECs. We suggest that the unrefined use of the $CD19^+$ pool makes the interpretation of which B cell population, for example $CD27^-$ and $CD27^+$ naive and memory B cells, respectively, or $FcRL4^+$ B cells, proliferates (or dies) following SGEC coculture challenging to gauge. Additionally, the accurate recapitulation of pSS-associated B cell/SGEC dynamics in this system can be questioned, considering the lack of crucial B cell stimuli to fully mimic complete B cell activation (TLR7 and/or TLR9 agonism, presence of IL-21 and BCR stimulation). In the same trend, the application of potential therapeutic agents (eg, LY294002 blocking the PI3K pathway) and stimuli (IFN-α, IFN-γ and Poly(I:C)) to B cell–SGEC cocultures and read out via B cell viability render the data challenging to interpret in terms of effects on the epithelium. Considering the likely crosstalk between the two cellular entities, we wonder whether the authors plan to probe this in future experiments.

Finally, regarding the study of SGECs alone, the authors employed CD326 (EpCAM) to select epithelial cells from digested LSGs. Transcriptomics analysis of these $CD326^+$ cells was performed. We were surprised to see expression of immune genes such as *IGHG1*, *BTK* (B cell/monocyte restricted), *CD8A* (NK/T cell restricted) in the $CD326^+$ SGEC fraction, and were wondering how the authors interpret these findings (Table S3). The lack of pathways reflecting changes in epithelial cell dynamics (cell cycle, proliferation, apoptosis) may also suggest that this SGEC system do not truly mirror the in vivo situation suggested by other authors.[5–9] The inference from the paper is that $CD326^+$ cells equate to ductal cells in the LSG, or least no indication to the contrary is stated. Acinar cells, epithelial cells present in LSGs, also express CD326 (figure 1). Considering their likely involvement in pSS pathogenesis, acinar cells may be partly responsible for the immune profile observed.[10–13] Using SGECs might well be an oversimplification of the SG, and

