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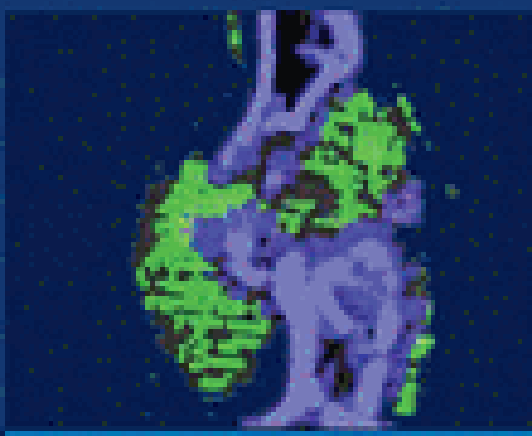

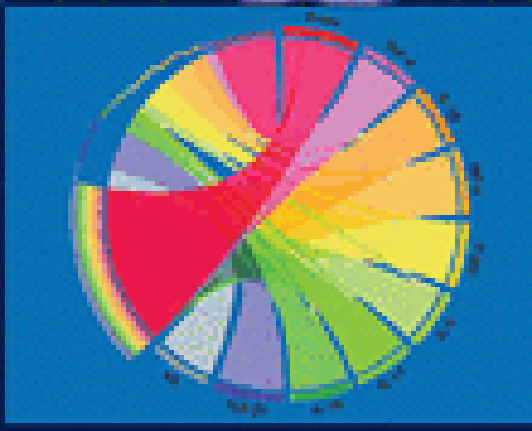

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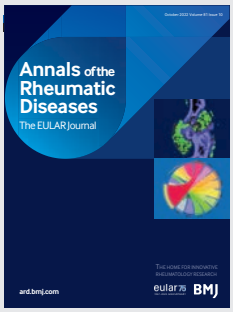




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# EULAR 75-year anniversary: commentaries on ARD papers from 50 years ago

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EULAR is celebrating its 75-year anniversary after its foundation in 1947 in Copenhagen. ARD is contributing to this celebration by presenting a series of previously published articles that highlight the development of rheumatology over these 75 years. After previously discussing some of the ARD papers from 75 years ago,[1] we now discuss five selected papers published 50 years ago, in 1972.

The year 1972 is marked as a dark year in history due to terrorism entering sport with the massacre of 11 Israel Athletes by Arab Gunman at the Munich Olympics. Also, 1972 was the beginning of a big political scandal in the USA, namely the start of the Watergate Scandal. In US cities, antiwar (Vietnam) demonstrations draw 100 000 demonstrators. In Europe, a worsening of the problems between the IRA and the British government led to the loss of innocent lives at Bloody Friday. However, also a positive signal, the USA and the Soviet Union signed the Anti-Ballistic Missile Treaty.

In hindsight for rheumatology important achievements in Medicine in 1972 were the discovery of the immunosuppressive effect of ciclosporin by a team of Sandoz in Basel, Switzerland and publication of Archie Cochrane on Effectiveness and Efficiency, an impressive plea for Evidence Based Medicine.[2] The Nobel Prize 1972 in Physiology or Medicine was shared between Robert Porter and Gerald Edelman for determining the chemical structure of an antibody! Also of major relevance was the report of Benacerraf and McDevitt on their discovery of major histocompability complex related immune response genes.[3] In the USA, rheumatology was formally identified in 1972 as a subspecialty of medicine, while it was already an independent specialism in a growing number of European countries.

In 1972, I was in my last years of training to become a physician, but more interested in Tropical Medicine than in Rheumatology. Returning from a few years in Tanzania I started training in Internal Medicine, and later switched to Rheumatology, attracted by the challenges of the expanding immunological science. I started reading rheumatological Journals only in 1982. For this special occasion I went therefore with great interest through the (six) issues of Annals of the Rheumatic Diseases of 1972. The Journal was published by the *British Medical Journal* and the editors were appointed by the British Medical Association and the Arthritis and Rheumatism Council for Research. Editor was TJ Scott, and associate editors were HLF Currey and V Wright. The address is still the same in 2022. The subjects of the articles were quite different from 2022. There was much attention for surgical procedures (synovectomies, biopsies, prostheses, cervical luxation treatment), for infections that might be related to the occurrence of rheumatic diseases (Corynebacterium acnes, Yersinia enterocolitica), for gout and for radiography (also already for pertechnetate joint scans and radiotherapy for osteoarthritis). Book reviews were standard, with sometimes really killing comments. There was a significant number of articles that would now be called basic research (dealing with fibrins, collagens, glycoproteins, immunoglobulins and human antiglobulins, lysosomal enzymes, cellular immunity, latex slide tests). There were hardly epidemiological studies reported and clinical trials, let alone RCTs, were missing. The only drugs that were described were glucocorticoids, gold, penicillamine and one non steroidal anti inflammatory drugs.

The Journal was related to the British Society for Rheumatology and in order to get a feeling of what was happening in rheumatology at that time the proceedings of the Heberden Society are very instructive. Already 50 years before the pandemic distal learning was introduced by V Wright and reported as follows[4]:

Little work has been done on the use of television in the teaching of rheumatology and as far as we know no attempt has been made to evaluate such work. The present study concerns a programme that was constructed for the period devoted to 'Introduction to Rheumatology'. Forty-eight students attending were divided into two groups, one of which watched the television presentation and the other received a lecture on the same topic. Immediately after the lecture a short test was given to determine immediate recall. A month later, after a lecture on nephrology given on a snowy morning at 8:30 hours, the same test was reapplied to ascertain delayed recall, the students being unaware that this was going to happen. This then produced three groups, those who had been to the lecture, those who had been to the television presentation and those who had been to neither (n=11)! Six questions were put to the students. The television group had a higher score on four of the questions, there was an equal score on 1, and the lecture group had a higher score on 1 question. The SD of marks was higher in the lecture group. For delayed recall the television group maintained their superiority of marks on three of the six

questions. On two questions the non-attenders equalled the marks of the lecture group.

An interesting discussion followed[4]:

Dr AGS Hill (Stoke Mandeville) Do you think that the bigger standard deviation in the lecture group represents those who were, in fact, asleep? PROF. WRIGHT Sir, students do not sleep during our lectures!

Dr K Lloyd (Cardiff) How does the television presentation differ from the lecture? Is it something more than a lecture on the television? PROF. WRIGHT Yes; it is important to use the full resources of the medium, otherwise there is no point in it. For instance, we demonstrated both interview and examination of two patients on the television.

Dr RN Maini (London) How did you control the intelligence of the three groups of students? The results suggest that the groups may not have been evenly matched for this, as those who failed to attend either of the two teaching sessions seemed to be the most intelligent! PROF. WRIGHT We failed to find any correlation with these results and the students' second M.B. marks. I shall remember in the final examination those who did not attend the lecture !

Unravelling Rheumatoid Arthritis: LE Glynn delivered the prestigious Roy Cameron lecture (for the Royal College of Pathology) on Pathology, pathogenesis and aetiology of rheumatoid arthritis.[5]

The three outstanding anatomical features of rheumatoid arthritis are inflammation with progressive deformity of joints, subcutaneous nodules especially over pressure points and sites of friction, and vascular lesions both necrotising and obliterative. The early stages of joint inflammation have for obvious reasons been much less well studied than the later. Nevertheless, a sufficient number of early lesions has now been documented to permit a clear picture of this early phase. By the end of the first week of clinical involvement, the histological changes of acute inflammation are well established. These include increased vascularity, endothelial swelling of vessels with some polymorphonuclear exudation, oedema and fibrin deposition both within the synovial membrane and on its surface, and hyperplasia of the lining cells. In the normal joint, these cells are of two kinds which can be readily distinguished with the electron microscope: (A) Cells with complex elongated processes, the filopodia and many membrane-bound vesicles rich in acid hydrolases, that is, lysosomes and (B) Cells less complex in their surface structure and much poorer in lysosomes but richly endowed with rough endoplasmic reticulum. The A cells are actively phagocytic and the B cells secretory in function.

He describes detailed histology of different tissues involved and experiments with antigens to evoke inflammatory responses. At the end, he concludes:

My present interpretation as a pathologist, based on the pure anatomical findings, of classical chronic rheumatoid arthritis is, therefore, that it is a two-phase disease in which by no means all patients enter the second phase. Phase one results from some systemic infection by an organism with a tendency to settle in joints, where it excites an inflammatory reaction largely as a result of a local immune response. This phase may last some 6 months, possibly even 12 months, but with the elimination of the antigen eventually subsides. Continuation of disease activity beyond this date could theoretically arise from reinfection with another initiating agent, but in most instances, it results from the development of autoimmunisation to some antigen or antigens engendered by the initial inflammation itself.

Glucocorticoids were the hallmark of many treatments, but the mechanism of action was not known. Lewis and Day, pharmacologists from Bath, obtained synovial tissue and synovial fluid from patients with RA and tested ex vivo the influence of glucocorticoids, with the changes in lysosomes as an important outcome.[6]

It is an accepted fact that corticosteroids at some concentrations stabilise lysosomes. Previous work has shown that steroid concentration is a critical factor in the stabilising action of these compounds on lysosomes. High concentrations of steroids damage lysosomes, but lower levels, which have more clinical significance, stabilise lysosomes. Although our findings suggest that steroids exert their anti-inflammatory action through a direct interaction with lysosomes, it must be borne in mind that the steroid concentration in the joint is constantly changing because of metabolism and other causes. It may well be that the distribution of steroids within the tissues and cells is a key factor in their action. Whether lysosomes are directly involved in producing inflammation, as suggested by the Hollander theory, or are indirectly involved as part of a sequence of events, as suggested by our findings with arthritic rats or the observations on levels of lysosomal enzyme levels in human synovial fluids, the interaction of steroids with lysosomes appears to be an important factor in their mode of action.

Clinical studies with glucocorticoids are sparse at that time. Carter and Day were interested whether alternate day therapy with glucocorticoids had less influence on suppression of the pituitary-adrenal axis. They treated two groups of 7 patients with 20–30 mg every second day and concluded that this alternate therapy might be feasible in some selected patients.[7]

The effect of alternate-day corticosteroid (prednisolone) therapy on pituitary-adrenal function has been studied in two groups of patients with active chronic polyarthritis. Doses of 20–30 mg. prednisolone every 48 hours were used, and responsiveness to the stress of insulin hypoglycaemia was tested at intervals in each patient during treatment. The first group of patients had not received any previous treatment with corticosteroids. Six of seven patients retained pituitary-adrenal responsiveness at, or near, the lower limit of normal; one patient showed a considerable degree of depression of response. The second group of patients were receiving corticosteroids daily and were converted to alternate day treatment. A slight reduction in dose was tolerated and improved pituitary-adrenal responses were observed. However, considerable variation was noted and, in some patients, several weeks elapsed before any marked improvement occurred. It is concluded that, when patients can tolerate alternate-day corticosteroid therapy, this is advantageous in producing less pituitary-adrenal suppression than does daily treatment.

Surgical therapy: Hill described over 1800 surgical interventions in a period of 15 years; it is most interesting to learn which operations were done[8]:

In the knee (433 procedures), the indications for arthrodesis (8) and osteotomy (19) are clear-cut and have not changed: mould arthroplasty (40) had its vogue; synovectomy (143) reached a peak of thirty a year and subsequently fell to 10–20, which now seems likely to remain fairly constant; synovectomy has only recently been recognised as an essential complement to excision of a popliteal cyst. In the hip (266 procedures, 112 major), the incidence of most minor operations has not changed, with the exception of iliopsoas release (85) in vogue for 3 years, reflecting the continual search for an effective remedy for chronic hip pain and deformity. Excision arthroplasty is now obsolete and total hip replacement (Charnley) provides eighteen to twenty new hips annually without making excessive demands on nursing care and physiotherapy. There has been no significant change

in the number of osteotomies (24) during the 15 years. In the wrist (231 procedures), arthrodesis (22) has been largely superseded by excision of the lower end of the ulna (84) providing relief of pain and a strong grip in addition to increased mobility. In the elbow (116 procedures, 71 major), synovectomy has replaced excision of the head of radius as the main indication for operation. Operations on other joints have been important for individual patients but have made little impact on the surgical treatment of rheumatoid arthritis. Cervical fusion has saved four patients from devastating disability or death.

It is stimulating to see how our specialty has moved forward the last 50 years, but it also makes us humble, realising what important steps our predecessors made with significant less research tools. We should not forget crediting our history.

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# Implementation of recommendations in rheumatic and musculoskeletal diseases: considerations for development and uptake

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## ABSTRACT

A clinical guideline is a document with the aim of guiding decisions based on evidence regarding diagnosis, management and treatment in specific areas of healthcare. Specific to rheumatic and musculoskeletal diseases (RMDs), adherence to clinical guidelines recommendations impacts the outcomes of people with these diseases. However, currently, the implementation of recommendations is less than optimal in rheumatology.

The WHO has described the implementation of evidence-based recommendations as one of the greatest challenges facing the global health community and has identified the importance of scaling up these recommendations. But closing the evidence-to-practice gap is often complex, time-consuming and difficult.

In this context, the implementation science offers a framework to overcome this scenario.

This article describes the principles of implementation science to facilitate and optimise the implementation of clinical recommendations in RMDs. Embedding implementation science methods and techniques into recommendation development and daily practice can help maximise the likelihood that implementation is successful in improving the quality of healthcare and healthcare services.

## INTRODUCTION

The dissemination of evidence-based recommendations is considered a key step for improving the quality of care. However, simple dissemination of information has rarely been effective in changing clinical practices and behaviour.[1 2] More specifically, in rheumatic and musculoskeletal diseases (RMDs), adherence to and uptake of recommendations are often suboptimal.[3 4] This is critical as it has been demonstrated the benefit of the adherence to clinical recommendations.[5 6]

Designing and conducting the implementation of recommendations are complex and daunting tasks, especially for those new to implementation and without specific training.[7] For this purpose, the implementation science provides methods, processes and strategies to promote and accelerate the systematic implementation of proven (evidence-based) practices,[7] for example, by developing an understanding of what influences implementation, or by testing behavioural, policy and health system interventions to overcome barriers to implementation.[8]

On the other hand, implementation also requires participation and interaction of multiple actors, organisations and care levels, and the provision of resources (human, time and economic).[9]

The aim of this article is to provide a brief guide to principles that facilitate the implementation of recommendations in RMDs. It will contribute to improve the quality and effectiveness of health services and reduce variations in care for RMDs.

### General principles of implementation

First of all, it is important to summarise the main general principles of implementation science.[10 11] Without this educational basis, it is not possible to put the implementation of a single or a set of recommendations into practice successfully. These general principles include the phases of implementation that will be described in detail.

Figure 1 outlines the general principles of implementation: (1) the multilevel approach, (2) the need to prioritise and adapt, (3) the implementation team, (4) the nature of the implementation process, (5) the need for resources and (6) the phases of implementation.

Connected to the multilevel approach, recommendations can influence three levels (macro, meso, micro), all of which might have an impact on implementation. The macro-level is the policy level. Depending on the country, health policymakers might decide, for example, which biological therapies are available nationally, or provide financial support in case of implementing specific recommendations.[12] National societies of rheumatology would be at this macro-level as well. The meso-level (primary care, regional organisations, patient charities or hospitals) addresses decentralisation, common in many health systems worldwide, and organisational aspects.[11] At this level, clinical protocols and pathways may 'encourage or promote' specific treatment alternatives over others and decisions on human resources allocation are also made (eg, nurses specialised in RMDs). The micro-level corresponds to the clinicians, healthcare professionals and patients, who will eventually decide, for example, which type of exercises is more

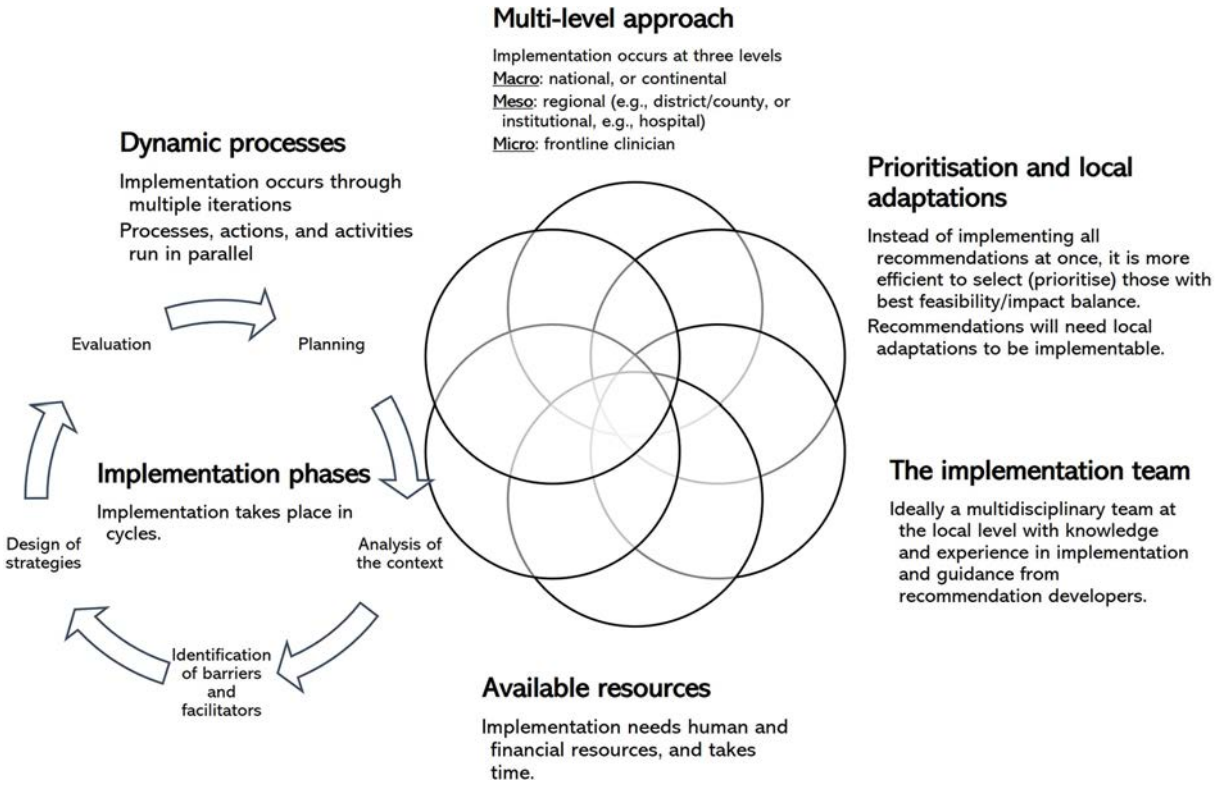


**Figure 1** Principles of implementation and its phases.

appropriate for individual patients with RMD or which joints to examine.

Implementation can be determined through prioritisation and local adaptations. Prioritisation refers to the selection of recommendations to put into practice, usually based on feasibility, potential for impact, patient and population need, etc. The adaptation of recommendations to local needs might be necessary, and how it is implemented may vary in different health systems where there may be different professional roles, access to drugs, etc. A recommendation can propose an intervention, for example, a joint education programme provided by occupational therapists, but in a specific setting, where occupational therapists are not available, this task can be offered by a specialised nurse or physiotherapist.

The implementation team is necessary at the local level and should be multidisciplinary, ideally with guidance from those who developed the recommendations and could vary depending on the recommendations to implement (eg, one may need a politician, another a pharmacist). Besides a team, other resources necessary for implementation can include time, financial support, patient and public involvement and engagement, and digital innovation.

Implementation requires specific knowledge mobilisation skills and training, not only the implementation team but also the clinical guideline developers. A minimum implementation knowledge includes the basis, methodology, and processes of implementation science and the practical application of theory.