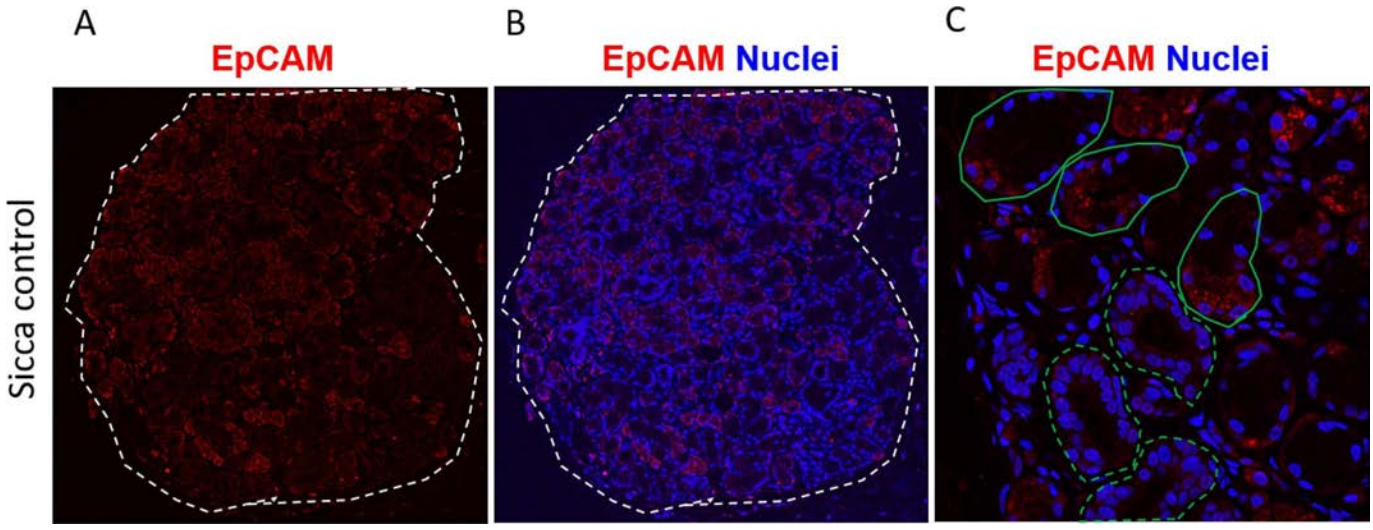

Figure 1 Both ductal and acinar epithelial cells express CD326 (EpCAM) in the LSGs. (A) EpCAM immunofluorescence staining of a sicca control LSG. (B) Merged EpCAM immunostaining with nuclear counterstain. (C) High-resolution microscopy of ductal cells (dashed green line) and acinar cells (solid green line), showing EpCMA expression by both. Methodology: paraffin sections of LSGs were dewaxed and rehydrated. Antigen retrieval was performed with a sodium citrate (pH 6.0) buffer containing 0.5% Tween. Sections were blocked in 1% bovine serum albumin (BSA), and incubated overnight at 4°C in mouse anti-human EpCAM conjugated to the fluorophore e660, at 1:100 dilution (eBioscience clone 1B7). Nuclei were counterstained with Hoechst and confocal microscopy performed with the Leica TCS Sp8 confocal microscope. LSG, labial salivary gland.

whether cultured SGECs are representative of in situ epithelial cells remains to be shown.

Although providing a platform for drug screening from which to further expand on and certainly representing an important contribution to the field, the applicability of two-dimensional epithelial cultures to in situ SG architecture remains to be validated. Three-dimensional approaches using SG organoids may represent a step towards true reflection of reality, and have been shown to provide valuable information about epithelial cell dynamics in pSS.[6] While acknowledging the novel and extensive work presented by Rivière *et al*, we look forward to seeing how the more intricate interactions between B cells and the epithelium can be teased apart, including those of LEL development.

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Response to: 'Role of interaction between B cells and epithelial cells in pSS' by Pringle *et al*

We thank Pringle *et al* for their interest and their comments[1] concerning our recent article demonstrating how salivary gland epithelial cells (SGECs) from patients with primary Sjögren's syndrome (pSS) can induce survival and activation of B cells.[2] They are rising six very interesting questions:

(1) F Kroese's group has extensively studied B cells expressing FcRL4 in pSS.[3 4] They have shown that this B cell subset is highly proliferating, express activation markers and participates to the formation of lymphoepithelial lesions. Interestingly, they have shown that FcRL4 mRNA was increased in parotid from patients with pSS with mucosa-associaed lymphoid tissue (MALT) lymphoma. FcRL4 could promote innate signalling in response to chronic antigenic stimulation. For all these reasons, it is tempting to speculate that FcRL4+ B cells could be involved in the cross-talk with SGECs.[5] We looked at FcRL4 expression in our RNA-seq data set (figure 1A). We did not detect FcRL4 mRNA in blood, nor in salivary gland biopsy. This could be explained by the fact that FcRL4 is likely to be more expressed within parotid[3] and our study exclusively involved minor salivary gland biopsies.

(2) Pringle *et al* wonders about the choice of total circulating B cells in the co-culture. We totally agree that co-culture with specific B cells subsets mainly present within the glands such as FcRL4+ B cells or CD27+ and CD27− B cells would be of interest. But sorting sufficient number of B cells from minor salivary gland that measures around 2–3 mm is, at this time, technically impossible. Plasma cells are another B cell subset infiltrating salivary glands in pSS.[6] We tried to differentiate blood B cells into plasmablasts and then performed co-culture with SGECs, but the viability was too low to allow fine assessment of the crosstalk.

(3) Pringle *et al* wondered why we did not used specific B cells stimulation in our co-culture. However, we made the hypothesis that SGECs could stimulate B cells by themselves without any stimulation, and thus we did not want to artificially stimulate them with the adjunction of cytokines or anti-µ. We used TLR3 stimulation in our co-culture models for mimicking a viral trigger, which could reinforce the ability of SGECs from patients with pSS to increase B-lymphocytes survival.

(4) Pringle *et al* have carefully analysed the profile of gene expression in SGECs provided in our study. We purified CD326+ SGECs, B lymphocytes, CD4 and CD8 T cells from salivary glands. In spite of this sorting, we found in some SGECs samples, some immunoglobulin genes due to a minor contamination by B cells expressing a high level of immunoglobulins genes. We excluded these samples from the analysis. The level of expression of some immune genes (BTK, CD8a and IGHG1) in SGECs, noticed by Pringle *et al*, was very low as shown in the figure 1B–D.

(5) Regarding CD326+ epithelial cells, we do agree that they can be either acinar or ductal. Based on the previous work by others on this type of culture,[7] we presume that they are rather ductal cells. However, it would be even more interesting if acinar cells that are the effective cells secreting saliva could also activate B cells.

(6) Finally, Pringle *et al* suggest that the two-dimensional co-culture might be too simplistic. We completely agree. This simple model just allows demonstrating the proof of concept that SGECs are able to activate B cells and that this phenomenon is increased with SGECs from patients with pSS. Development of three-dimensional approaches with organoids is promising. Of note, our group is currently working on a simpler model, which is the culture of a whole minor salivary gland that will also help to unravel from the inside lymphocyte infiltration within salivary glands.[8]

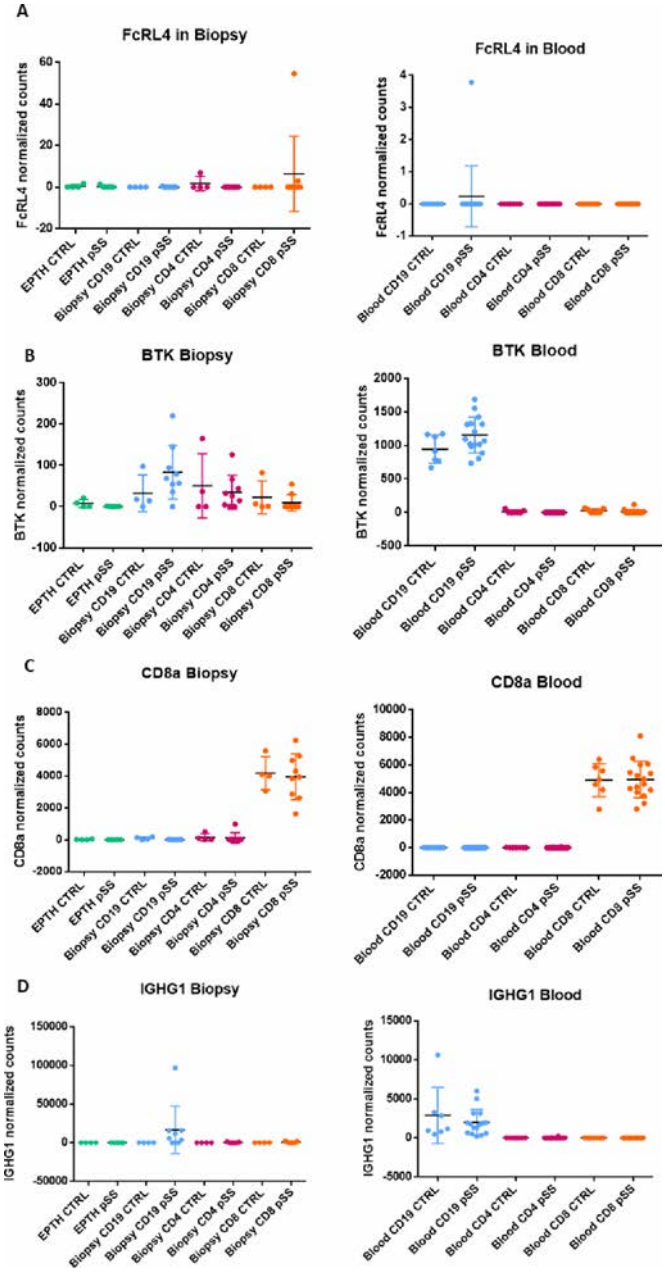

Figure 1 Representation of the normalised counts of FcRL4 (A), BTK (B), CD8a (C) and IGHG1 (D) in salivary gland epithelial cells, CD19+ B, CD4+ and CD8+ T lymphocytes sorted from biopsies (left panel) and CD19+ B, CD4+ T and CD8+ T lymphocytes sorted from blood (right panel), in controls and in primary Sjögren's syndrome (pSS).

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Elucidation of disease mechanisms underlying rheumatic immune-related adverse events may lead to novel therapeutic strategies for autoimmune diseases

We read with great interest the article 'EULAR points to consider for the diagnosis and management of rheumatic immune-related adverse events due to cancer immunotherapy with checkpoint inhibitors' by Kostine *et al*.[1] In this article, the authors performed a systematic literature review and generated the first set of recommendations for the diagnosis and management of rheumatic immune-related adverse events (irAEs) induced by checkpoint inhibitors.