Although implementation is better apprehended in its phases (table 1), it is critical to acknowledge that many processes and actions will run in parallel and circles based on immediate feedback from the field; as implementation is an iterative and dynamic process.

A final educational point is the **terminology** used, which will be new to many. The Effective Practice and Organisation of Care of the Cochrane Collaboration provides terms and definitions.[13] Here, for example, 'continuity of care' is defined as 'Interventions to reduce fragmented care and undesirable consequences of fragmented care, for example, by ensuring the responsibility of care is passed from one facility to another so the patient perceives their needs and circumstances are known to the provider'.

## Implementation phases

Regarding the phases of implementation (table 1), the implementation of any recommendation starts with an implementation plan. Usually, implementation planning starts upon guideline completion.[14] However, implementation is more successful if planning occurs concurrently rather than consecutively to recommendations development, or even before sometimes so that the recommendations issued are clear and usable, target users are primed for adoption, and their needs and preferences are taken into account.[15] Implementation plan templates are abundant on the internet, most of which only highlight the actions and actors involved. It is important to determine in this plan which is the recommendation's implementation objective (eg, to increase uptake of core treatment, to implement exercise in spondyloarthritis or having rheumatologists perform synovial fluid aspiration in patients with undiagnosed inflammatory arthritis).

An analysis of context will afterwards assess the organisational, community and individual readiness for change.[16] This analysis should identify the care level/s and their relationships (eg, at what level are specific decisions related to the recommendation taken), the organisational culture and climate (eg, whether the national societies have the power to homogenise behaviours), which teams will be likely involved in the implementation (eg, whether a primary care physician should be included), and which are the human, material, economic and time resources available, including a precise description of the information systems. The latter will be critical to both evaluate and ensure that the recommendation is implemented. The analysis of the context requires

**Table 1** Clinical recommendation implementation phases

| Phase | Description | Practicalities |
|---|---|---|
| 1. Planning | The implementation plan is reflected in a protocol that includes the following headings:<br>► Background<br>► Objectives<br>► Implementation team<br>► Contact and involved stakeholders<br>► Milestones<br>► Budget<br>► Evaluation plan | ► Templates<br>► Abundance of examples on the internet |
| 2. Analysis of the context | It should identify and describe at a minimum:<br>► the care level/s and their relationships (from policies to hospital and public), interactions, mediators or determinants (eg, human and economic resources)<br>► the organisational culture and climate<br>► the teams to be involved in the implementation process<br>► the human, material, economic and time resources available<br>► the information systems | Narrative review based on interviews with local stakeholders and organisational data.<br>An analysis can be developed by each country or region and then be reviewed:<br>► with each set of recommendations, that may require specific items<br>► periodically |
| 3. Identification of barriers and facilitators | These should reflect factors related to:<br>► health professionals<br>► social context (including patients)<br>► organisational context<br>► the recommendations itself | Use brainstorming, Delphi, nominal or focus groups, qualitative interviews, communities of practice or surveys (qualitative research techniques). |
| 4. Design of strategies | These can be tools, actions or activities.<br>Will imply economic, organisational or regulatory aspects.<br>The focus can be on clinicians, health professionals or patients. | Examples are leaflets, courses, clinical sessions, local consensus documents, changes in regulation, recruitment of health professionals, checklists, standards of care, decision rules or algorithms in electronic medical records, protocols, clinical pathways, etc. |
| 5. Evaluation | It implies the definition of quality indicators. These include:<br>► what to measure<br>► how to measure it<br>► sources and timing | Whenever possible, use quality indicators already developed in rheumatology. |
| 6. Review | Evaluation of the implementation process and related decisions. | Periodical meetings of the implementation team to check on plan and quality indicators. |

accurate knowledge of current clinical practice in the setting.[16] For example, in the recommendations dealing with the transition of care from paediatrics to adult rheumatology, the age at which children become adults in the different health systems varies across countries.[17]

The following phase is the identification of barriers and facilitators. These are factors that hinder or facilitate, totally or partially, the implementation of a change in clinical practice, which are related to health professionals, social (including patients) and organisational context or to the recommendations.[18 19] Many techniques can be used to identify them, such as Delphi, nominal groups, qualitative interviews, surveys, communities of practice, etc.[20] The Eumusc.net project identified several facilitators and barriers in European rheumatology.[21]

Next is the design or selection of implementation strategies, that is, the interventions that will facilitate the implementation of recommendations.[22 23] Implementation needs to be adjusted for the various target populations and organisations and to offer educational and practical tools. Therefore, strategies include economic, organisational, or regulatory tools, actions and activities focused on clinicians, health professionals and patients. A non-exhaustive list includes leaflets, courses, clinical sessions, local consensus documents, decision rules, checklists, standards of care, electronic medical records or decision-making programmes.[22 24–26] However, the efficacy of these strategies is variable.[25 26]

The evaluation of the implementation is the subsequent step,[27] and is not only related to the outcome of the implementation but also the implementation process. Selected recommendations can be transformed into quality measures (ie, indicators and standards of indicators), which are observed before and after the implementation (eg, waiting list, time to access rheumatologist, time to remission).[28] There are examples of quality indicators in rheumatology.[4 28–30] The whole implementation process can also be evaluated with checklists.

The final phase is the review or replanning. This phase includes taking into consideration the evaluation of the whole implementation process and, if necessary, to redesign or redefine a new implementation plan or even de-implement strategies that do not produce the expected outcome.

## CONCLUSIONS

The adherence to and uptake of clinical recommendations impact on outcomes of patients with RMDs. However, clinical recommendations' simple dissemination (journal publication, congress communication, etc) has rarely been effective in changing clinical practices and behaviour. Implementation science provides a framework to facilitate the implementation of recommendations. Implementation should start early, even before the clinical guideline developmental processes and complete all of the phases of the implementation.

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TRANSLATIONAL SCIENCE

# Assessing acceptability and identifying barriers and facilitators to implementation of the EULAR recommendations for patient education in inflammatory arthritis: a mixed-methods study with rheumatology professionals in 23 European and Asian countries

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## ABSTRACT

**Objectives** To disseminate and assess the level of acceptability and applicability of the European Alliance of Associations for Rheumatology (EULAR) recommendations for patient education among professionals in rheumatology across Europe and three Asian countries and identify potential barriers and facilitators to their application.

**Methods** A parallel convergent mixed-methods design with an inductive approach was used. A web-based survey, available in 20 different languages, was distributed to health professionals by non-probability sampling. The level of agreement and applicability of each recommendation was assessed by (0–10) rating scales. Barriers and facilitators to implementation were assessed using free-text responses. Quantitative data were analysed descriptively and qualitative data by content analysis and presented in 16 categories supported by quotes.

**Results** A total of 1159 completed the survey; 852 (73.5%) were women. Most of the professionals were nurses (n=487), rheumatologists (n=320), physiotherapists (n=158). For all recommendations, the level of agreement was high but applicability was lower. The four most common barriers to application were lack of time, lack of training in how to provide patient education, not having enough staff to perform this task and lack of evaluation tools. The most common facilitators were tailoring patient education to individual patients, using group education, linking patient education with diagnosis and treatment and inviting patients to provide feedback on patient education delivery.

**Conclusions** This project has disseminated the EULAR recommendations for patient education to health professionals across 23 countries. Potential barriers to their application were identified and some are amenable to change, namely training patient education providers and developing evaluation tools.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Patient education is an integral part of the management of inflammatory arthritis. An international task force of health professionals, researchers and patients, developed evidence-based European Alliance of Associations for Rheumatology recommendations for patient education in inflammatory arthritis in 2015.

### WHAT THIS STUDY ADDS

⇒ This study disseminated the recommendations for patient education to healthcare professionals in rheumatology across Europe, India, Hong Kong and Japan.

⇒ The levels of agreement with the recommendations among healthcare professionals were very high, the level of applicability was lower for each corresponding recommendation.

⇒ The top three barriers to application were lack of time, lack of training in how to provide patient education and not having enough staff to perform this task.

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

⇒ Patient education delivered according to the recommendations can support patients to make informed choices about how to manage their inflammatory arthritis and optimise their health.

## BACKGROUND

Patient education (PE) is recommended as an integral part of standard care for patients with inflammatory arthritis (IA).[1–3] PE has been defined as *'a*

**Box 1 Recommendations for patient education for people with inflammatory arthritis.**

**Overarching principles**

⇒ Patient education is a planned interactive learning process designed to support and enable people to manage their life with inflammatory arthritis and optimise their health and well-being.

⇒ Communication and shared decision-making between people with inflammatory arthritis and their health professionals are essential for effective patient education.

**Recommendations**

1. Patient education should be provided for people with inflammatory arthritis as an integral part of standard care in order to increase patient involvement in disease management and health promotion.
2. All people with inflammatory arthritis should have access to and be offered patient education throughout the course of their disease including as a minimum; at diagnosis, at pharmacological treatment change and when required by the patient's physical or psychological condition.
3. The content and delivery of patient education should be individually tailored and needs based for people with inflammatory arthritis.
4. Patient education in inflammatory arthritis should include individual and/or group sessions, which can be provided through face-to-face or online interactions, and supplemented by phone calls, written or multimedia material.
5. Patient education programmes in inflammatory arthritis should have a theoretical framework and be evidence based, such as self-management, cognitive behavioural therapy or stress management.
6. The effectiveness of patient education in inflammatory arthritis should be evaluated and outcomes used must reflect the objectives of the patient education programme.
7. Patient education in inflammatory arthritis should be delivered by competent health professionals and/or by trained patients, if appropriate, in a multidisciplinary team.
8. Providers of patient education in inflammatory arthritis should have access to and undertake specific training in order to obtain and maintain knowledge and skills

*planned interactive learning process designed to support and enable people to manage their life with a disease and optimise their health and wellbeing*'.[4] It can include health education, self-management programmes, psychoeducational programmes (such as stress management, relaxation techniques, strategies to manage psychological distress and social functioning), and health promotion by healthcare providers.[4]

Using an evidence-based and expert opinion-based approach, European Alliance of Associations for Rheumatology (EULAR) recommendations for PE[4] were developed in 2015 to increase the awareness of and improve the quality of PE for people with IA across Europe. The recommendations comprised two overarching principles and eight recommendations, which address the content of PE, when and how this should be provided, the need for evaluation of PE and training of the providers (box 1).

While developing evidence-based recommendations is essential, successful implementation in practice is crucial to obtain the desired improvements in quality of care and patient outcomes.[5–7] Implementation is a dynamic, iterative process comprising planning, analysis of the context, assessing barriers and facilitators, designing strategies and evaluation.[7–10] It occurs at three levels, the micro level (individual clinicians, clinical teams and patients or carers), the meso level (institution, organisation or local government) and the macro level (national or regional/continental). Dissemination of the recommendations to all stakeholders and assessing acceptability, feasibility and identifying barriers and facilitators to implementation is the first crucial step in the implementation process.[6–10]

To facilitate implementation, it is essential to assess acceptability to various stakeholders, feasibility in different health systems, the cost and sustainability if applied in practice.[11] PE is usually organised by rheumatology nurses[12–15] although all professionals in the care of people with IA (rheumatologists, physiotherapists, occupational therapists, psychologists and social workers) deliver PE as part of their role in a multidisciplinary team.[16–18] Patients with IA have also been successfully involved in the design and delivery of PE to other patients.[19–21]

Therefore, all these groups are the target of the dissemination and implementation.[4] We have disseminated these recommendations to patients with IA in Europe and overall, their agreement levels were very high, suggesting that they reflect patients' preferences for engaging in collaborative care.[22]

The objectives of this study were to: (1) disseminate the recommendations to professionals in the care of people with IA across Europe and three countries in Asia, (2) assess the level of acceptability and applicability and (3) identify potential barriers and facilitators to implementation of the recommendations.

## METHODS

### Design

We applied a parallel convergent mixed methods research design with an inductive approach. Quantitative and qualitative data were collected concurrently and then merged and integrated during analysis and interpretation. Since both quantitative and qualitative methods can provide complementary data on the same research problem, a mixed methods design was used to provide a more comprehensive understanding of the dissemination including awareness, and barriers and facilitators to the implementation of the recommendations across Europe.[23]

The study was conducted in 20 European countries, Hong Kong, India and Japan. The research team comprised 31 multidisciplinary members, including a methodologist, patient research partners, researchers and/or health professionals within each collaborating country.

### Quantitative data collection

The survey developed by authors comprised two sections: (1) personal characteristics (age, sex, country) and professional background (profession, qualification, work setting and experience in rheumatology) and (2) items regarding eight recommendations. For each of the recommendations, numerical (0 to 10) rating scales were used to assess participants' level of agreement and application of the recommendations. Example:

Recommendation 1. PE should be provided for people with IA as an integral part of standard care in order to increase patient involvement in disease management and health promotion

- *Do you agree with this recommendation? (Please indicate the level of your agreement: 0 'I do not agree at all' and 10 'I agree completely'*)
- *Do you provide patient education as it is advocated in this recommendation? 0 'No, not at all' and 10 'Yes, entirely'*.

All the items are presented in online supplemental material.

### Qualitative data collection

The two overarching principles were stated using bullet points, and for each of the eight recommendations, respondents were invited to add free text comments on reasons for not agreeing entirely and/or barriers to application of the recommendation.

### Translation of the survey

Investigators in each country translated the survey into their national language using a dual panel approach.[24–26] This approach involves a consensus translation produced by a primary (professional) panel of bilingual people familiar with the target language, followed by review by a second panel who speak the target language, in order to ensure acceptability and understanding of the wording for prospective participants. Any discrepancies in translation were resolved using a group consensus approach. This approach has been shown to produce translations that are easier to understand, compared with the forward–backward translation approach.[24–26] In total, 20 different language options were available for the survey respondents to select from a drop-down menu.

After data collection was complete, investigators in each collaborating country were sent the free-text responses from their corresponding languages. These were translated back into English and sent to the study coordinator for analysis.

### Participants

The target participants for this survey were all professionals involved in the care of people with IA. From July to September 2019, collaborators from the 23 countries disseminated the web-based survey to their colleagues and national rheumatology organisations using a snowball sampling technique.[27]

## DATA ANALYSIS

### Quantitative analysis

Descriptive statistics were used to summarise the levels of agreement and application of each of the recommendations. IBM SPSS Statistics V.20 (IBM, New York) software was used.

### Qualitative analysis

Translated free text responses were imported into NVivo V.12 (QSR International, Melbourne, Australia) and analysed with a manifest qualitative content analysis with an inductive approach. This qualitative method involved coding, creating categories and data abstraction.[28] Each translated data set was read through repeatedly by the first author (SB) to gain a greater understanding of the whole data.[29]

The text was first divided into barriers and facilitators for each of the eight EULAR recommendations, and into positive and negative opinions, relating to the overarching principles.[29] Although the survey items asked about barriers to implementation of the recommendations, many participants gave examples of instances where they had successfully implemented recommendations in their practice, and exemplars of how they had achieved this. These were coded as facilitators for each recommendation. Phrases and words containing information relevant to the aims of the study were identified, extracted and labelled with a code.[29] For each barrier and facilitator, codes with similar underlying meanings were grouped into subcategories. Each subcategory was organised and named using words and phrases characteristic of the data, such as ‘not enough time’. Subcategories with similar content and incidences were grouped together into broader main categories, giving a two-level hierarchy.[28] Data analysis was conducted by the first author (SB), with a critical discussion of codes, subcategories and main categories with the principal investigator (MN) and input of a qualitative methodologist (IL).

### Mixed-method analysis

After independent analyses of the quantitative and qualitative data, the results were paired side by side for comparison and identification of similar and different categories between and within the eight recommendations in order to validate the results.[23] The categories were correlated and thereafter ranked within each recommendation (figure 1).

### Ethical considerations

Participating in this study was voluntary. Survey respondents were advised that completing and submitting the survey implied that they had read the information sheet and consented to taking part. The study was approved by the Faculty of Health and Applied Sciences Research Ethics Committee at the University of the West of England, Bristol, UK (UWE REC REF No: HAS.18.11.066).

## RESULTS

### Participants

A total of 1510 responses were received, 1159 of which were complete responses. This may be due to the in-built feature of Qualtrics survey, where incomplete responses were saved automatically after 2 weeks. The respondents comprised 487 nurses, 320 rheumatologists, 158 physiotherapists, 75 occupational therapists, 22 pharmacists, 8 nutritionists, 8 medical assistants, 3 psychologists and 78 ‘other’ professionals. Most were women (852; 73.5%) and median duration of clinical experience was 13 (IQR: 6–23) years of which 5 (IQR: 1–7) years were in rheumatology. Table 1 presents the number of respondents by country.

### Cross-cultural adaptation

The adaptation of the questionnaire into target languages was largely seamless except for professional characteristics, training and educational background, which differs across countries. In Hong Kong, the term ‘theory’ in the context used in recommendation 5 was difficult to understand, therefore this was modified to ‘scientific-based approved information as a component in PE’. In Spain, the word ‘designed’ in recommendation 3 was substituted for ‘tailored’ as this was considered more personal. In addition, examples of ‘personal needs’ in recommendation 2 were expanded to give examples of the nature of those needs (such as work or pregnancy). As the recommendations were often described in long sentences, it was necessary in some languages to break into two sentences in order to retain the intended meaning. In the Norwegian translation, the adaptation included shortening the number of words in the information section.

## QUANTITATIVE RESULTS

### Level of agreement and application of the recommendations

Table 2 presents the level of agreement and application of the recommendations. Overall, there was high agreement (median=10, IQR: 8–10) across all recommendations. However, the level of applicability was generally lower compared with each corresponding agreement level, especially for recommendation 6, which states that the effectiveness of PE should be evaluated (median=6, IQR: 4–8). Lack of an effective evaluation tool was the most often mentioned barrier to implementation for recommendation 6. For recommendation 4, the most cited barrier was limited access to phone or internet-based PE.

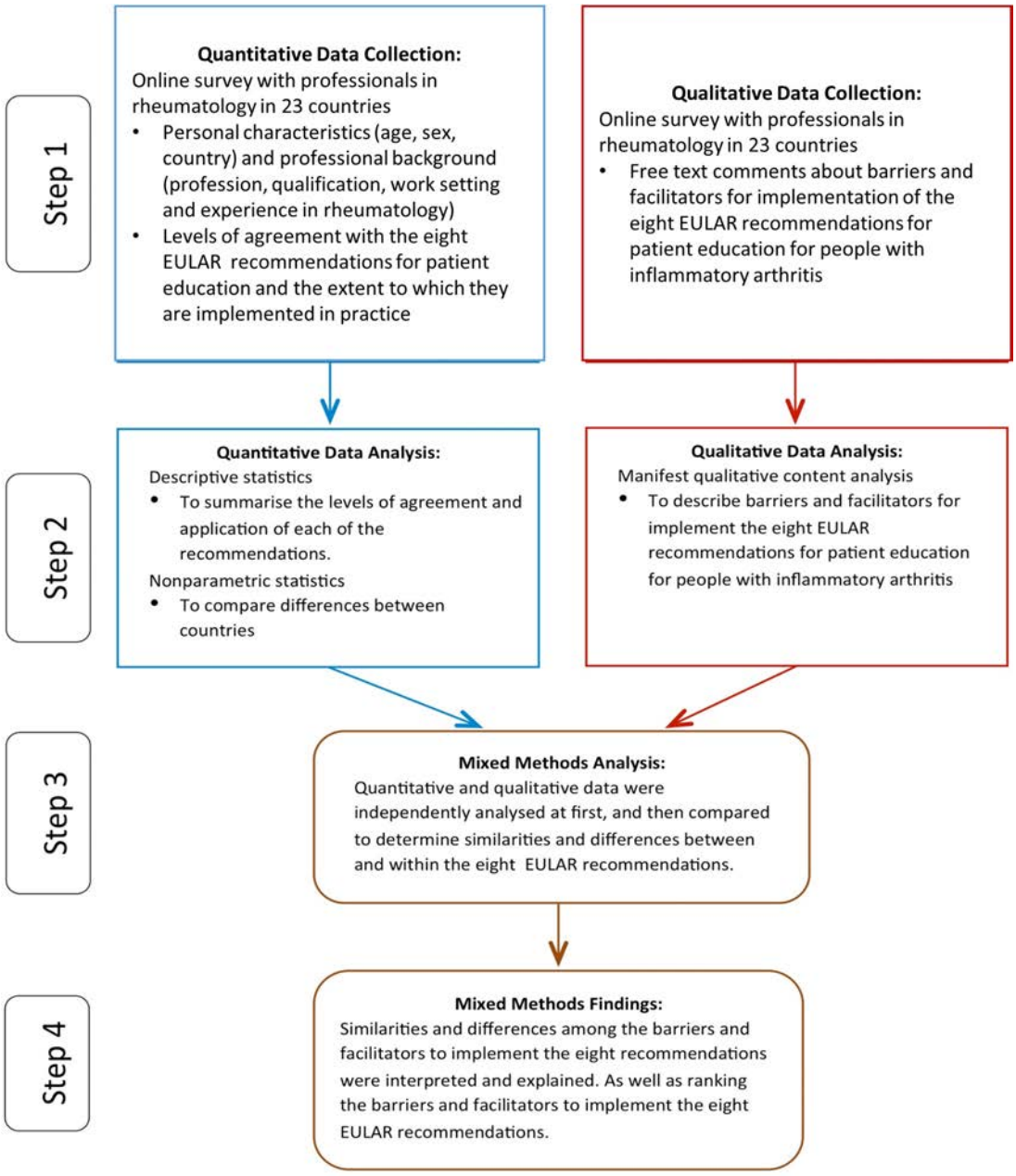


**Figure 1** Parallel convergent mixed-methods model. EULAR, European Alliance of Associations for Rheumatology.

For recommendation 5, a lack of training in theoretical frameworks, self-management or cognitive–behavioural therapy was a common barrier.