[1] As the authors noted, the paucity of literature on this issue has led to empirical treatment that is not based on evidence. Their points to consider provide a rationale for the management of rheumatic irAEs, as well as help rheumatologists engage with oncologists, thereby enabling patients with cancer to maintain a better quality of life. We have reported on the role of the programmed cell death protein-1 (PD-1) checkpoint in various vascular pathologies[2–4] and thus wanted to comment on this article, since vasculitis can occur as a type of rheumatic irAEs.[5 6]

First, the article stated that irAEs can affect any organ and cause a wide variety of autoimmune disease-like pathologies, with an estimated prevalence of 1.5%–22%; however, the risk factors for rheumatic irAEs remain unclear. Risk factors for exacerbation in patients with pre-existing rheumatic disease also remain unidentified. Recent studies have reported that variation in human leucocyte antigen is associated with the development of irAEs, such as colitis and adrenal insufficiency.[7 8] Further studies are needed to identify risk factors for rheumatic irAEs.[9]

Second, three treatment escalations for rheumatic irAEs were defined in the article: local/systemic glucocorticoids, conventional synthetic disease-modifying antirheumatic drugs (DMARDs) and biological DMARDs.[1] However, there are still many unanswered questions. For example, how do we reduce or discontinue these immunomodulators or when do we resume cancer immunotherapy? It is also of importance to monitor whether these immunosuppressive agents interfere with checkpoint inhibitors.

Third, and most importantly, as the authors highlighted in their research agenda, a better understanding of the pathophysiology of rheumatic irAEs is crucial. Early reports of fulminant myocarditis due to checkpoint inhibitors demonstrated that activated T cells were the main players in irAE pathology.[10 11] Using an experimental mouse model of large vessel vasculitis, we have previously reported that blockade of PD-1 signalling not only exacerbated vascular inflammation through the infiltration of activated T cells but also caused intimal thickening and adventitial neovascularisation, indicating that activated T cells play a central role in vascular remodelling.[2 3] What we want to emphasise here is that the disease mechanisms underlying rheumatic irAEs should be investigated, which may lead to novel therapeutic strategies for autoimmune diseases.

Despite the many unsolved problems, this article raised awareness of rheumatic irAEs among rheumatologists and created a blueprint for therapeutic strategies. Further studies are needed to better define basic and clinical aspects of rheumatic irAEs.

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Management of immune checkpoint inhibitor-induced polymyalgia rheumatica

We read with interest the relevant article by Kostine *et al*[1] describing the specific concerns for the diagnosis and management of rheumatic immune-related adverse events (irAEs), which are caused by immune checkpoint inhibitor (ICI) therapy. The authors recommend to consider glucocorticoid treatment if rheumatic irAEs are not sufficiently controlled by symptomatic treatment. The authors propose that systemic glucocorticoids should be promptly tapered to ≤10 mg predniso(lo)ne equivalent per day, since higher doses might potentially limit the efficacy of ICI therapy. One of the the most common rheumatic irAEs is a polymyalgia rheumatica-like syndrome (ICI-PMR). Prior reports indicate that ICI-PMR is typically treated with 12.5–25 mg prednisolone per day,[2 3] which is standard practice for regular PMR.[4] We here propose to use lower doses of prednisolone (ie, 5–7.5 mg per day) for patients presenting with ICI-PMR.

We evaluated the treatment requirements of six consecutive patients with ICI-PMR (online supplementary table 1), of whom the clinical and imaging findings were recently reported.[5] ICI therapy led to complete cancer remission (n=1), partial remission (n=4) or stable disease (n=1). ICI therapy was eventually discontinued in one patient (*patient 6*) due to cancer progression. Another patient (*patient 2*) showed oligoprogression of the cancer. This could be managed with radiotherapy, while ICI therapy was continued. The other patients showed a sustained tumour response ranging from >10 to >24 months after initiation of ICI therapy.

ICI therapy was briefly interrupted in one patient on the diagnosis of ICI-PMR, but continued in all other patients. One subject (*patient 4*) could be managed with nonsteroidal anti-inflammatory drug (NSAID) treatment only (figure 1A). Two subjects (*patients 2 and 3*) required a prednisolone dose of 5–7.5 mg per day (figure 1A; online supplementary figure 1, showing *patients 3*, *5* and *6*). Two other subjects (*patients 1 and 5*) briefly required 15 mg of prednisolone per day. *Patient 1* started with 15 mg prednisolone per day, but this could be tapered to 7.5 mg within 2 weeks. *Patient 5* was initially treated with 7.5 mg prednisolone per day; but this dose was promptly increased to 15 mg after 1 day and could already be decreased to 7.5 mg after 3 days. One subject (*patient 6*) received methotrexate as steroid-sparing disease-modifying antirheumatic drug (DMARD) (online supplementary figure 1). *Patient 6* initially received 7.5 mg prednisolone equivalent per day due to hypophysitis/adrenal insufficiency; and this was raised to 10 mg prednisolone equivalent per day when ICI-PMR was diagnosed. Due to ongoing disease activity and concerns regarding the potential effect of higher glucocorticoid doses on the efficacy of ICI therapy, a decision was made to add methotrexate to the treatment. The patient eventually died of infection after initiation of chemotherapy.

Prednisolone-free remission was obtained in three subjects (figure 1A). The ICI-PMR went into remission in *patient 4* despite continuation of ICI therapy. *Patient 2* reached prednisolone-free remission shortly after completion of ICI therapy. *Patient 1* reached prednisolone-free remission at 1 year after the final ICI infusion. A [18F]-fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT) scan was performed during prednisolone-free remission in two subjects (figure 1B). These follow-up scans showed markedly lower FDG uptake scores when compared with the previously reported scans obtained at the diagnosis of ICI-PMR.[5] Thus, the FDG-PET/CT scan confirmed that the ICI-PMR had subsided in both patients.

In conclusion, ICI-PMR may require lower doses of prednisolone than recommended by clinical guidelines for regular PMR.[4]