## QUALITATIVE FINDINGS

### Barriers to implementation

Table 3 presents the 10 categories and selected quotes that illustrate perceived barriers to implementation of the recommendations.

#### Lack of time

The most cited barrier to the implementation of PE as part of standard care was a lack of time. Patient consultations were subjected to competing demands (Q1) and health professionals found it difficult to tailor information (Q2). While it was good to meet the needs of patients, this created additional work (Q3). Activities such as evaluation of PE were not always prioritised due to lack of time (Q4).

#### Lack of training

Many described a lack of knowledge and training (Q5–Q7), which prevented participants from offering self-management training or cognitive behavioural therapy (Q8 and Q10). Whether patients received PE varied, depending on the experience of the provider (Q9). Similarly, identifying and training patients to deliver PE could be challenging (Q11).

#### Lack of staff

Often, there were not enough staff with specialised expertise, such as trained nurses, to provide PE to patients. Many indicated that there was a lack of psychological support such as cognitive behavioural therapy (CBT) or stress management interventions to support self-management in patients with IA (Q12).

**Table 1** Number of respondents by country

| Country | Number attempted survey | Number completed survey |
|---|---|---|
| 1. Austria | 17 | 11 |
| 2. Belgium | 99 | 71 |
| 3. Bulgaria | 9 | 8 |
| 4. Czech Republic | 1 | 0 |
| 5. Denmark | 57 | 45 |
| 6. Estonia | 1 | 0 |
| 7. Finland | 70 | 61 |
| 8. France | 156 | 128 |
| 9. Germany | 32 | 26 |
| 10. Hong Kong | 14 | 12 |
| 11. Hungary | 90 | 75 |
| 12. India | 17 | 13 |
| 13. Ireland | 24 | 18 |
| 14. UK | 51 | 41 |
| 15. Italy | 85 | 63 |
| 16. Japan | 214 | 169 |
| 17. Latvia | 3 | 3 |
| 18. Netherlands | 36 | 28 |
| 19. Norway | 55 | 42 |
| 20. Poland | 36 | 29 |
| 21. Portugal | 245 | 171 |
| 22. Slovenia | 1 | 1 |
| 23. Spain | 60 | 46 |
| 24. Sweden | 136 | 97 |
| 25. Switzerland | 1 | 1 |
| Total | **1510** | **1159** |

### Lack of assessment tools

The lack of a reliable evaluation tool was cited as a significant barrier. Some had evaluation activities in place for the effect of PE, but no tool to evaluate whether PE had been successfully achieved (Q13). Staff had difficulties remembering to evaluate PE, and a lack of structure made it difficult to check-up with patients (Q14–Q15).

### Limited resources

Respondents cited a lack of resources to provide patients with PE during the course of their disease. Examples of resources mentioned were both patient-facing (lack on internet access) and staff-facing (online support, telephone and institutional internet restrictions (Q16–Q18).

**Table 2** Levels of agreement and applicability of each recommendation

| | Agreement | | Applicability | |
|---|---|---|---|---|
| | Median | IQR | Median | IQR |
| Recommendation 1 | 10 | 10 to 10 | 8 | 7 to 10 |
| Recommendation 2 | 10 | 10 to 10 | 8 | 6 to 10 |
| Recommendation 3 | 10 | 9 to 10 | 8 | 7 to 10 |
| Recommendation 4 | 10 | 8 to 10 | 7 | 5 to 10 |
| Recommendation 5 | 10 | 8 to 10 | 7 | 5 to 9 |
| Recommendation 6 | 10 | 8 to 10 | 6 | 4 to 8 |
| Recommendation 7 | 10 | 9 to 10 | 8 | 5 to 8 |
| Recommendation 8 | 10 | 10 to 10 | 8 | 5 to 8 |

### Concerns about online PE

Some respondents raised their reservations about delivering PE online as written information could be misunderstood. They preferred face-to-face interactions for PE delivery (Q19–Q21).

### Concerns about patient-delivered PE

Others felt that health professionals should be the only providers of PE. Some had concerns that non-healthcare providers (such as patients) could risk providing misinformation (Q22–23).

### Lack of systematic PE

Health professionals described some PE as lacking in organisation. Monitoring of PE was unusual, and patients were not always referred sufficiently (Q24–Q27). The need for participants to attend training was not always recognised or seen as a priority. Many had to rely on 'self-study' instead (Q28). As a result, their practice may not be as informed as it could be (Q29).

### Lack of funding

A lack of funding was cited as a barrier in terms of employing enough staff (to evaluate PE) as well as for supporting training (Q30–32).

### Lack of patient participation in disease management

Lack of patient involvement was cited as a barrier as patients had to be open and willing to engage with PE. Some responded described patients as 'uninterested' when PE was offered (Q32–Q34).

## Facilitators for implementation

Table 4 presents the six categories and selected quotes that illustrate facilitators of implementation of the recommendations.

### Tailoring PE

Respondents cited tailoring PE to individual patients' needs as important (Q35, table 4). Providing one-to-one PE enables patients to ask questions and gain information (Q36).

The need for flexibility in patient access to PE was emphasised (Q37). Offering PE when required supported patient independence (Q38). Others described adapting PE with brochures and education materials tailored to patients' needs (Q39). The need to support each patient to manage their mental and physical health was recognised (Q40). Others suggested providing standardised PE as a baseline and offer extra elements that could be personalised and tailored to individual patients according to the need (Q41 and Q42).

### Using group education

Some respondents described how they used a combination of group education alongside one-to-one (Q43) as patients could learn from, and support each other in a group setting.

### Linking PE with diagnosis, treatment and multidisciplinary care

Many agreed that PE should be scheduled regularly (Q44). PE was often offered at the start of drug interventions, with annual review clinics cited as an excellent opportunity for education. The need for flexibility in patient access to PE was emphasised (Q45). Successful PE included regularly organised programmes (Q46). PE was cited as fundamental to increasing patient knowledge and understanding (Q47).

**Table 3** Quotes for respective category supporting barriers to implementation

| Quote number (Q) | Category /illustrative quotes | Quoted by |
|---|---|---|
| | Lack of time | |
| 1 | 'Medical file, medical history, clinical assessment, lab tests, imaging, medication … there is often a lack of time, consequently, patient education is provided but in a less optimal way'. | Rheumatologist, Belgium |
| 2 | 'Not all the needs of patients can be extracted within the set time of current PE'. | Nurse, Japan |
| 3 | 'It is ideal to meet various needs, but on the other hand, increasing the burden on the provider side is an issue'. | Rheumatologist, Japan |
| 4 | 'Evaluation is never performed, no time is allocated to it'. | Registered Nurse, Belgium |
| | Lack of training | |
| 5 | 'Lack of training in the area on my part; little time available'. (Recommendation 1) | Registered Nurse, Portugal |
| 6 | 'Inflammatory chronic disease nursing and nurse specialist in this field have not been established. Therefore, as information, and knowledge and skills of nurses are insufficient, nurses may not be able to take care of patients based on the personal situation'. (Recommendation 3) | Nurse Educator, Japan |
| 7 | 'Ignorance of the [EULAR] recommendations' (Recommendation 1) | Registered Nurse, Portugal |
| 8 | 'I think we don't do it because we don't know how to do it. Especially [CBT] and stress management' (Recommendation 3) | Rheumatologist, France |
| 9 | PE [may] varies depending on the years of experience of the nurse. (Recommendation 1) | Nurse, Japan |
| 10 | 'Not enough training providers in our country' (Recommendation 6) | Rheumatologist, Bulgaria |
| 11 | 'Finding appropriate patients and training them to be trainers are all challenges' (Recommendation 7). | Registered Nurse, Hong Kong |
| | Lack of staff | |
| 12 | 'We do not currently have the resources to incorporate CBT or stress management strategies into patient self management. We do refer some patients to the pain team service …however waiting lists are very lengthy' (Recommendation 5) | Registered Nurse, UK |
| | Lack of assessment tools | |
| 13 | 'At follow up with the patient it will emerge what the patient needs to be re-informed about and what is missing, but we don't use any tool for this evaluating…' (Recommendation 6) | Registered Nurse, Sweden]. |
| 14 | 'No framework for follow-up' (Recommendation 6) | Rheumatologist, Belgium |
| 15 | 'Lack of time to organize follow-up and evaluation consultations' (Recommendation 6). | Family Doctor, Portugal |
| | Limited resources | |
| 16 | 'Not all patients have access to the Internet' (Recommendation 4) | Nurse, Finland |
| 17 | 'Face-to-face online support and telephone support at a general hospital like ours are not possible' (Recommendation 4) | Nurse, Japan |
| 18 | 'Group sessions and online cannot be used due to institutional restrictions'. (Recommendation 4) | Occupational Therapist, Japan |
| | Concerns about online PE | |
| 19 | 'My preferred method to answer patients' questions is absolutely individually and face-to-face, online contact and written material can be misunderstood; however, this (online/written) is possible for most patients in case of sharing more general information' (Recommendation 3) | Rheumatologist, Belgium |
| 20 | 'Online interaction seems not an ideal approach in my opinion. For example, information shared via email could be misinterpreted wrongly'. (Recommendation 4) | Rheumatologist, Belgium |
| 21 | 'Online self-learning can be misleading' (Recommendation 3) | Rheumatologist, Japan |
| | Concerns about patient-delivered PE (Recommendation 7) | |
| 22 | 'It is mandatory that the physician should control over the information provided to the patient'.(Recommendation 7) | Rheumatologist, France |
| 23 | 'The presence of non-healthcare personnel would open the door to dubious situations'. (Recommendation 7) | Rheumatologist, Italy |
| | Lack of systematic PE | |
| 24 | 'Not systematic' (Recommendation 2) | Occupational Therapist, Norway |
| 25 | 'Very rare monitoring of patients with [IA)' (Recommendation 2) | Registered Nurse, Portugal |
| 26 | 'The focus is on newly diagnosed patients, there is no organised PE aside from ordinary doctor- and nurse visits' (Recommendation 2) | Rheumatologist, Sweden |
| 27 | 'Patients come often spontaneously to PE after reading a poster, receiving a flyer etc… Not enough on doctor's initiative…(not) according to a defined agenda'. (Recommendation 2) | Pharmacist, France |
| 28 | 'It's up to me to keep me updated about appropriate pedagogics' (Recommendation 8) | Nurse, Sweden |
| 29 | 'I do not think we do [PE] according to the most up-to date research findings' (Recommendation 8). | Physiotherapist, Hungary |
| | Lack of funding | |
| 30 | 'The money for training costs is reduced year by year' (Recommendation 8). | Nurse, Finland |
| 31 | 'Do not have the money' (Recommendation 8). | Physiotherapist, Hungary |
| | Lack of patient participation in disease management | |
| 32 | 'Patient with incorrect beliefs, patient thinking that only treatment is important, patient not wanting or unable to change their everyday life activities' (Recommendation 1). | Occupational Therapist, France |
| 33 | 'The patient is not willing to come to the nurse's office. All patients do not understand that there is something to be done by the caregiver in treating the patient'. (Recommendation 1). | Nurse, Finland |
| 34 | I always offer it, and the rheumatologist always offers this, however, when the patient indicates that he or she does not want to be ready for this, it will not happen. We do not see all patients with inflammatory arthritis, so [PE] is not standard care(Recommendation 1). | Specialist Nurse, The Netherlands |

**Table 4** Quotes to illustrate the respective category supporting facilitators to implementation

| Quote number (Q) | Category/illustrative quotes | Quoted by |
|---|---|---|
| | Tailoring PE | |
| 35 | 'Some [are] more in need of information than others and are more "dependent" on information to move forward' (Recommendation 1). | Occupational Therapist, Norway |
| 36 | 'Informed… on their disease(s) and treatment(s) and options' (Recommendation 1). | Rheumatologist, Belgium |
| 37 | 'Life's situations are changeable, which the teaching should be targeted for' (Recommendation 1). | Authorised Nurse, Denmark |
| 38 | 'Therapy compliance, self-management and treatment objectives' (Recommendation 1). | Nurse, The Netherlands |
| 39 | 'PE must…always be customized to the patients' needs and resources and limitations. The feasibility for the different platforms for the patient education must always be considered'. (Recommendation 4) | Occupational Therapist, Sweden |
| 40 | 'We have psychologist, group therapy… nurses and physiotherapists trained in pain and trained in drug education'. (Recommendation 5). | Rheumatologist, France |
| 41 | 'Common basis for all patients and a personalized part, 50/50' (Recommendation 3) | Rheumatologist, Belgium |
| 42 | 'General instructions … After that, individual instructions will be given' (Recommendation 3) | Physiotherapist, Finland |
| | Using group education | |
| 43 | 'Group interaction and experience sharing can be very enriching' (Recommendation 3) | Nurse, France |
| | Linking patient education with diagnosis and treatments | |
| 44 | 'We provide education at diagnosis, at the start of pharmacological and non pharmacological interventions and periodically depending on individual patient needs. Sometimes limited clinic time can act as a barrier, however, I believe, as a department, we do strive to give good quality education via a multi-disciplinary approach'. (Recommendation 2) | Registered Nurse, UK |
| 45 | 'Life's situations are changeable, which the teaching should be targeted for' (Recommendation 2). | Authorised Nurse, Denmark |
| 46 | 'Regularly organised education programs (by and for patients)' (Recommendation 1). | Rheumatologist, The Netherlands |
| 47 | 'Patient education is … the basis for standard treatment"…I want to think of patient education like "soil ploughing" for standard treatment to "grow" or develop'. (Recommendation 1). | Physiotherapist, Japan |
| | Maintaining face-to-face PE delivery and inviting feedback | |
| 48 | 'Asking the patient verbally … not by means of questionnaires' (Recommendation 6). | Rheumatologist, Belgium |
| 49 | '(This method] makes it possible to check whether the information is understood, the other forms do not' (Recommendation 4) | Nurse, The Netherlands |
| | Accessing multidisciplinary teams and patient organisations | |
| 50 | 'Patients are being asked to take care of [PE] especially if we are moving towards general health education that does not require very specialized knowledge' (Recommendation 7) | Rheumatologist, France |
| 51 | 'More awareness about avenues for patients to get trained in PE should be created' (Recommendation 7) | Educationist, India |
| 52 | 'The patient organizations are important players and should have a more eminent role, both for the patients but also for education of the professionals' (Recommendation 7) | Rheumatologist, Sweden |
| | Accessing training from different providers | |
| 53 | 'For me, it is the same as for the patients: competencies need to be maintained over time' (Recommendation 8) | Rheumatologist, France |
| 54 | 'I had a training course with the support of private funding (pharma companies)' (Recommendation 8) | Nurse, France |
| 55 | 'Specific training is …provided by the physiotherapy association' (Recommendation 8) | Physiotherapist, Belgium |

### Maintaining face-to-face PE delivery and inviting feedback

Benefits of face-to-face PE were acknowledged. In addition to allowing tailoring PE and patients to learn from one another in group setting, face-to-face delivery facilitated PE evaluation by inviting feedback and checking whether the information is understood (Q48 and Q49). To facilitate evaluation participants also suggested sending out evaluation forms, planning follow-up sessions and providing telephone support as needed.

### Accessing multidisciplinary teams and patient organisations to deliver PE

Ability of patients to provide PE was acknowledged together with training opportunities (Q50–Q51). Patient organisations were identified as important players in providing PE and also in training patients as PE providers (Q52).

### Accessing training from different providers

Participants acknowledged the importance of obtaining and maintaining knowledge and skills (Q53) and accessed training from a variety of sources, including private and professional organisations (Q54, and Q55).

### Mixed methods results

The mixed-methods analysis revealed similarities in barriers and facilitators for implementation across the recommendations. For example, lack of time, lack of training was seen in 6/8 recommendations. In the suggested facilitators, tailoring PE was suggested in 5/8 recommendations (table 5).

## DISCUSSION

This study disseminated the recommendations for PE in IA and assessed their acceptability and barrier and facilitators for implementation across 23 countries. This substantial project achieved good dissemination of the recommendations, providing a total of 20 translations of the recommendations. The responses (including textual data) suggest an expansive awareness and engagement with the recommendations and identify issues of implementation across the countries.

The findings suggested a very high level of agreement with all recommendations (median 10), but the self-reported application in clinical practice was rated consistently lower (median scores between 6 and 8). This difference illustrates the commonly known gap between knowledge or agreeing with the evidence

**Table 5** Similarities in the barriers and facilitators to implementation by recommendation

| Barriers | Lack of time | Lack of training | Lack of staff | Lack of assessment tools | Limited resources | Concerns about online PE | Concerns about patient-delivered PE | Lack of systematic PE | Lack of funding | Lack of patient participation in disease management |
|---|---|---|---|---|---|---|---|---|---|---|
| Recommendation 1 | ● | ● | ● | | | | | ● | | ● |
| Recommendation 2 | ● | ● | ● | | ● | | | ● | | |
| Recommendation 3 | ● | ● | ● | | ● | | | | | |
| Recommendation 4 | ● | | ● | | ● | ● | | | ● | |
| Recommendation 5 | | ● | ● | | | | | | | |
| Recommendation 6 | ● | | | ● | | | | ● | ● | |
| Recommendation 7 | | ● | ● | | ● | | ● | | | |
| Recommendation 8 | ● | ● | | | | | | ● | ● | |

| Facilitators | Tailoring PE | Using group PE | Linking PE with diagnosis, treatment and multi-disciplinary care | Maintaining face-to-face PE delivery and inviting feedback | Accessing multi-disciplinary teams and patient organisations to deliver PE | Accessing training from different providers |
|---|---|---|---|---|---|---|
| Recommendation 1 | ● | | ● | | | |
| Recommendation 2 | ● | | ● | | | |
| Recommendation 3 | ● | ● | | | | |
| Recommendation 4 | ● | ● | | ● | | |
| Recommendation 5 | ● | | | | ● | |
| Recommendation 6 | | | | ● | | |
| Recommendation 7 | | | | | ● | ● |
| Recommendation 8 | | | | | | ● |

The dots indicate how the barriers and facilitators relate to the recommendations.
PE, patient education.

and application in practice, the latter requiring efforts to address individual, organisational and societal barriers to change.[7–10]

The common barriers to implementation were lack of time, lack of training and inadequate staff. This agrees with the literature, which suggests that work pressure, lack of time and perceived lack of training are the common reasons why clinicians find it hard to apply recommendations into clinical practice.[30 31] While those three factors interact with each other, efforts directed towards (cross-disciplinary) training of professionals and patients to deliver PE may help improve the perceived lack of time and staff. However, it is important to highlight that training also needs funding, time and effort, thus needing a change at all (individual professional, institution and policy) levels. Training of PE providers was also identified as an education agenda of the current recommendations.[4]

The mixed-methods approach has made it possible for the qualitative findings to explain the quantitative results. For example, recommendation 6 (the requirement for outcomes of PE to be evaluated) was rated the lowest in applicability to practice and the corresponding qualitative findings explain the possible reasons for this such as perceived lack of time, lack of structure and oversight about the effectiveness of PE, including a lack of a reliable assessment tool. This meant that evaluation of PE was often overlooked.

There were notable differences in responses across countries, in terms of applicability of the recommendations. For example, participants from Ireland, Denmark, Hong Kong, Japan and Portugal indicated that the technology and internet access provided by hospitals might not be sufficient to offer supplementary online PE support. A previous UK study found while internet-based video consultations in outpatient care were found to be safe, time-efficient and convenient, there was strong resistance from hospital information/technology departments, as videoconferencing was anticipated to require costly updates and increased technical support.[32] In light of changes to service delivery as a result of COVID-19, hospitals across the world have quickly adopted virtual (video or phone-based) appointments in response to restrictions in face-to-face interactions, therefore showing potential for faster development in the delivery of PE in virtual environment. Evaluation of how departments adopt these changes will inevitably inform future training and developments in the delivery of PE.

Interestingly, some responses on recommendation 7 from France, Italy, Portugal and Japan expressed concerns that there would not be enough trained patients to deliver PE, or patients might give inaccurate information and who would be responsible for this information. A study with general practitioners in the UK[33] highlighted similar tensions between supporting increased patient self-management and professional responsibility. It took confidence from both the doctor and the patient to ensure that control and responsibility were shared.[33] Developing targeted training for patients who deliver PE may help address some of the above concerns and this could be championed by patient and professional organisations.

The main strength of this study is its extensive reach across 23 countries, including those with less established rheumatology multidisciplinary team care or focus on PE. Collaborating with leaders of professional organisations in these countries facilitated the dissemination. Second, the response from such a number of diverse health professionals suggests multidisciplinary engagement with the recommendations. Third, efforts were made to gain textual responses, which ensured rich data on specific

barriers or facilitators for implementing each recommendation. The mixed-methods design has provided a unique opportunity to obtain a deeper understanding of the issues needed to address for a successful implementation of these recommendations. Last, our data can be used to develop practitioner-informed quantitative scales to measure the level of applicability of future recommendations.

This study has four key limitations. First, there is limitation of external validity, as the voluntary nature of the study meant that the responses were not uniform across countries, with some countries having higher response rates than others. Therefore, the results can only represent the views of respondents to our survey and may not be representative of all professionals in rheumatology across all 23 countries. Further work will be required to assess country-specific barriers and facilitators, especially in the regions that were under-represented in this study. Second, data were collected between July and September 2019, a typical summer vacation time in some countries, which could have affected the response rates. Third, some participants started the online survey but did not complete. Our analysis focused on completed data only as our survey platform (Qualtrics) captures all the data and it is impossible to tell if participants with incomplete data went ahead to complete the survey using a different device. All these suggest that a degree of selection bias cannot be excluded. Last, this study identified the barriers and facilitators to implementation at the individual practitioners and institutional (micro and meso) levels. Further study of the wider policy context (macro) level in each country will be required to ensure sustainable implementation and improvements in the quality PE.[6–9]

In conclusion, the EULAR recommendations for PE in IA have been disseminated across 23 countries and a range of barriers and facilitators to their implementation has been identified. A high level of agreement with all the recommendations is encouraging although addressing the barriers at the individual, organisation and societal level will be important to ensure successful application to practice. Some barriers to application are amenable to change, such as addressing training needs of providers and developing evaluation tools for PE. Further targeted implementation activities may be required in different countries, taking account of their healthcare systems to promote integration of the recommendations in practice and, thus, improve the outcome of patients with IA.

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**Contributors** SB, coordinated the study, collected the data, undertook the analysis, drafted the manuscript and revised it for intellectual content. MN is the Principal Investigator, designed the study, led the grant application, oversaw the project and interpretation of the results and revised the study report for intellectual content. HAZ codesigned the study, contributed to the grant application, interpretation of the results and revised it for intellectual content. AvT is the Methodologist for this project, co-designed the study, contributed to the grant application, interpretation of the results and revised it for intellectual content. IL, provided methodological advice, contributed to adaptation of the questionnaire in Sweden, interpreting the data and reviewing the manuscript for intellectual content. The following co-investigators were involved in the dual-panel adaptation of the questionnaire from English into their respective countries, disseminating the survey, interpreting the results, and revising the manuscript for intellectual content: CBo, Sweden; AD, Hungary; JP, Denmark; PM, Ireland; TN, Italy; RJOF, Portugal; MF, Japan; KvdE, Belgium; MdCHM, Spain; RR, India; CBe, France; MG-P, Bulgaria; KH, Germany; YvE-H, Netherlands; MLK, Finland; MSi, Poland; MS-M, Austria; GK, Hong Kong; HZ, Norway. MN and SB had access to the data. MN is responsible for the overall content as guarantor, controlled the decision to publish and accepts full responsibility for the finished work and the conduct of the study.

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**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 conducted in accordance with the Declaration of Helsinki. Participants were informed that their participation was voluntary and that by submitting a completed survey this implied their consent to participate. The ethics approval was obtained from Faculty of Health and Applied Sciences Research Ethics Committee, University of the West of England, Bristol, UK (UWE REC REF No: HAS.18.11.066). Participants gave informed consent to participate in the study before taking part.

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EPIDEMIOLOGICAL SCIENCE

# Effectiveness of TNF-inhibitors, abatacept, IL6-inhibitors and JAK-inhibitors in 31 846 patients with rheumatoid arthritis in 19 registers from the 'JAK-pot' collaboration

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## ABSTRACT

**Background** JAK-inhibitors (JAKi), recently approved in rheumatoid arthritis (RA), have changed the landscape of treatment choices. We aimed to compare the effectiveness of four current second-line therapies of RA with different modes of action, since JAKi approval, in an international collaboration of 19 registers.

**Methods** In this observational cohort study, patients initiating tumour necrosis factor inhibitors (TNFi), interleukin-6 inhibitors (IL-6i), abatacept (ABA) or JAKi were included. We compared the effectiveness of these treatments in terms of drug discontinuation and Clinical Disease Activity Index (CDAI) response rates at 1 year. Analyses were adjusted for patient, disease and treatment characteristics, including lines of therapy and accounted for competing risk.

**Results** We included 31 846 treatment courses: 17 522 TNFi, 2775 ABA, 3863 IL-6i and 7686 JAKi. Adjusted analyses of overall discontinuation were similar across all treatments. The main single reason of stopping treatment was ineffectiveness. Compared with TNFi, JAKi were less often discontinued for ineffectiveness (adjusted HR (aHR) 0.75, 95% CI 0.67 to 0.83), as was IL-6i (aHR 0.76, 95% CI 0.67 to 0.85) and more often for adverse events (aHR 1.16, 95% CI 1.03 to 1.33). Adjusted CDAI response rates at 1 year were similar between TNFi, JAKi and IL-6i and slightly lower for ABA.

**Conclusion** The adjusted overall drug discontinuation and 1 year response rates of JAKi and IL-6i were similar to those observed with TNFi. Compared with TNFi, JAKi were more often discontinued for adverse events and less for ineffectiveness, as were IL-6i.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ For patients with rheumatoid arthritis with an inadequate response or contraindications for conventional synthetic disease-modifying antirheumatic drugs (DMARDs), several second-line therapy options exist from which rheumatologists and patients can choose.

⇒ Only a limited number of small studies have evaluated the effectiveness of targeted-synthetic DMARDs and biological DMARDs in the real world.

### WHAT THIS STUDY ADDS

⇒ This large comparative effectiveness analysis, involving 19 registers and over 30 000 treatment courses, is the first to evaluate real life effectiveness and safety outcomes among four common available treatment alternatives and found similar discontinuation rates and Clinical Disease Activity Index response, although discontinuation reasons tended to differ between treatments, with more discontinuation for safety with JAK-inhibitors, but less for effectiveness.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Considering similar effectiveness among these treatments, this study calls for the evaluation of other outcomes that could influence treatment choice, such as patient-reported outcomes, comorbidities, tolerability, safety or cost-effectiveness.

## INTRODUCTION

Since the development of tumour necrosis factor-inhibitors (TNFi) in the nineties, treatment options for rheumatoid arthritis (RA) have greatly increased with the emergence of other classes of biological disease-modifying antirheumatic drugs (bDMARDs), such as interleukin-6 inhibitors (IL-6i) and abatacept (ABA), and more recently the

targeted-synthetic DMARDs (tsDMARDs), with Janus kinase inhibitors (JAKi).[1–9] While all bDMARDs and tsDMARDs have demonstrated efficacy in randomised controlled trials,[1–9] these results are not always relevant to 'real-world patients', because of very restrictive inclusion criteria, numerous exclusion criteria and limited follow-up.[10] In addition, bearing in mind the number of current options available, head-to-head trials including several of the alternative treatment options would be impractical to realise. However, considering the number of available treatment options for second-line therapy in RA, a representative estimation of their relative effectiveness in the real world would be useful to help patients and rheumatologists to choose an appropriate treatment. Registers provide a unique opportunity to compare available treatment options and understand the effectiveness of these therapies in clinical situations, which is becoming even more important as we move towards personalised clinical care. The objective of this study was thus to evaluate and compare the real-world effectiveness of four different second-line therapies namely TNFi, ABA, IL-6i and JAKi.

## METHODS

### Patient sample

The JAK-pot collaboration is an investigator-initiated observational study, which aims to evaluate clinical aspects of bDMARDs and tsDMARDs in RA. Patients were included since JAKi were commercially available in each country (earliest being 2013 for Switzerland and Russia) until March 2021. To avoid confounding by time-trends, we excluded patients who initiated treatment of interests (bDMARDs or JAKi) before JAKi were commercially available in each country. Patients with a clinical diagnosis of RA and starting treatment with a JAKi (baricitinib, tofacitinib or upadacitinib at that time), a TNFi, ABA or an IL-6i during the study period, were included from the following registers: ATTRA from the Czech Republic, ARBITER from Russia, BIOBADASER from Spain, BIOREG from Austria, biorx.si from Slovenia, BSRBR-RA from the UK, DANBIO from Denmark, GISEA from Italy, I-RECORD from Israel, METEOR from the Netherlands, NORDMARD from Norway, RABBIT from Germany, REUMA.PT from Portugal, RHUMADATA from Canada, ROB-FIN from Finland, RRBR from Romania, SCQM from Switzerland, TARDIS from Belgium, TURKBIO from Turkey. ARBITER, biorx.si, BSRBR-RA and RABBIT did not contribute ABA treatment courses, and RABBIT did not contribute IL-6i treatment courses. All registers contributed individual treatment course-level data (eg, non-aggregated) to this collaborative analysis, except DANBIO and TARDIS that provided only aggregated data and results of analyses. Filgotinib was not included because it was not marketed during most of the study period in participating registers. Rituximab was also not included as discontinuation is often difficult to assess.

### Time point definitions and treatment groups

Baseline was defined as the initiation date of each of the treatment courses under investigation. Each treatment course was operationally defined as the period between drug initiation to treatment discontinuation, the switch to another treatment, the end of participation in the register, or the end of the study period (March 2021), whichever came first. Durations between visits depend on the register design and national recommendations on frequency of clinical contact, but most registers usually include at least an annual visit.

### Exposure of interest

The exposure of interest was the type of treatment (TNFi, ABA, IL-6i or JAKi).

### Study outcomes

The primary outcome of effectiveness was treatment discontinuation, which was evaluated in all registers. As secondary outcomes of effectiveness, we evaluated (1) reasons for discontinuation by treatment and (2) treatment response using the Clinical Disease Activity Index (CDAI) defined as reaching low disease activity (LDA, CDAI ≤10) and remission (CDAI ≤2.8) at 12 months.[11 12] We used CDAI as disease activity measure as it does not include acute phase reactants and is less skewed by agents having a strong effect on acute phase reactants, such as IL-6i and probably JAKi.

### Covariates of interests

For multivariable adjustments, we chose baseline covariates considered a priori as potential confounding factors according to clinical knowledge and current literature.[13] We included gender, age, disease duration, seropositivity, number of previously used bDMARDs/tsDMARDs (0, 1, 2, ≥3), concomitant conventional synthetic DMARDs (csDMARDs) treatment (none; methotrexate; other csDMARDs without methotrexate; methotrexate and at least one other csDMARDs), concomitant glucocorticoids (presence/absence), tobacco smoking, functional status (Health Assessment Questionnaire Disability Index -HAQ-DI), CDAI (or Disease Activity Score on 28 joints - DAS28 - if CDAI was not available), C reactive protein (CRP) and year of treatment initiation. For seropositivity, patients were classified as being seropositive if rheumatoid factor (RF) and/or anticyclic citrullinated peptide antibodies (ACPA) were positive, negative if both were negative, and missing if one was missing and the other was negative, to limit misclassification. In BSRBR-RA, only RF was available and seropositivity was defined as positive if RF was positive, negative if RF was negative, and missing if RF was missing. In the TARDIS register, concomitant csDMARDs treatment, HAQ, CDAI and seropositivity were not available, and DAS28 was used for adjustment for disease activity. Other sporadically missing data by registers are shown in online supplemental table 1.

### Statistical methods

We performed analyses and reported results according to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines and the European Alliance of Associations for Rheumatology points to consider on comparative effectiveness research.[14 15]

We analysed baseline characteristics using standard descriptive statistics and indicated number of patients with valid values. Since two of the registers could only provide aggregated results due to local regulations with respect to cross border data sharing, all adjusted analyses were performed within each individual register and combined using random-effect meta-analysis methods, which also allowed to account for possible heterogeneity between registers. Heterogeneity was evaluated using $I^2$. Several treatment courses from a single patient could be included if the patient had been treated by more than one second line treatment during the follow-up period. Thus, we added a cluster term for the patient identity, thereby allowing the estimation of robust SEs, in a manner similar to generalised estimating equation models. The investigators of the two registers that did not provide individual treatment course-level data, received a detailed description of the analyses, as well as the code used for

**Table 1** Characteristics of the patients at the start of their 20 837 treatment courses (17 registers, individual treatment course-level data)

| | TNFi | | ABA | | IL-6i | | JAKi | |
|---|---|---|---|---|---|---|---|---|
| | N valid | Value | N valid | Value | N valid | Value | N valid | Value |
| N | | 11 376 | | 1877 | | 2517 | | 5067 |
| N visits (median (IQR)) | | 2 (1–4) | | 2 (1–4) | | 2 (1–4) | | 2 (1–3) |
| Total patient-years | | 18 072 | | 2589 | | 3508 | | 4218 |
| Treatment duration, years (median (IQR)) | 11 376 | 0.9 (0.4, 1.9) | 1877 | 0.8 (0.4, 1.9) | 2517 | 0.9 (0.3, 1.9) | 5067 | 0.6 (0.2, 1.1) |
| Age, years (mean (SD)) | 11 353 | 55.6 (13.3) | 1873 | 59.6 (12.5) | 2514 | 57.1 (12.8) | 5067 | 58.2 (12.5) |
| Gender (female) (%) | 11 354 | 8699 (76.6) | 1874 | 1469 (78.4) | 2516 | 1977 (78.6) | 5066 | 4108 (81.1) |
| Disease duration, years (mean (SD)) | 10 771 | 9.2 (8.5) | 1820 | 11.1 (9.3) | 2429 | 11.2 (9.0) | 4939 | 11.8 (9.2) |
| Seropositivity (RF and/or ACPA), (%) | 9396 | 7419 (79.0) | 1575 | 1304 (82.8) | 2103 | 1723 (81.9) | 4271 | 3471 (81.3) |
| No of previous b/tsDMARDs (%) | 11 146 | | 1811 | | 2456 | | 4922 | |
| 0 | | 6538 (58.7) | | 685 (37.8) | | 783 (31.9) | | 1556 (31.6) |
| 1 | | 2119 (19.0) | | 469 (25.9) | | 730 (29.7) | | 1010 (20.5) |
| 2 | | 1409 (12.6) | | 322 (17.8) | | 517 (21.1) | | 872 (17.7) |
| ≥3 | | 1080 (9.7) | | 335 (18.5) | | 426 (17.3) | | 1484 (30.2) |
| Concomitant csDMARDs (%) | 11 376 | | 1877 | | 2517 | | 5067 | |
| None | | 3397 (29.9) | | 600 (32.0) | | 712 (28.3) | | 1694 (33.4) |
| MTX | | 4257 (37.4) | | 213 (11.3) | | 238 (9.5) | | 426 (8.4) |
| MTX associated with other than MTX | | 1611 (14.2) | | 601 (32.0) | | 1070 (42.5) | | 2064 (40.7) |
| Other than MTX | | 2111 (18.6) | | 463 (24.7) | | 497 (19.7) | | 883 (17.4) |
| GC (yes/no) | 10 449 | 4213 (40.3) | 1643 | 829 (50.5) | 2211 | 1076 (48.7) | 4760 | 2293 (48.2) |
| GC dose (median (IQR)) | 3386 | 5.0 (4.0, 7.5) | 720 | 5.0 (5.0, 10.0) | 912 | 5.0 (5.0, 10.0) | 2074 | 5.0 (4.0, 7.5) |
| CRP (mg/L) (mean (SD)) | 7842 | 11.8 (23.6) | 1269 | 13.8 (22.2) | 1818 | 16.3 (27.4) | 3849 | 13.9 (25.4) |
| CDAI (mean (SD)) | 4002 | 20.7 (12.7) | 833 | 21.8 (12.3) | 969 | 22.8 (13.2) | 2358 | 23.7 (13.3) |
| DAS28 (mean (SD)) | 4176 | 4.4 (1.5) | 839 | 4.5 (1.5) | 1029 | 4.8 (1.5) | 2461 | 4.8 (1.5) |
| HAQ (mean (SD)) | 3660 | 1.0 (0.7) | 674 | 1.1 (0.7) | 976 | 1.1 (0.7) | 1741 | 1.2 (0.7) |
| Smoking (%) | 8271 | | 1202 | | 1737 | | 3177 | |
| Current | | 1642 (19.9) | | 223 (18.6) | | 288 (16.6) | | 644 (20.3) |
| Never | | 4927 (59.6) | | 834 (69.4) | | 1192 (68.6) | | 1976 (62.2) |
| Past | | 1702 (20.6) | | 145 (12.1) | | 257 (14.8) | | 557 (17.5) |
| Body mass index kg/m$^2$ (mean (SD) | 7181 | 27.0 (5.7) | 1097 | 27.2 (5.7) | 1389 | 26.9 (5.5) | 3218 | 27.1 (5.6) |
| Any comorbidity, % | 7772 | 3306 (42.5) | 1571 | 793 (51.2) | 2032 | 926 (45.6) | 4336 | 2158 (49.8) |

ABA, abatacept; ACPA, anticitrullinated protein antibody; bDMARDs, biological disease-modifying antirheumatic drugs; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; csDMARDs, conventional DMARDs; DAS28, Disease Activity Score on 28 joints; GC, glucocorticoids; HAQ, Health Assessment Questionnaire; IL-6i, Interleukin 6 inhibitors; JAKi, Janus kinase inhibitors; MTX, methotrexate; RF, rheumatoid factor; RF, rheumatoid factor; TNFi, tumor necrosis alpha inhibitors; tsDMARDs, targeted synthetic DMARDs.

the analyses (in R programming language), and replicated these analyses as closely as possible.