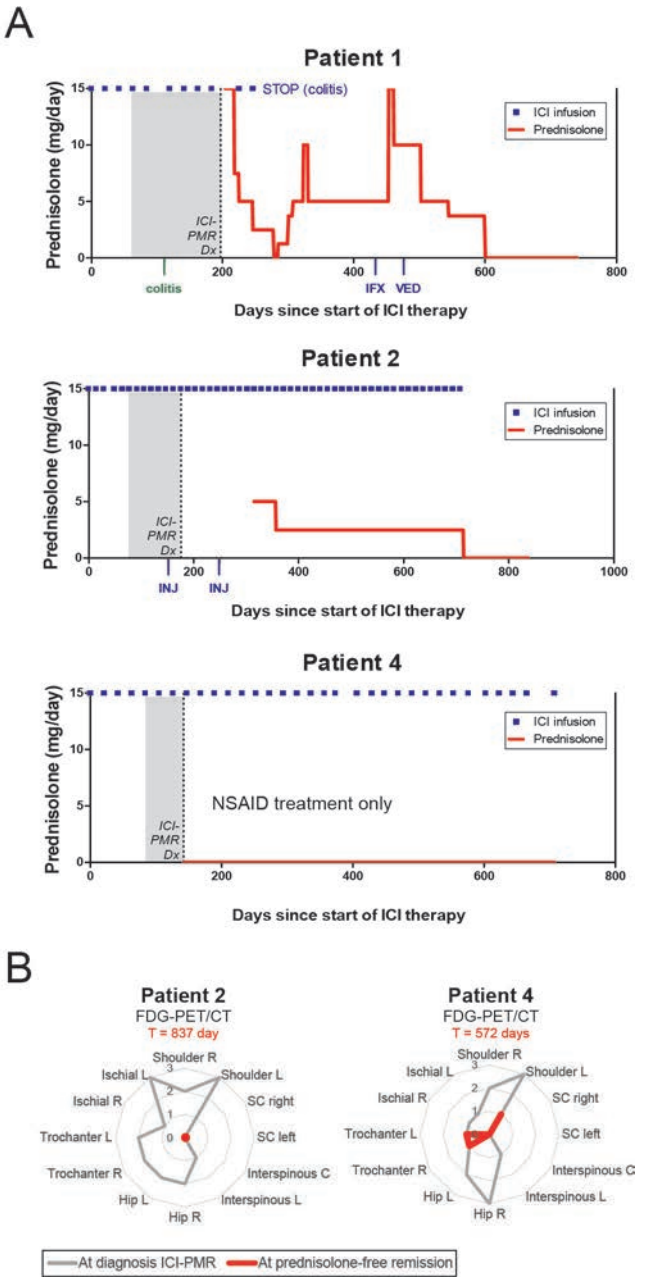

Figure 1 Treatment and long-term outcomes of patients with ICI-PMR. (A) Overview of immune checkpoint inhibitor (ICI) infusions, prednisolone doses and use of additional therapies in three patients with the longest follow-up. In *patient 1*, prednisolone doses were increased due to ICI-PMR rather than the autoimmune colitis. The dashed line indicates the time point at which ICI-PMR was diagnosed. The grey box depicts the period during which symptoms of ICI-PMR were already present. (B) FDG uptake at the shoulders, sternoclavicular (SC) joints, cervical (C) and lumbar (L) interspinous bursae, hip joints, hip trochanters and ischial tuberosities in two patients reaching prednisolone-free remission. Grading: 0, no uptake; 1, uptake lower than liver; 2, uptake equal to liver; 3, uptake higher than liver.[7] For comparison, the previously reported FDG uptake scores at diagnosis of ICI-PMR are also shown.[5] Dx, diagnosis; FDG, [18F]-fluorodeoxyglucose; ICI, immune checkpoint inhibitor; IFX, infliximab (for autoimmune colitis); INJ, injection of shoulder (for ICI-PMR); NSAID, nonsteroidal anti-inflammatory drug; PET, positron emission tomography; PMR, polymyalgia rheumatica; VED, vedolizumab (for autoimmune colitis).

If systemic glucocorticoids are needed, a starting dose of 5–7.5 mg prednisolone per day might be sufficient in a substantial part of patients. Prompt response evaluation will identify patients in which the treatment should be stepped up. This approach will limit the use of prednisolone doses that might possibly compromise the efficacy of ICI therapy. As recently proposed by others,[6] ICI therapy should not necessarily be discontinued in patients with ICI-PMR.

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