### Main outcome

For the primary outcome (drug retention), we used Kaplan-Meier and Cox models. The Cox models were adjusted for all the baseline covariates as described above. TNFi was used as the comparison group, comprising the most treatment courses. Missing covariates were imputed using multiple imputations with chained equations (50 samples, predictive mean matching algorithm, see supplementary methods). A cluster term was added for the patient identity, as each patient could provide information for each treatment arm. As a sensitivity analysis, we excluded TARDIS, as some covariates were not available for adjustment in this register.

### Secondary outcomes

For the secondary outcomes, only registers with individual treatment course-level data (non-aggregated) were included (17 registers, online supplemental figure 1).

For the analysis of discontinuation reasons, we used a Fine-Gray model for adverse events, considering lack of effectiveness and other reasons of discontinuation as competing risks. To avoid overadjustment, and as this was a secondary analysis, we pooled the registers and adjusted for fewer a priori selected baseline variables than for the main outcome namely: gender, age, disease duration, seropositivity, previous treatment with a b/tsDMARD as a binary variable (presence/absence), CRP, CDAI and presence of a concomitant treatment with csDMARDs as a binary variable (presence/absence) with a strata term for the country and the year of treatment initiation. Missing covariates were imputed using multiple imputations.

For the other secondary outcome (CDAI treatment response at 1 year), we additionally excluded BSRBR-RA, I-RECORD, RRBR and TURKBIO registers, as information on CDAI was not available for follow-up visits (online supplemental figure 1). When no CDAI assessments were present at 1 year at the individual level, the means of their values within a ±1.5 months window were used. Values that were still missing for patients on drug after 12 months were imputed using the nearest available neighbour, as advised by a recent simulation study.[16] We estimated the proportions of patients reaching remission or LDA by treatment group using a method correcting for attrition (patients lost to follow-up or stopping treatment), and adjusting for confounders (confounder-adjusted response rate with attrition correction).[17] Briefly, this method discards values of CDAI at 12

months for treatment courses discontinued before 12 months, due to potential influence of new, subsequent treatments. It then uses multiple imputation with chained equations to estimate the difference of CDAI remission or LDA between treatments, adjusting for covariates. These covariates include those used for the discontinuation model but also the reason for treatment discontinuation (ineffectiveness, adverse events, other reasons). When using this method, the adjusted response rates correspond to the response rates that the whole population would have had if all had been treated with the treatment of interest. We also calculated adjusted difference in response rates using TNFi as comparator.

## RESULTS

We included a total of 31 846 treatment courses: 17,522 TNFi, 2775 ABA, 3863 IL6-i and 7,686 JAKi. Two registers provided only aggregated data, while the rest of the 17 registers provided individual treatment course-level data, for a total of 20 837 treatment courses (table 1). In these 17 registers, patients were on average 56.8 years old, with a mean disease duration of 10.2 years, mostly seropositive (80%), female (78%) and with moderate disease activity at treatment initiation. Forty-one per cent of the JAKi treatment-courses were baricitinib and 59% tofacitinib. There were no patients included during the study period with upadacitinib. Overall baseline characteristics were similar between registers (online supplemental table 1). Patients starting TNFi were younger, had a shorter disease duration, less previous b/tsDMARD experience, and were less often on monotherapy. JAKi and IL-6i were more often given as monotherapy, and JAKi were more often prescribed after several treatment failures. Treatment groups were comparable for gender, seropositivity and disease activity.

### Treatment retention

Crude median drug retention for registers with individual treatment course-level data was 1.68 years (IQR 1.62–1.74) for TNFi, 1.58 years (IQR 1.48–1.73) for ABA, 1.88 years (IQR 1.76 to 2.02) for IL-6i and 1.19 (IQR 1.10–1.26) years for JAKi. Crude HR of discontinuation for ABA compared with TNFi was 1.16 (95% CI 0.97 to 1.39), 1.05 (95% CI 0.91 to 1.21) for IL6-i and 1.48 (95% CI 1.20 to 1.83) for JAKi. When adjusting for confounding factors, we no longer found any significant difference in the adjusted HRs (aHR) for discontinuation for ABA (aHR 0.96, 95% CI 0.86 to 1.07), IL-6i (0.91, 95% CI 0.82 to 1.01) and JAKi (1.00, 95% CI 0.83 to 1.22), compared with TNFi (figure 1). In the sensitivity analysis excluding TARDIS, aHR for discontinuation were not significantly different for ABA (0.94, 95% CI 0.84 to 1.06) and JAKi 0.96 (95% CI 0.82 to 1.12) compared with TNFi but tended to be slightly lower for IL-6i (0.89, 95% CI 0.81 to 0.98). The adjusted hazard of JAKi discontinuation compared with TNFi was heterogeneously distributed across the countries (figure 2, $I^2$=92.7%). We obtained less discrepant results for IL-6i vs TNFi ($I^2$=64.1%) and ABA vs TNFi ($I^2$=58.4%).

### Discontinuation reasons

In the 17 registers with individual treatment course-level data, the main unique reason of stopping treatment was rather ineffectiveness than adverse events, and the order was similar for all treatments. Sixty-two per cent of treatment-courses specified the reason of discontinuation. When analysing the reason for discontinuation by treatment, no differences existed between ABA and TNFi for any of the discontinuation reason, while IL-6i (aHR 0.76, 95% CI 0.67 to 0.85) and JAKi (aHR 0.75, 95% CI 0.67 to 0.83) were less frequently discontinued for ineffectiveness compared with TNFi (online supplemental figure 2), but tended to be discontinued more often for adverse events (JAKi aHR 1.16, 95% CI 1.03 to 1.33; IL-6i: 1.09, 95% CI 0.85 to 1.03). Female gender (aHR 1.22, 95% CI 1.08 to 1.38) increased the hazard of discontinuation for adverse events, but not for ineffectiveness or for other reasons. Age also increased the hazard of discontinuation for adverse events (aHR 1.01, 95% CI 1.01 to 1.02 per additional year of age), but decreased the hazard of discontinuation for ineffectiveness and other reasons.

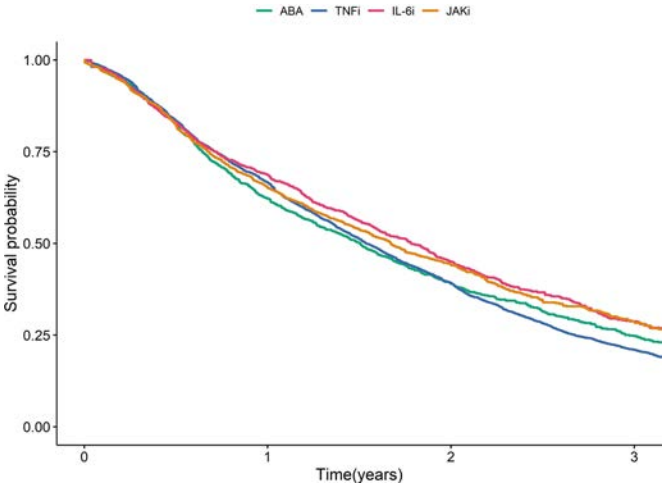


**Figure 1** Multivariable Cox model of drug discontinuation in patients from 16 registers with individual treatment course-level data. Analysis was adjusted for age, gender, disease duration, seropositivity, number of previous treatments, concomitant treatment with csDMARDs, concomitant treatment with glucocorticoids, CRP, HAQ, CDAI, comorbidities, smoking and BMI, and stratified by country and year of treatment initiation. ABA, abatacept; BMI, body mass index; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; csDMARDs, conventional synthetic disease-modifying anti-rheumatic drugs; HAQ, Health Assessment Questionnaire; JAKi, Janus kinase inhibitors.

### Response rates

In the 13 registers with individual treatment course-level data and available CDAI information during follow-up (8,404 TNFi, 1523 ABA, 1843 IL-6i, 3,925 JAKi), the overall adjusted 1 year response rates was generally similar (figures 3 and 4). The adjusted response rates were slightly lower for ABA (50% for LDA and 12% for remission, figures 3 and 4) compared with the other groups (54% LDA and 16% remission for TNFi, 55% and 16% for IL-6i and 56% and 15% for JAKi), and the difference reached significance when comparing the proportion of patients on ABA to TNFi for remission (difference in LDA −3.9%, 95% CI −8.9 to −1.1%; difference in remission −4.6%, 95% CI −6.7 to −1.3%). No significant differences existed in response rates at 1 year between JAKi, IL-6i and TNFi (difference in LDA 0.9, 95% CI −2.8 to 4.7% for IL-6i vs TNFi; −0.9%, 95% CI −2.8 to 4.6% for JAKi vs TNFi; difference in remission 0.3%, 95% CI −2.4 to 3.0% and −1.8%, 95% CI −4.4 to 1.0%).

## DISCUSSION

In this large international collective of registers, we found similar overall drug retention rates between treatment groups. Compared with TNFi, IL-6i and JAKi were less frequently discontinued for ineffectiveness, while JAKi and IL-6i tend to

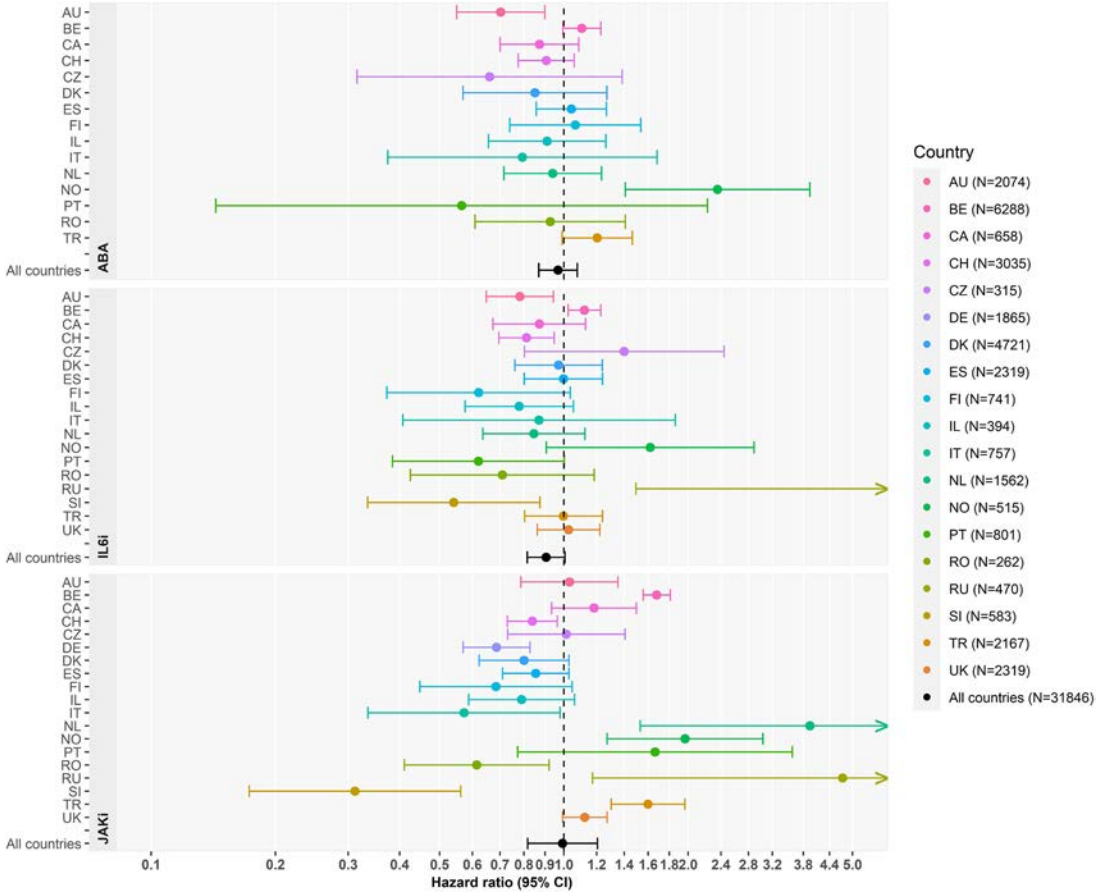


**Figure 2** HR of discontinuation compared with TNF-inhibitors by country and treatment. Adjusted for age, gender, disease duration, seropositivity, number of previous treatments, concomitant treatment with csDMARDs, concomitant treatment with glucocorticoids, CRP, HAQ, CDAI, comorbidities, smoking and BMI, and stratified by country and year of treatment initiation. All countries HR combined using a meta-analysis with random effect. AU, Austria; BE, Belgium; CA, Canada; CH, Switzerland; CZ, Czech Republic; DE: Germany, DK, Denmark; ES, Spain; FI, Finland; IL, Israel, IT, Italy; NL, Netherlands, NO, Norway, PT, Portugal, RO, Romania; RU, Russia; SI, Slovenia; TR, Turkey. DE, SI, RU and UK do not have or provide data on ABA. DE did not provide data on IL-6i. Due to lack of information on these variables, BE did not adjust for concomitant treatment with csDMARDs, concomitant treatment with glucocorticoids, HAQ, CDAI, smoking, BMI, comorbidities and seropositivity and adjusted for DAS28. HR for RU is out of bound: 5.0 (95% CI 1.0 to 22.9) for IL6-i, 3.7 (1.2 to 11.4) for JAKi. HR for nl is out of bound: 4.0 (95% CI 1.6 to 10.0) for JAKi. ABA, abatacept; BMI, body mass index; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; HAQ, Health Assessment Questionnaire; IL6i, interleukin 6 inhibitors; JAKi, Janus kinase inhibitors.

be more frequently discontinued for adverse events, furthermore, disease activity, adjusted for confounders and treatment discontinuation, was similar. More than half of patients with RA reached LDA state at 1 year and one out of seven, remission.

In this study, we compared the effectiveness of four classes of second-line treatment for RA with different mechanisms of action, including JAKi. JAKi are the latest class of advanced therapies and have the advantage of oral administration. Various randomised controlled trials have demonstrated JAKi being more efficacious than methotrexate, and non-inferior or even better on some outcomes than certain bDMARDs.[9 10 18] Beyond their proven efficacy, safety issues have raised questions on the impact these drugs would have on the management of patients with RA in routine care.[19] Contrasting with the short duration and the relatively limited number of patients included in randomised controlled trials, our study, conducted on a large sample of patients seen in daily clinical practice, enables to better appraise the persistence of therapy, a composite endpoint incorporating clinical effectiveness and safety, in comparing TNFi vs JAKi, ABA or IL-6i. We found a similar retention rate between these four treatment groups. These results are in line with recent evidence from a couple of smaller observational studies.[10] Recently, data from the Swiss RA register found in a real-world setting that the persistence on tofacitinib did not differ from ABA or IL-6i, and was slightly better than TNFi.[20] Like in this study, TNFi was discontinued more often for ineffectiveness and less for safety reasons compared with JAKi and treatment with other modes of action (including IL-6i).

The retention rates were heterogeneous among the participating countries, possibly reflecting national differences in physician's treatment choices and prescribing patterns. An investigation of national treatment guidelines or access to second line therapies, which could explain some of the national discrepancies, did not identify specific differences in access, eligibility criteria or prescription patterns among participating countries.[21] Other researchers have described the wide variability of drug retention among countries and found that it is in general not related to disparities in patient or disease characteristics, but to differences in health systems or surrogates thereof, such as national gross domestic product per capita.[22] To avoid biasing or wrongly assuming overly precise estimates, all main analyses reported herein were meta-analysed using random effect. The main single reason for discontinuation was ineffectiveness for all treatments, but JAKi tended to be discontinued more frequently

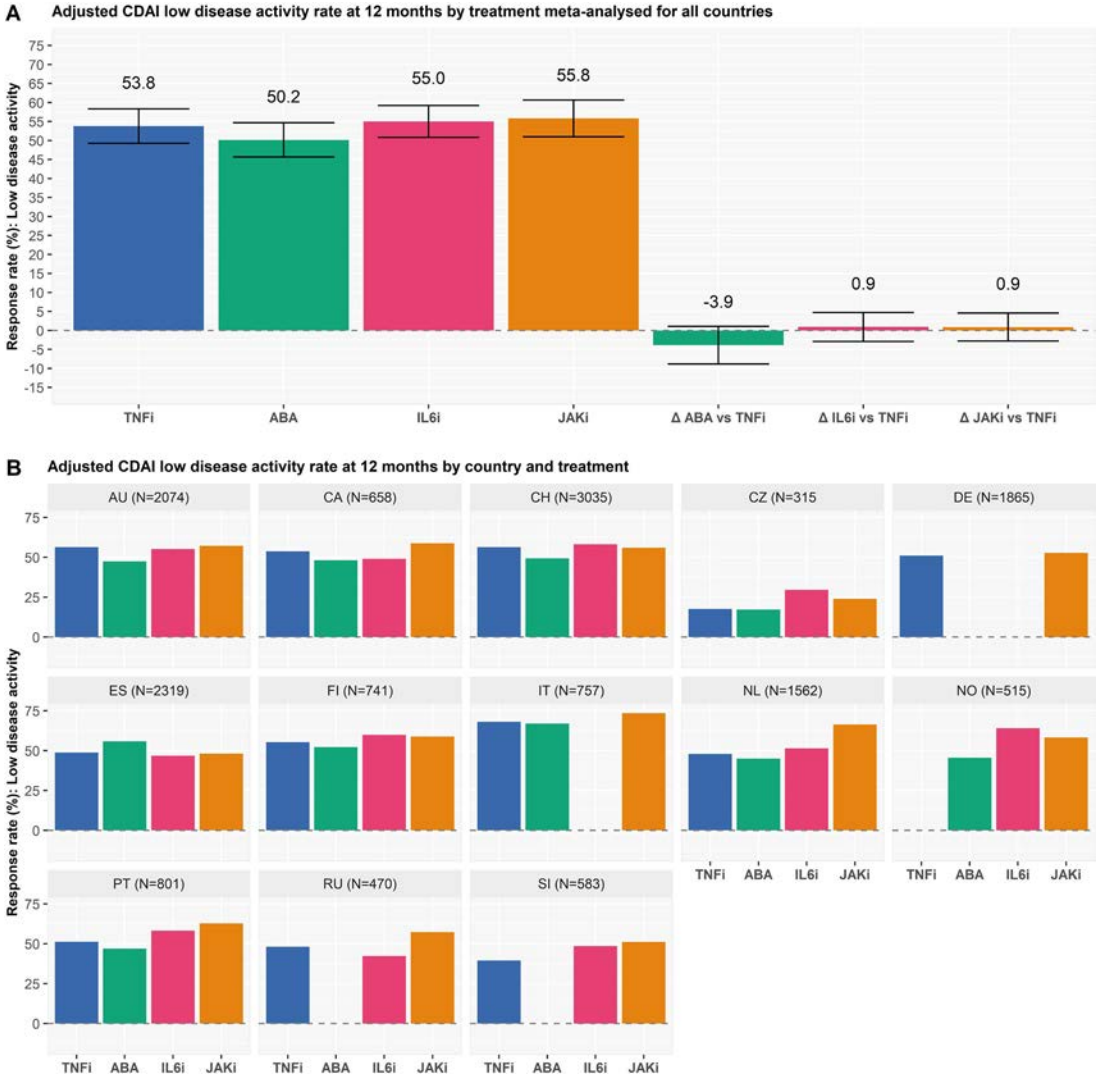


**Figure 3** Adjusted CDAI low disease activity rates at 12 months for the 14 registers with individual treatment course-level data and CDAI information during follow-up (A) by treatment meta-analysed for all countries (B) by country and treatment. analysis was adjusted for age, gender, disease duration, seropositivity, number of previous treatments, concomitant treatment with csDMARDs, concomitant treatment with glucocorticoids, CRP, HAQ, CDAI at baseline, comorbidities, smoking and BMI. DE, SI and RU do not have or provide data on ABA. DE did not provide data on IL6i. All countries rates are combined using a meta-analysis with random effect. TNFi: TNF inhibitors, ABA: abatacept, IL6i: IL6 inhibitors, JAKi: Janus kinase inhibitors, Δ ABA vs TNFi: difference in the response rate between abatacept and TNF inhibitors, Δ IL6i vs TNFi: difference in the response rate between IL6 inhibitors and TNF inhibitors, Δ JAKi vs TNFi: difference in the response rate between JAK inhibitors and TNF inhibitors. AU: Austria, CA: Canada, CH: Switzerland, CZ: Czech Republic, DE: Deutschland, ES: Spain, FI: Finland, IT: Italy, NL: Netherlands, no: Norway, Pt: Portugal, Ro: Romania, RU: Russia, SI: Slovenia. BMI, body mass index; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; csDMARDs, conventional synthetic disease-modifying anti-rheumatic drugs; HAQ, Health Assessment Questionnaire.

for adverse events and less for ineffectiveness compared with TNFi, although the effect size was small. It is possible that less experience in the treatment with JAKis and differences in the perceived or factual utility and safety of JAKi, in particular considering the warnings of medicine agencies, could contribute to this finding. However, most of our observations predate these communications and the publication of the results of the ORAL surveillance study.[19] To what extent this phenomenon mirrored a real 'biological' behaviour of JAKi remains to be further investigated.

Our study has some limitations. First, treatment was not randomly assigned opening the possibility for confounding. Though we used robust methods and statistical techniques to draw causal inferences from observational data, there is certainly some residual and/or unmeasured confounding, which could change the estimated associations. Nevertheless, only observational studies with large sample size can detect small effect sizes for safety concerns[23 24] and the adjustment was relatively comprehensive, except for a low granularity on type of comorbidities. Second, use of meta-analysis to combine national estimates limits the evaluation of the factors associated with effectiveness. Third, we did not fully evaluate safety in this study, though it was one of the reasons for discontinuation, as we do not currently have details on the specific adverse events that led to discontinuation nor their severity in this dataset. Indeed, as for all observational register studies, detailed quality check of the recorded adverse events and careful consideration of potential confounders for each adverse event will be necessary to produce trustworthy results. Moreover, for several treatment courses, discontinuation reasons other than ineffectiveness or adverse events were

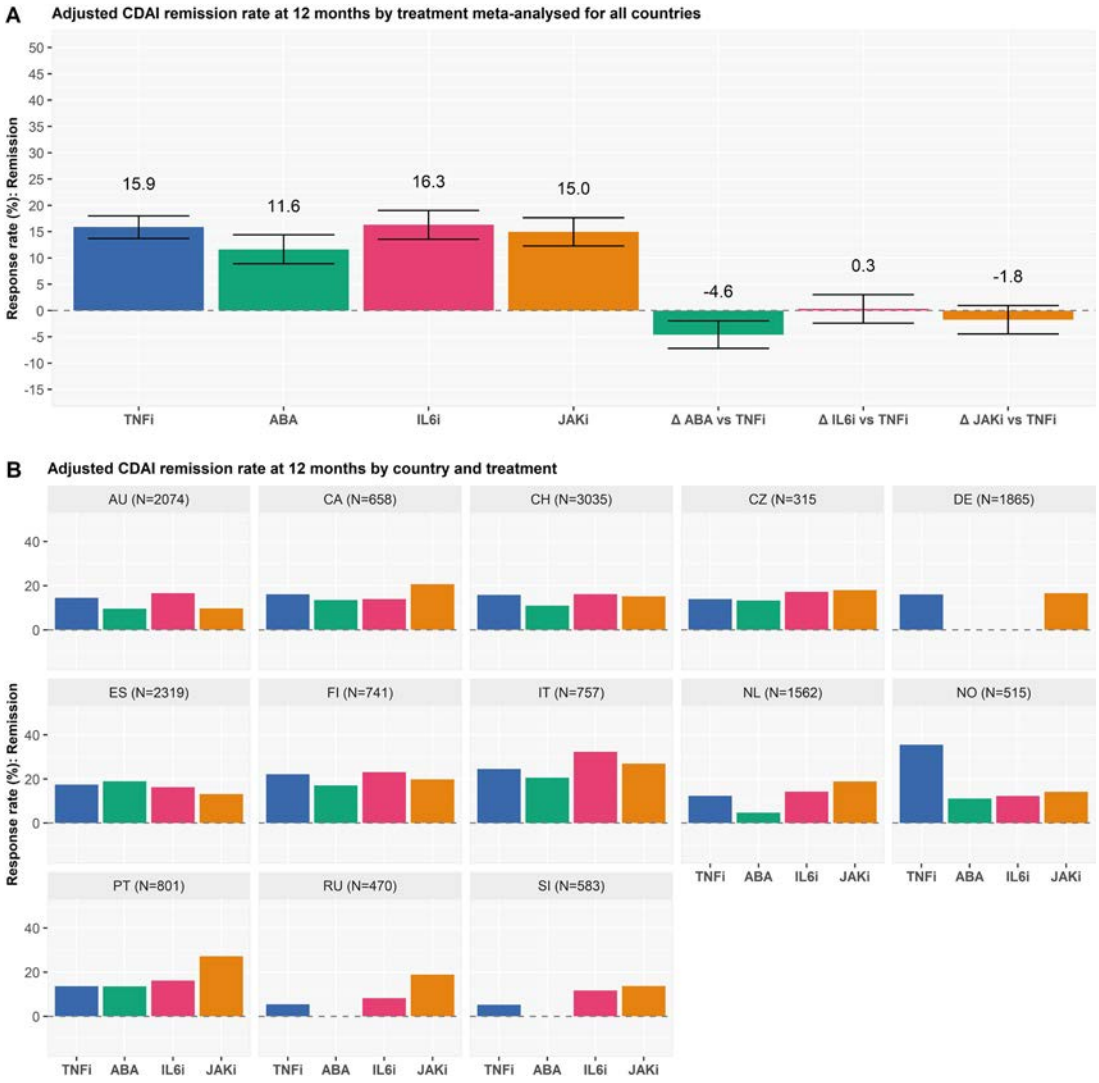


**Figure 4** Adjusted CDAI remission rates at 12 months for the 14 registers with individual treatment course-level data and CDAI information during follow-up (A) by treatment meta-analysed for all countries (B) by country and treatment. analysis was adjusted for age, gender, disease duration, seropositivity, number of previous treatments, concomitant treatment with csDMARDs, concomitant treatment with glucocorticoids, CRP, HAQ, CDAI at baseline, comorbidities, smoking and BMI. All countries rates are combined using a meta-analysis with random effect. DE, SI and RU do not have or provide data on ABA. DE did not provide data on IL6i. TNFi: TNF inhibitors, ABA: abatacept, IL6i: IL6 inhibitors, JAKi: JAK inhibitors, Δ ABA vs TNFi: difference in the response rate between abatacept and TNF inhibitors, Δ IL6i vs TNFi: difference in the response rate between IL6 inhibitors and TNF inhibitors, Δ JAKi vs TNFi: difference in the response rate between JAK inhibitors and TNF inhibitors. AU: Austria, CA: Canada, CH: Switzerland, CZ: Czech Republic, DE: Deutschland, ES: Spain, FI: Finland, IT: Italy, NL: Netherlands, NO: Norway, PT: Portugal, Ro: Romania, RU: Russia, SI: Slovenia. BMI, body mass index; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; HAQ, Health Assessment Questionnaire.

recorded, without more granularity to explore, as the categories chosen had to match all the registers; for several treatment courses, no reasons of stopping were recorded, and they could not be evaluated for this outcome. Finally, we grouped all JAKi agents into one category and did not explore potential differences in effectiveness among them. It is possible that individual JAKi have different effectiveness and safety profiles.

The strength of our study relies on the availability of the data of the largest collaborative international effort to date aiming at providing information on the real-world management of patients with RA in different countries. The clinical relevance of these results should therefore not be undermined by issues of low generalisability, as occurs in randomised controlled trials. Moreover, we provide an evaluation and a comparison of the effectiveness across different routinely prescribed alternative drugs.

In conclusion, our results support the use of these four treatments for treating patients with RA in 'real-world' clinical care, underscoring their similar effectiveness, as assessed by retention and response rates, which were comparable. However, we found an increased discontinuation of JAKi for safety reasons compared with TNFi, which could be due to a combination of real differences in safety profile and heightened concerns from physicians and patients, causing them potentially to be more careful with this newer treatment.

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**Correction notice** This article has been corrected since it published Online First. The second author's name and affiliation number 7 has been corrected and minor errors in the text at the end of the "Response rates" paragraph, where the numbers do not correspond to the images.

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CLINICAL SCIENCE

# Tumour necrosis factor inhibitor use during pregnancy is associated with increased birth weight of rheumatoid arthritis patients' offspring

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## ABSTRACT

**Objectives** To study pregnancy outcomes in a closely monitored, well-defined cohort of women with rheumatoid arthritis (RA). In particular, pregnancy outcomes of women that used a TNFi during pregnancy.

**Methods** Patients were derived from a prospective study on pregnancy and RA (Preconception Counseling in Active RA study) and treated according to a treatment protocol aimed at minimal disease activity. Multivariate linear regression analysis was used to describe which variables influenced birth weight.

**Results** 188 patients were included, 92 (48.9%) patients with RA used a TNFi during pregnancy. Disease Activity Score in 28 joints C reactive protein (DAS28CRP) was low at all time points during pregnancy (DAS28CRP in the third trimester: 2.17 (SD 0.73). TNFi use was not associated with an increase of adverse pregnancy outcomes such as low birth weight (<2500 g), (emergency) caesarian section, hypertensive disorders or congenital malformations. TNFi use resulted in less children born small-for-gestational age (p=0.05), however, did not increase the risk of large-for-gestational age (p=0.73). Mean birth weight was 173 g higher in women that used a TNFi during pregnancy (3.344 kg vs 3.171 kg, p=0.03). In the multivariate analysis, maternal age (β −0.023, 95% CI −0.040 to −0.0065, p=0.007), TNFi use (β 0.20, 95% CI 0.066, 0.34, p=0.004), diabetes mellitus (β 0.37, 95% CI 0.12, 0.63, p=0.004) and gestational age (β 0.18, 95% CI 0.15, 0.2, p<0.001) were statistically significant associated with birth weight.

**Conclusions** This is the first study to show that TNFi use during pregnancy is associated with increased birth weight of offspring of women with well-controlled RA. The underlying mechanism of TNF-inhibition on birth weight and the long-term consequences for the offspring should be explored in future research.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Although tumour necrosis factor-inhibitors use (TNFi) during pregnancy is not associated with an increased risk of congenital malformations, data on other pregnancy outcomes is contradictory.

### WHAT THIS STUDY ADDS

⇒ The current study is the first to show that the use of TNFi during pregnancy in women with rheumatoid arthritis (RA) is associated with increased birth weight of the offspring after correcting for all relevant confounders and less children with small for gestational age.

⇒ TNFi use during pregnancy in women with RA does not increase the risk of adverse pregnancy outcomes such as prematurity, low birth weight, hypertensive disorders and emergency caesarean section.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The exact mechanisms behind the increase in birth weight after TNFi use during pregnancy should be the focus of future studies.

⇒ TNFi might be a future therapeutic option in preventing and treatment of intrauterine growth restriction.

⇒ The long-term effects of the increase in birth weight on the offspring should be further explored.

## INTRODUCTION

Rheumatoid arthritis (RA) is one of the most common chronic diseases in women in the reproductive age.[1] Pregnancy outcomes, like small-for-gestational age (SGA) and hypertensive disorders, in women with RA are impaired compared with healthy women, especially in women with active disease during pregnancy.[1] In recent years, more treatment options during pregnancy became available, including tumour necrosis factor (TNF)-inhibitors (TNFi), resulting in improved disease outcomes in women with RA during pregnancy.[2] Therefore, TNFi are now important in the management of RA during pregnancy.[2]

Research on pregnancy outcomes after TNFi exposure during pregnancy have mainly focused on exposure to TNFi's in the first trimester and congenital malformations.[1] Multiple studies have shown that TNFi do not increase the risk of birth defects.[3] However data on other pregnancy outcomes is contradictory, some studies report that TNFi are associated with increased risks of preterm birth, caesarean section (CS), low birth weight and SGA whereas others do not.[2–5]

These diverse findings across different studies may however indicate an association related to confounding variables, such as underlying disease, disease activity and the use of other certain medication (eg, glucocorticoids), rather than to the



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TNFi itself. Moreover, the use of a TNFi has an impact on these other variables by decreasing disease activity[6] and preventing the need for use of glucocorticoids.[7] A majority of previous studies that examined associations between TNFi use and pregnancy outcomes were performed in patients with different underlying diseases and without information on disease activity, making it difficult to interpret associations between TNFi use and pregnancy outcomes.

In the Preconception Counseling in Active RA (PreCARA) study, women with RA were prospectively followed-up, closely monitored and treated during pregnancy according to a modern treatment approach aimed at minimal disease activity including the use of TNFi.[2] The objective of the current study was to describe pregnancy outcomes of offspring born to patients with RA included in the PreCARA-cohort, and in particular to describe pregnancy outcomes of women that used a TNFi during pregnancy when correcting for relevant confounders.

## METHODS

### Patient population

Patients were included in the PreCARA-study (2011–ongoing).[2] The PreCARA study is an ongoing, prospective cohort study on inflammatory rheumatic diseases before and during pregnancy. Data up to May 2021 was used for the current manuscript. The PreCARA study (ClinicalTrials.gov reference NCT01345071) is solely performed in the Erasmus MC, a tertiary referral hospital (Rotterdam, the Netherlands). All patients in this hospital are treated within a dedicated specialised healthcare pathway for rheumatic diseases during pregnancy.

### PreCARA treatment protocol

The PreCARA-treatment protocol was extensively described previously.[2] In brief, patients in the PreCARA cohort were treated according to a modified treat-to-target approach aimed at minimal disease activity. Treatment was, if needed, intensified at every study visit. If treatment was intensified in the PreCARA-protocol, first, sulfasalazine and/or hydroxychloroquine were started, followed by the addition of prednisone (preferably in a maximum daily dosage of 7.5 milligram) and/or a TNFi, preferably certolizumab-pegol. Patients could get pregnant using the TNFi which they used when they enrolled in the study. During pregnancy, TNFi was stopped at the gestational age (GA) as recommended by the European-Alliance-of-Associations-for-Rheumatology: adalimumab and infliximab were stopped at GA 20 weeks, etanercept at GA 28–32,[8] certolizumab-pegol was discontinued at GA 38 weeks to prevent maternal infections during delivery, based on expert opinion. After stopping a TNFi a switch to certolizumab-pegol or prednisone was considered.

### Data collection

Participants entered the PreCARA-study preferably before they got pregnant. Visits were scheduled every 3 months before conception, in the first, second and third trimester of pregnancy and at 6, 12 and 26 weeks after delivery. At every visit, patients were seen by their rheumatologist and a rheumatology nurse, they underwent joint examination, filled in questionnaires electronically (including on frequencies and dosages of conventional synthetic DMARDs and biologic DMARDs) and a blood sample was collected.

Information on characteristics such as smoking, body mass index (BMI), previous pregnancies, presence of rheumatoid factor (RF) or anticitrullinated protein antibodies (ACPA), medical history, education, race and previous medication use were collected at inclusion.

Data on pregnancy outcome included birth weight, GA at delivery (as calculated by their attending gynaecologist and/or midwife), sex of the child and mode and location of the delivery. Information on the mode of delivery was categorised to spontaneous birth, induced birth or CS. CSs were recorded as elective or emergency. Pregnancy complications were collected as well: premature birth (delivery at <37 weeks (259 days) of the GA, low birth weight (<2500 g), high birth weight (>4000 g), hypertensive disorders (gestational hypertension and preeclampsia, both as reported by their attending gynaecologist), Diabetes mellitus (DM) (analysed as both preexisting DM and gestational DM combined) and congenital malformations. These data was collected at various visits during pregnancy and postpartum (by interview, questionnaires and careful investigation of the electronic medical charts). If patients gave birth outside our tertiary centre, data on pregnancy outcomes was collected from their attending physicians.

### Data analysis

Disease activity was calculated using the Disease Activity Score in 28 joints (DAS28)[9 10]: the DAS28 using three variables: the number of swollen joints, the number of tender joints, and the C reactive protein (CRP) level (DAS28CRP),[11] this disease activity measure is validated for use during pregnancy.[12]

Birth weight SDS were calculated using the sex-specific formulas as previously described in literature,[13] infants SGA (birth weight <p10) and large for GA (LGA) (birth weight >p90) were determined based on Dutch growth charts.[14] For the current analysis, pregnancies carried beyond week 20 were included in the study, twin pregnancies and diagnosis other than RA were excluded.

### Statistical analysis

Descriptive statistics are presented as numbers (n) and percentages (%). Continuous variables are given as mean±SD or median ±IQR as appropriate. We tested categorical data using $\chi^2$ and Fisher's exact tests. Continuous data were checked for the distribution of the data and analysed using (paired) t-test and Wilcoxon-rank as appropriate. We considered a two-sided $p<0.05$ significant.

To describe which variables influenced birth weight, univariate and multivariate linear regression were performed. The following covariates were considered: disease activity (DAS28CRP), GA at delivery, maternal age, prednisone use during pregnancy, TNFi use during pregnancy, nulliparity, pregnancy through assisted reproductive technology (combined: ovulation induction, intrauterine insemination in vitro fertilisation and intracytoplasmic sperm injection), smoking, (gestational) DM. An interaction between TNFi use and disease activity and TNFi use and prednisone use were considered in the multivariate analysis separately.

Kaplan-Meier survival analysis was performed to visualise whether GA at delivery was dependent on TNFi use during pregnancy, significance was tested using the Wilcoxon-Gehan statistic.

Statistical analyses were performed using Stata V.17 by StataCorp. Patient involvement and ethics are provided in online supplemental file 1.

## RESULTS

An overview of patients included in the PreCARA study (n=188) is presented in table 1. 48.9% of the women with RA used a

**Table 1** Clinical and demographic features from patients with RA within the PreCARA cohort (n=188) who conceived

| | PreCARA-cohort (n=188) | TNFi use during pregnancy (n=92) | No TNFi use during pregnancy (n=96) | P value, for difference yes/no TNFi use during pregnancy |
|---|---|---|---|---|
| Mean age at delivery, years (SD) | 32.6 (4.0) | 33.3 (3.9) | 31.9 (4.1) | **0.023** |
| Median disease duration at first visit, years (IQR) | 6.8 (3.6–10.9) | 6.0 (2.9–10.7) | 7.3 (4.1–11.5) | 0.21 |
| Rheumatoid factor positive, n (%) | 130/186 (69.9) | 67/90 (74.4) | 63 (65.6) | 0.19 |
| ACPA positive, n (%) | 129/184 (70.1) | 70/90 (77.8) | 59/94 (62.8) | **0.026** |
| Caucasian race, n (%) | 157 (83.5) | 77 (83.7) | 80 (83.3) | 0.94 |
| Nulliparity, n (%) | 100 (53.2) | 42 (45.7) | 58 (60.4) | **0.043** |
| Education level, median no of years of education (IQR) | 16 (14–18) | 16 (14–18) | 17 (14–18) | 0.78 |
| Conception through assisted reproduction technique, n (%) | 26/185 (14.1) | 12/90 (13.3) | 14/95 (14.7) | 0.78 |
| DAS28CRP in the first trimester of pregnancy (SD)* | 2.21 (0.80) | 2.18 (0.81) | 2.24 (0.79) | 0.64 |
| DAS28CRP in the second trimester of pregnancy (SD)* | 2.30 (0.77) | 2.35 (0.85) | 2.24 (0.67) | 0.34 |
| DAS28CRP in the third trimester of pregnancy (SD)* | 2.17 (0.73) | 2.22 (0.70) | 2.14 (0.76) | 0.49 |
| DAS28CRP<2,6 1 st trimester, n (%) | 120/161 (74.5) | 59/79 (74.7) | 61/82 (74.4) | 0.96 |
| DAS28CRP<2,6 2 nd trimester, n (%) | 130/178 (73.0) | 63/89 (70.8) | 67/89 (75.3) | 0.49 |
| DAS28CRP<2,6 3 rd trimester, n (%) | 130/169 (80.7) | 59/84 (70.2) | 71/85 (83.5) | **0.04** |
| Smoking during pregnancy, n (%) | 6 (3.2) | 3 (3.3) | 3 (3.1) | 0.96 |
| Medication use during pregnancy (any use), n (%)†: | | | | |
| ► Hydroxychloroquine | 105 (55.9) | 45 (48.9) | 60 (62.5) | 0.061 |
| ► Sulfasalazine | 110 (58.5) | 52 (65.5) | 58 (60.4) | 0.58 |
| ► Prednisone | 79 (42.0) | 42 (45.7) | 37 (38.5) | 0.32 |
| ► TNF inhibitor‡ | 92 (48.9) | 92 (100) | | |
| ► Certolizumab-pegol | 62 (33.0) | 62 (67.4) | | |
| ► Adalimumab | 8 (4.3) | 8 (8.7) | | |
| ► Etanercept | 25 (13.3) | 25 (27.2) | | |
| ► Infliximab | 12 (6.4) | 12 (13.0) | | |

Bold values denote statistical significance at the p < 0.05 level.
*Number of missing data for disease activity: 27/188 (14.4%) trimester 1, 10/188 (5.3%) trimester 2, 19/188 (10.1%) trimester 3.
†Either alone or in combination with other medication.
‡The sum of TNFi exceeds 100%, because some patients switched from etanercept, adalimumab or infliximab to certolizumab-pegol during pregnancy.
ACPA, anticitrullinated protein antibody; DAS28CRP, Disease Activity Score in 28 joints C reactive protein; PreCARA, Preconception Counseling in Active Rheumatoid Arthritis.

TNFi during pregnancy. Disease activity was low at all pregnancy trimesters, for example DAS28CRP in the third trimester of pregnancy: 2.17 (SD 0.73). 119 (63.3%) women were included before there were pregnant.

Maternal age, the number of patients with ACPA-antibodies and nulliparity were statistically significant different between women that used a TNFi during pregnancy and women who did not.

## Pregnancy outcomes

Pregnancy outcomes are shown in table 2. Pregnancy cholestasis was observed in 2 (1.1%) women. One patient developed a villoglandular carcinoma of the cervix during pregnancy. Twelve out of a total of 49 CS (24.5%) were emergency procedures, children born via an emergency CS had a median APGAR score after 5 min of 9 (range: 2–10).

Pregnancy outcomes stratified for TNFi use during pregnancy showed that birth weight, birth weight SDS and the number of emergency CS were different between both groups (table 2). The absolute difference in mean birth weight compared between women that used a TNFi during pregnancy and women who did not was 173 g (3.344 kg vs 3.171 kg, p=0.03). Survival analysis showed that GA was not different between women that used a TNFi during pregnancy and women who did not (online supplemental figure 1). Information on SGA, prematurity, gestational hypertension and pre-eclampsia stratified for DAS28CRP <2.6 and TNFi use is presented in online supplemental table 1).

## Influence of different factors on birth weight

In the univariate analysis, disease activity, GA at delivery, maternal age and TNFi use during pregnancy were significantly associated with birth weight (table 3).

In the multivariate analysis (table 3), maternal age had a significant negative effect on birth weight (kg). In addition, a positive effect on birth weight of TNFi use during pregnancy, DM and GA at delivery was observed. When the analysis were repeated with birth weight standard deviation score (SDS) instead of the actual birth weight as dependent variable, similar results were found (data not shown).

Since TNFi use and disease activity and TNFi use and the use of prednisone might have interaction, two additional analyses were performed, in which interaction terms (TNFi use and disease activity and TNFi use and prednisone use) were introduced into the model. These interaction terms showed no significant effect (interaction term TNFi use and disease activity (p=0.67), interaction term TNFi use and prednisone use (p=0.59).

To get more insight whether the effect of the use of TNFi during pregnancy on birth weight was depended on the trimester additional analysis were performed. When TNFi use was stratified for use per trimester, the following results for the outcome birth weight (corrected for DAS28CRP, GA at delivery, maternal age, nulliparity, sex of the newborn, type of conception, DM, prednisone use, and smoking) were observed: TNFi use in the first trimester β 0.081 (p=0.26), difference in mean birth weight 90.6 g. TNFi use in the second trimester β 0.16 (p=0.021), difference in mean birth weight 180.5 g. TNFi use in the third

**Table 2** Pregnancy outcomes of women with RA included in the PreCARA cohort (n=188) that conceived stratified for (any) TNFi use during pregnancy

| | PreCARA-cohort (n=188) | TNFi use during pregnancy (n=92) | No TNFi use during pregnancy (n=96) | P value, for difference yes/no TNFi use during pregnancy |
|---|---|---|---|---|
| Sex of the child (male), n (%) | 95 (50.8) | 53 (57.6) | 42/95 (44.2) | 0.07 |
| Birth weight, kg (SD) | 3.256 (0.56) | 3.344 (0.51) | 3.171 (0.59) | **0.03** |
| Gestational age at delivery, weeks (IQR) | 39.1 (37.8–40.1) | 39.0 (38–39.9) | 39.2 (37.7–40.4) | 0.53 |
| Birth weight SDS (SD) | - 0.096 (1.04) | 0.064 (0.99) | - 0.25 (1.06) | **0.04** |
| SGA (birth weight <p10), n (%) | 28/187 (15.0) | 9/92 (9.8) | 19/95 (20.0) | **0.05** |
| LGA (birth weight >p90), n (%) | 13/187 (7.0) | 7/92 (7.6) | 6/95 (6.3) | 0.73 |
| APGAR score (IQR) | 10 (9–10) | 10 (9–10) | 10 (9–10) | 0.99 |
| Location of delivery, n (%) | | | | |
| ► Hospital (including outpatient clinic) | 177/185 (95.7) | 89/91 (97.8) | 88/94 (93.6) | 0.16 |
| ► Home | 8/185 (4.3) | 2/91 (2.2) | 6/94 (6.4) | 0.16 |
| Mode of delivery, n (%) | | | | |
| ► Spontaneous vaginal delivery | 77/185 (41.6) | 35/91 (38.5) | 42/94 (44.7) | 0.39 |
| ► Induced labour | 59/185 (31.9) | 27/91 (29.7) | 32/94 (34.0) | 0.52 |
| ► Caesarean section | 49/185 (26.5) | 29/91 (31.9) | 20/94 (21.3) | 0.10 |
| Emergency caesarean section | 12/49 (24.5) | 2/29 (6.9) | 10/20 (50.0) | **0.001** |
| Pregnancy complications, n (%) | | | | |
| ► Prematurity (<37 weeks) | 23 (12.2) | 8 (8.7) | 15 (15.6) | 0.15 |
| ► Birth weight less than 2500 grams | 15 (8.0) | 4 (4.4) | 11 (11.6) | 0.069 |
| ► Birth weight over 4000 grams | 10 (5.4) | 3 (3.3) | 7 (7.4) | 0.21 |
| ► Diabetes mellitus* | 14 (7.5) | 10 (10.9) | 4 (4.2) | 0.080 |
| Hypertensive disorders, n (%) | | | | |
| ► Pre-eclampsia | 11 (5.9) | 5 (5.4) | 6 (6.3) | 0.81 |
| ► Gestational hypertension | 12 (6.4) | 4 (4.4) | 8 (8.3) | 0.26 |
| Congenital malformations, n (%) | 13 (6.9) | 7 (7.6) | 6 (6.3) | 0.71 |

Bold values denote statistical significance at the p < 0.05 level.
*Combined variable of both pre-existing diabetes mellitus and gestational diabetes mellitus.
APGAR, APGAR score; LGA, large for gestational age; PreCARA, Preconception Counseling in Active RA; RA, rheumatoid arthritis; SDS, standard deviation score; SGA, small for gestational age.

trimester β 0.22 (p=0.002); difference in mean birth weight 191.5 g.

Figure 1 shows that maternal TNFi use during pregnancy results in a decreased number of children born SGA compared with no maternal use of TNFi during pregnancy.

### TNFi use during pregnancy and CSs

A total of 49/185 (26.5%) children were born via CS, 12/49 (24.5%) were emergency CS. Women that used a TNFi during pregnancy had a trend towards an increase in delivery via CS (TNFi use during pregnancy: CS in 29/91 (31.9%) women vs no TNFi use during pregnancy CS in 20/94 (21.3%) women, p=0.10), however, emergency CS was more common in women that did not use a TNFi during pregnancy: (TNFi use during pregnancy: emergency CS in 2/29 (6.9%) women versus no TNFi use during pregnancy emergency CS in 10/20 (50.0%) women, p=0.001).

**Table 3** Findings of univariate and multivariate regression analyses of actual birth weight (kilogram) of RA patients (n=188) included in the PreCARA cohort

| | Univariate linear regression analysis for birth weight (kg) | | | Multivariate linear regression analysis for birth weight (kg)* | | |
|---|---|---|---|---|---|---|
| | Coefficient | 95% CI | P value | Coefficient | 95% CI | P value |
| Disease activity in third trimester (DAS28CRP) | – 0.20 | –0.31 to 0.085 | **0.001** | –0.091 | –0.19 to 0.0058 | 0.065 |
| Gestational age at delivery (weeks) | 0.17 | 0.14 to 0.21 | **<0.001** | 0.18 | 0.15 to 0.22 | **<0.001** |
| Maternal age (years) | – 0.013 | – 0.033 to 0.0074 | 0.21 | –0.023 | –0.040 to 0.0065 | **0.007** |
| Parity (nulliparity) | – 0.21 | – 0.36 to 0.047 | **0.011** | –0.13 | –0.26 to 0.0042 | 0.057 |
| Sex of the newborn (female) | 0.034 | – 0.13 to 0.20 | 0.67 | 0.081 | –0.053 to 0.21 | 0.24 |
| Type of conception (assisted reproduction) | – 0.075 | – 0.31 to 0.16 | 0.53 | –0.051 | –0.26 to 0.15 | 0.62 |
| Diabetes Mellitus (yes)† | 0.22 | – 0.084 to 0.53 | 0.15 | 0.37 | 0.12 to 0.63 | **0.004** |
| TNF inhibitor use during pregnancy (yes) | 0.17 | 0.014 to 0.33 | **0.033** | 0.20 | 0.066 to 0.34 | **0.004** |
| Prednisone use during pregnancy (yes) | – 0.064 | – 0.23 to 0.99 | 0.44 | – 0.99 | – 0.23 to 0.039 | 0.16 |
| Smoking during pregnancy (yes) | – 0.17 | – 0.62 to 0.29 | 0.47 | – 0.028 | – 0.41 to 0.35 | 0.89 |

*Corrected for all the other variables listed in this table.
†Combined variable of both preexisting diabetes mellitus and gestational diabetes mellitus.
DAS28CRP, Disease Activity Score in 28 joints C reactive protein; preCARA, Preconception Counseling in Active RA; RA, rheumatoid arthritis.

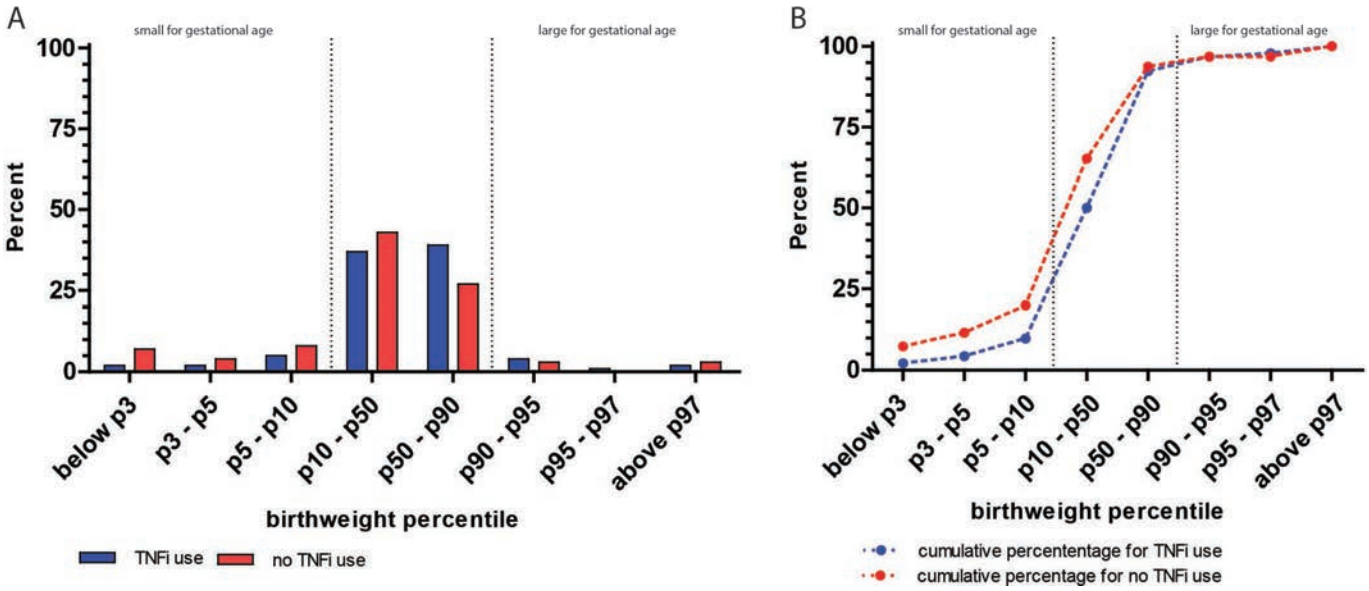


**Figure 1** Bar charts showing the percentage of children born in different birth weight percentile groups stratified for maternal TNFi use during pregnancy (A). (B) shows the cumulative percentage of these birth weight percentiles. Small for gestational age (SGA) is defined as p<10, large of gestational age (LGA) as p>90, TNFi use during pregnancy is associated with less children born SGA (p=0.05), however the risk of LGA was not increased (p=0.73).

### Congenital malformations

In the PreCARA-cohort, 13 (6.9%) women had a child born with a congenital malformation, of which 7 (7.6% of the women that used a TNFi at any time point during pregnancy) used a TNFi during pregnancy. One child had two congenital malformations: both undescended testis and an umbilical hernia. The congenital malformations that were observed in women that used a TNFi during pregnancy were: umbilical hernia n=1, haemangioma n=1, pupil anomaly n=1, salmon patch n=1, epispadias n=1, port-wine stain n=1 and different stance of both ears and teeth which required further investigation by a geneticist n=1. Congenital malformations in women that did not use a TNFi during pregnancy included: clubfoot n=2, heel foot n=1, undescended testis n=2, umbilical hernia n=1 and polydactyly n=1.

## DISCUSSION

In the current manuscript, we describe pregnancy outcomes of women with RA that were prospectively followed up and treated according to a modern treatment approach, including the use of TNFi. We showed that the use of TNFi during pregnancy was associated with a clinically significant increased fetal birth weight even when corrected for major confounders such as disease activity and resulted in less children born SGA. The risk of adverse pregnancy outcomes did not differ between patients that used an TNFi during pregnancy and patients who did not. In addition, the use of TNFi during pregnancy showed a trend towards an increase in birth via CS, however, no increased risk for an emergency CS was observed.

Shimada *et al* reported in a small, retrospective study that birth weight is increased in women that used a TNFi during pregnancy.[15] In their study, women that continued therapy with their TNFi (etanercept or certolizumab-pegol) during pregnancy had children with a higher birth weight compared with women that stopped their TNFi at conception. However, their study has several limitations; besides a low number of included patients, the authors were not able to perform additional analysis to correct for relevant confounders, such as disease activity. Our study is the first to show a strong association between TNFi use during pregnancy and an increased birth weight of the offspring when corrected for major confounders.

Increasing evidence shows that slight changes in birth weight (corrected for gestational-age) may have lifelong consequences for these children; both low and high birth weight are associated with complications throughout life.[16 17] RA is for example associated with SGA, which itself is associated with an increased risk on metabolic and cardiovascular disease later in life.[18 19] On the other hand, children born with a high birth weight have an increased risk of becoming overweight, on metabolic syndrome and giving birth to a child that is LGA as well. Our study shows a decreased risk of children born SGA and no increased risk of children born with high birth weight or LGA when a TNFi was by the mother used during pregnancy.

It is challenging to determine in which trimester the effect of TNFi was the largest, due to highly correlated exposure; women that used a TNFi in the third trimester most often used these biologics in the first and second trimester of pregnancy as well. Our study shows the largest effect in the third trimester of pregnancy, these results should be interpreted with caution and require replication by others research groups.

In RA pathology, high levels of circulating proinflammatory cytokines such as TNF alpha and interleukine (IL)-6 and a lower number of regulatory T-cells (Tregs) are observed in patients with active disease.[20] Importantly, on treatment with a TNFi a decrease of these proinflammatory cytokines and increase the number and function of Tregs and IL-10 can be observed.[21 22] The immune system is not only important in the pathogenesis of RA, but also for ensuring and maintaining a normal pregnancy. For pregnancy to avoid rejection of the semi allogenic fetal-placental unit and to ensure proper development of the placenta and hence fetal growth, local expansion of leukocytes with unique regulatory properties, including Tregs is required.[23 24] In addition a tight balance between proinflammatory (eg, TNF and IL-6) and anti-inflammatory (eg, IL-10) cytokines is necessary, in which the anti-inflammatory cytokines prevail.[24] Many disease of pregnancy, including recurrent miscarriages, intrauterine growth restriction, SGA and hypertensive disorders of pregnancy, like

preeclampsia, are thought to arise from inadequate development and growth of the placenta and hence, if pregnancy continues, impaired fetal growth.[23 24] Interestingly, in these conditions an increase in proinflammatory cytokines like TNF and IL-6 can be found.[24] It is tempting to speculate that treatment with TNFi during pregnancy promotes placentation and thereby fetal growth and birth weight by changing the balance between proinflammatory and anti-inflammatory cytokines and by increasing the number and function of Tregs. As stated previously, several diseases of pregnancy are thought to arise from impaired placentation and are characterised by an immunological imbalance, whether treatment with TNFi is beneficial in these conditions should be the focus of future research.

An alternative, but not mutually exclusive, hypothesis could be that treatment with TNFi during pregnancy is able to induce epigenetic changes in the fetus, which positively influence fetal growth. In this respect, it has been shown that RA during pregnancy, either related to the disease itself, disease activity or medication, is associated with marked epigenetic changes in the offspring.[25] In addition, in several populations of healthy mothers and their children, it has been shown that DNA-methylation in the newborn is associated with birth weight.[26]

Previous literature shows that TNFi use during pregnancy in patients with a chronic inflammatory conditions is associated with an increased risk of birth via elective and emergency CS.[5] In the current study, patients that used a TNFi during pregnancy had trend towards a higher risk of an elective CS, but not for emergency CS compared with patients that did not use TNFi during pregnancy. The exact reasons behind this phenomenon are unknown. One possible explanation for this observation could be that gynaecologist/obstetricians sooner tend to plan a CS when patients are exposed to biologic DMARDs during pregnancy to avoid emergency situations in pregnancies at risk, however, this is speculation and should be further explored. The overall rate of CS in our study (26.5%) is probably comparable to the overall percentage of birth via CS in Europe (25.7%).[27]

In line with previous reported literature, we did not observe an association between TNFi use during pregnancy and an increased risk of congenital malformations.[28 29] Although the risk of congenital malformations in infants born to women with RA is probably not increased,[30] directly comparing percentages of congenital malformations observed in our study to that of safety studies that use different definitions, inclusion criteria and study designs is probably not appropriate.

Our study has several strengths: our study comprise a well-defined, large, prospectively followed up cohort of women with homogeneous disease making us able to correct for confounding factors when studying the effect of TNFi on pregnancy outcomes such as birth weight. Some limitations of the current study should however be acknowledged. Our study did not have sufficient power to perform multivariate analysis on outcomes such as CS. Furthermore, our study was not designed to detect congenital malformations, since not all confounders for these outcome were collected and children born to women included in this study were not examined by a paediatrician. In addition, we were not able to correct our multivariate analysis for pre-pregnancy BMI since women were both included before pregnancy and already pregnant. Separate multivariate analysis of women that were included before pregnancy showed that BMI was not a significantly associated with birth weight and did not affect the effect size of TNFi on birth weight (data not shown).

In conclusion, our study shows that the use TNFi during pregnancy is associated with increased birth weight of offspring of women with well-controlled RA. Interestingly TNFi use during pregnancy results in less children born SGA, however, it does not increase the risk of born LGA. Our results might pave the way towards new clinical indications for the use of TNFi during pregnancy such as intrauterine growth restriction. Future research should focus on understanding the underlying mechanism of TNF-inhibition on birth weight and the long-term consequences for the offspring.

**Contributors** All authors met the authorship criteria, they had a substantial contribution to the conception or design of the work (HTWS, ER, RJEMD) or the acquisition (RJEMD), analysis (HTWS, AGMGJM, RJEMD) or interpretation of data for the work (all authors) and were involved in revising a draft of this work, gave final approval of this version to be published, and are accountable for all aspects of the work in ensuring accuracy and integrity. The guarantor (RJEMD) accepts full responsibility for the work and controlled the decision to publish.

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**Competing interests** None declared.

**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** Consent obtained directly from patient(s)

**Ethics approval** This study involves human participants and was approved by MEC-2011-032. Participants gave informed consent to participate in the study before taking part.

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CLINICAL SCIENCE

# Impact of pre-existing background therapy on placebo responses in randomised controlled clinical trials of rheumatoid arthritis

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## ABSTRACT

**Objectives** Various hypotheses exist for the explanation of placebo response rates in randomised controlled trials (RCTs) of patients with rheumatoid arthritis with IR to methotrexate (MTX). We hypothesised that placebo responses may be related to more consequent intake of MTX during the tightly monitored trial period.

**Methods** We conducted a post hoc analysis of placebo-treated patients included in two RCTs that had allowed inclusion of patients with and without ongoing MTX: the GO-AFTER and the SIRROUND-T trials. We pooled placebo patients of both trials and compared American College of Rheumatology (ACR) 20%/50%/70% response rates and Clinical Disease Activity Index (CDAI) low disease activity (LDA; ie, CDAI ≤10) responses between those receiving placebo on top of continued MTX and those receiving placebo without any background disease modifying antirheumatic drugs (DMARDs).

**Results** Of 398 placebo patients, 285 continued MTX and 113 had no background DMARDs. Baseline characteristics were similar. At week 16, ACR20 response was achieved by 72/285 (25.3%) of placebo+continued MTX and 14/113 (12.4%) of placebo only patients (nominal p=0.005); for ACR50 these numbers were 25/285 (8.4%) versus 1/113 (0.9%; nominal p=0.003) and for ACR70 they were 8/285 (2.8%) versus 0/113 (0%; nominal p=0.112). Also, more patients with placebo+continued MTX achieved CDAI-LDA at week 16 (25/285; 8.8%) compared with placebo only (2/113; 1.8%; nominal p=0.013).

**Conclusion** Clinical responses to placebo are higher in patients who continue an insufficient MTX background therapy. This suggests an inadvertently more consequent intake of background therapy during the trial. Background therapy should therefore be effectively aligned before enrollment into a clinical trial.

### WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

Randomised controlled clinical trials investigating biological disease modifying antirheumatic drugs (DMARDs) and targeted synthetic DMARDs in rheumatoid arthritis (RA) show considerable placebo response rates, which were increasing over the past decade. More consequent intake of background therapies after study inclusion was hypothesised to have an impact on placebo rates.

### WHAT DOES THIS STUDY ADD?

Placebo-treated patients with ongoing methotrexate (MTX) therapy from the pre-trial period had higher American College of Rheumatology 20%, 50% and 70% as well as Clinical Disease Activity Index-low disease activity response rates compared with placebo-treated patients without DMARD background therapy.

### HOW MIGHT THIS IMPACT ON CLINICAL PRACTICE OR FUTURE DEVELOPMENTS?

Background therapies are a potentially important confounder in RA clinical trials and should be effectively aligned before recruitment of patients into a clinical trial, possibly also through introducing 'run-in' phases in future clinical trials. This may lead to lower numbers of patients necessary to be recruited into a clinical trial and re-emphasises the importance of adequate MTX treatment and improving compliance in patients diagnosed with RA.

## INTRODUCTION

Therapies targeting molecules involved in the inflammatory pathways, such as tumour-necrosis-factor (TNF) alpha and interleukin (IL)-6 have proven effective in patients with rheumatoid arthritis (RA) with insufficient response (IR) to conventional synthetic (cs) and biological (b)disease modifying antirheumatic drugs (DMARDs).[1 2] While generally well designed and conducted, many of these trials show considerable placebo (PBO) response rates, which are even found to be increasing over the past decade.[3 4] Several generic reasons exist for an observable response in individuals treated with PBO, including over-incentivising inclusions of patients or generally more limited access to newer drugs, all leading to a phenomenon known as 'regression to the mean'.[5] However, additional hidden confounders of a PBO response may be the trial setting as such, which requires patients to be strict with the intake of their prescribed or allocated drugs including respective documentation. This may also affect pre-existing therapies, which are continued into the trials, the intake of which may be more scrutinised in the trial than in clinical practice before recruitment into the study.

Methotrexate (MTX) is the standard first-line csDMARD for RA, and in patients who had an insufficient response to MTX it is typically continued as background therapy.[1 2] Trial protocols usually



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dictate that the regimens (ie, the compounds given at a certain dose and route) remain unchanged during the trial to avoid noise created by effects of background therapy, since the interest lies solely on the intervention itself. Here, we investigate the hypothesis that such background medication may partly explain PBO responses observed in contemporary clinical trials. This hypothesis receives support from a recent meta-analysis showing that background csDMARD treatment may constitute a potential factor associated with higher PBO response rates in RA clinical trials,[6] although differences were not significant in multivariate analyses. However, hitherto, no analysis using patient level trial data have been performed to prove this hypothesis.

## METHODS

### Study design and patient data

We included patient level data from two randomised, PBO-controlled trials, both conducted in patients in whom bDMARDs had previously failed. In both trials, patients taking csDMARDs prior to enrolment were required to continue these drugs into the trial, while patients who had stopped even csDMARDs prior to recruitment were not allowed to start such therapy after screening. Ethical reasons usually preclude inclusion of patients without any DMARD therapy. However, patients who had an previous IR to biologicals as monotherapy often do not receive any DMARD medication when stopping the bDMARD and also such patients were allowed to enter these two trials with the provision of rescue medication in case of an IR; indeed, ethical committees of all trial sites accepted this design in those days.

For the purpose of our comparative analysis, these inclusion criteria were an ideal constellation, since they allowed the stratification of patients in the PBO arms into those with and without continued background therapy. GO-AFTER investigated the efficacy of golimumab, a monoclonal antibody targeted against TNF, in patients with bDMARD-IR,[7] while SIRROUND-T investigated sirukumab, a monoclonal antibody targeting the cytokine IL-6, also in patients with bDMARD-IR.[8] The primary reports of both studies have been previously published and details are available in the respective manuscripts and supplementary appendices.[7 8] Access to the data was provided through the YODA (Yale Open Data Access) Project, a Yale University project to promote open data in clinical research.[9] The study protocol and analysis plan were submitted as a project proposal to the YODA committee before start of the analyses and can be found in the online supplemental file 1.

Only patients randomised to the PBO arms of the GO-AFTER and SIRROUND-T studies were included in the analysis. Active treatment arms of the trials were not of interest to the study question and were therefore not further analysed. Since among those with background csDMARDs, 80% had received MTX monotherapy, we focused on this patient group for the reason of homogeneity. Both studies included a screening period of 4–6 weeks and required stable background medication (stable dose of MTX for 4 weeks and of oral corticosteroids for 2 weeks). Also, patients had to tolerate MTX for at least 12 weeks (GO-AFTER) or 24 weeks (SIRROUND-T) prior first administration of the study agent. To be eligible for inclusion in the GO-AFTER study, patients must have received at least one dose of infliximab, adalimumab or etanercept and could not be included if infliximab was administered within 12 weeks (or 8 weeks in the case of etanercept and adalimumab) before first administration of the study agent. In SIRROUND-T, patients were not eligible for inclusion if they received rituximab within 7 months; tocilizumab, infliximab, abatacept or golimumab (intravenous) within 8 weeks; golimumab (s.c.), adalimumab or certolizumab-pegol within 6 weeks; etanercept within 4 weeks or anakinra within 1 week. Detailed information on therapy relevant eligibility criteria of both trials is shown in online supplemental table S1 and online supplemental table S2. Since baseline demographic and clinical data were quite similar between the two studies (with the exception of a slightly longer disease duration in SIRROUND-T)[7 8] and no comparison between active therapies was done, we pooled the PBO arms of both studies.

### Statistical analysis

The primary endpoint in GO-AFTER was the ACR20 response at week 14, while in SIRROUND-T it was the ACR20 response at week 16; in both trials patients could switch to early escape active therapy if they did not achieve a greater than 20% decrease in either tender joint count (TJC) or swollen joint count (SJC) at week 16 in the GO-AFTER study and at week 18 in the SIRROUND-T study. Therefore, the primary endpoint of the present analysis was defined as the difference of the American College of Rheumatology (ACR) 20% response rate between PBO-treated patients with continued MTX therapy and PBO-treated patients without DMARD therapy between baseline and week 16. We also investigated differences in the Simplified Disease Activity Index and Clinical Disease Activity Index (SDAI and CDAI, respectively), core-set components of these composite scores and changes from baseline in CDAI core-set components as well as CDAI low disease activity (LDA) (ie, $\leq 10$) response rates.

Similar to the primary analyses of the individual studies, non-responder imputation for state variables (ACR responses, CDAI LDA) or last observation carried forward imputation for continuous variables (CDAI and core set variables as described above) was applied in patients who had their MTX or corticosteroid dosage increased during the study or discontinued the study.

$\chi^2$ were used to nominally test the differences between the two groups (PBO+continued MTX vs PBO) for ACR20, ACR50, ACR70 and CDAI LDA. Further, changes from baseline and nominal differences in core-set variables (Swollen Joint Count 66, SJC66; Tender Joint Count 68, TJC68; Patient Global Assessment, PGA; Evaluator Global Assessment, EGA; Patient Assessment of Pain; the Health Assessment Questionnaire Disability Index (HAQ) and C reactive protein, CRP) were calculated via Student's t-test. Additionally, longitudinal linear mixed models were used, including SJC, TJC, Pain, EGA, PGA, HAQ, CRP and CDAI scores as dependent variables in separate models, with group, visit and baseline values (to adjust for baseline differences of disease activity) as independent variables. All statistical analyses are considered exploratory and both trials were not powered to show differences in the strata of PBO patients specified here, therefore all results are shown with nominal p values.

R (V.3.6.3) and Python (V.3.8.3) were used for conduction of all statistical analyses.

## RESULTS

Baseline characteristics of the patients analysed are shown in table 1. In total, 285 patients randomised to PBO who received ongoing MTX monotherapy, and 113 PBO-treated patients without any csDMARD therapy were included in our analyses. Baseline characteristics were largely similar across the trials and the strata with most patients having a high disease burden.

Concomitant continued background therapy with MTX led to consistently higher ACR 20%/50%/70% and CDAI LDA response rates, compared with patients treated with PBO

**Table 1** Baseline characteristics of patients randomised to placebo arms receiving either MTX monotherapy or no concomitant background DMARD

| | GO-AFTER | | | | SIRROUND-T | | | | Combined | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Placebo+continued MTX | | Placebo | | Placebo+continued MTX | | Placebo | | Placebo+continued MTX | | Placebo | |
| n | 91 | | 48 | | 194 | | 65 | | 285 | | 113 | |
| SJC 66 (0–66) | 16.9 | (11.7) | 17.1 | (10.7) | 15.8 | (10.4) | 15 | (9.4) | 16.2 | (10.8) | 15.9 | (9.9) |
| TJC 68 (0–68) | 28.8 | (17.1) | 28.9 | (16) | 25.8 | (15) | 29.3 | (17.5) | 26.7 | (15.7) | 29.1 | (16.8) |
| PGA (VAS 0–10) | 6.3 | (2.1) | 6.7 | (2.3) | 6.6 | (2.1) | 6.9 | (2.1) | 6.5 | (2.1) | 6.8 | (2.2) |
| EGA (VAS 0–10) | 6 | (1.9) | 6.3 | (2) | 6.1 | (1.9) | 6.4 | (1.8) | 6.1 | (1.9) | 6.4 | (1.8) |
| Pain (VAS 0–10) | 6.5 | (2) | 7.1 | (1.9) | 6.6 | (2.1) | 7 | (2) | 6.6 | (2.1) | 7 | (1.9) |
| HAQ-DI (0–3) | 1.6 | (0.6) | 1.7 | (0.7) | 1.6 | (0.6) | 1.6 | (0.8) | 1.6 | (0.6) | 1.6 | (0.7) |
| CRP (mg/dL) | 2 | (3.2) | 2.1 | (3) | 2.2 | (2.7) | 2.8 | (3.4) | 2.1 | (2.9) | 2.5 | (3.2) |
| CDAI | 37.7 | (12.7) | 38.5 | (14.5) | 38.8 | (12.9) | 40 | (12.9) | 38.5 | (12.8) | 39.4 | (13.5) |
| SDAI | 39.7 | (13.1) | 40.5 | (15.7) | 41 | (13.3) | 42.8 | (13.3) | 40.6 | (13.2) | 41.8 | (14.4) |
| Concomitant GC intake (%) | 52 | (57%) | 24 | (50%) | 122 | (63%) | 43 | (66%) | 174 | (61%) | 67 | (59%) |
| MTX monotherapy | 91 | (100%) | 0 | (0%) | 194 | (100%) | 0 | (0%) | 285 | (100%) | 0 | (0%) |
| MTX dosage (mg/week) | 16.5 | (5.4) | 0 | (0%) | 15.4 | (6.3) | 0 | (0%) | 15.8 | (6) | 0 | (0%) |
| MTX dosage ≥12.5 mg (%) | 74 | (81%) | 0 | (0%) | 148 | (76%) | 0 | (0%) | 222 | (78%) | 0 | (0%) |
| MTX dosage <12.5 mg (%) | 17 | (19%) | 0 | (0%) | 46 | (24%) | 0 | (0%) | 63 | (22%) | 0 | (0%) |

Data are shown as mean (SD) or n (%).
.ACPA, anticitrullinated protein antibody; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; csDMARD, conventional synthetic disease modifying antirheumatic drug; EGA, Evaluator Global Assessment; GC, glucocorticoids; HAQ-DI, Health Assessment Questionnaire Disability Index; MTX, methotrexate; PGA, Patient Global Assessment; SDAI, Simplified Disease Activity Index; SJC, swollen joint count; TJC, tender joint count; VAS, visual analogue scale.

only (figure 1, table 2). Patients with continued MTX therapy compared with those without background csDMARD therapy showed significantly higher rates of ACR 20% (25.3% vs 12.4%, nominal p=0.004) and ACR 50% (8.4% vs 0.9%, nominal p=0.003) responses; ACR 70% response rates were numerically higher (2.8% vs 0%), but the difference was not statistically significant (nominal p=0.110); 8.8% of patients receiving PBO+MTX monotherapy compared with 1.8% of PBO-treated patients without DMARD background therapy achieved CDAI LDA at week 16 (nominal p=0.013). Similar differences in outcomes were observed when comparing both trials separately (online supplemental table S3 and figure S1).

Figure 2 shows outcomes of core-set parameters from baseline to week 16. Differences were observed already early on and the separation of the groups peaked already at 12 weeks for some of the measures. Differences were observed for SJC and TJC, to a smaller degree for PGA and EGA, but not for Pain or HAQ. CRP levels remained constant in PBO-treated patients with continued MTX background therapy but worsened in PBO-treated patients without DMARD over the course of the trials. Also, significant and clinically meaningful differences in CDAI (29.3±15.5 vs 35.2±14.8, respectively; nominal p<0.001) and changes from baseline in CDAI were achieved when comparing the two groups.

Longitudinal mixed models also showed significant differences in changes from baseline across all outcomes (online supplemental table S4 and figure S2). Data were missing at random and a table depicting the amount of missing data per endpoint is included in online supplemental table S5.

## DISCUSSION

High PBO response rates are a continuous challenge in the conduction of modern RA clinical trials, complicating the interpretation of their results. While early trials investigating TNF blocking agents showed ACR 20% PBO response rates of 15%–20%,[10 11] these response rates mostly doubled in recent drug development programmes, especially in patients characterised as having previously insufficiently responded to MTX treatment.[1 12–14] MTX, one of the oldest csDMARDs in rheumatology

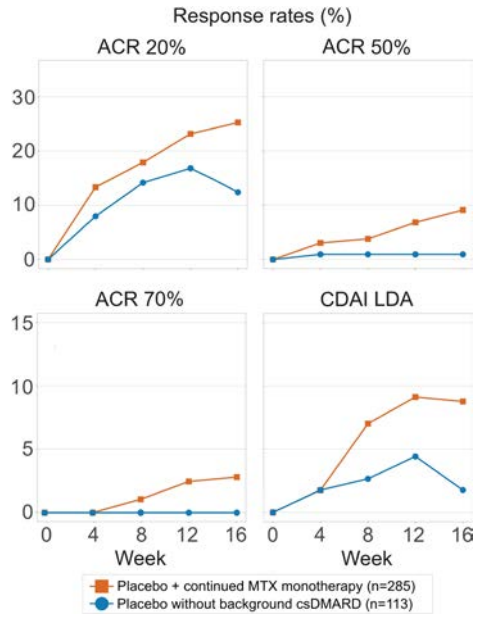


**Figure 1** Patients randomised to the placebo arm of the GO-AFTER and SIRROUND-T studies (pooled) achieving an American College of Rheumatology 20%, 50%, 70% or Clinical Disease Activity Index low disease activity response. Orange squares and lines show placebo-treated patients receiving continued methotrexate monotherapy. Placebo treated patients without concomitant conventional synthetic disease modifying drug therapy are shown as blue circles/lines. ACR, American College of Rheumatology; CDAI LDA, Clinical Disease Activity Index low disease activity; csDMARD, conventional synthetic disease modifying antirheumatic drug; MTX, methotrexate.

**Table 2** Impact of continued MTX monotherapy treatment in patients randomised to the placebo arms of the GO-AFTER and SIRROUND-T studies on composite outcomes and core set parameters. Pooled results are shown at week 16

| | Placebo+continued MTX | | Placebo | | Nominal p value |
|---|---|---|---|---|---|
| n | 285 | | 113 | | |
| ACR20 | 72 | (25%) | 14 | (12%) | 0.004 |
| ACR50 | 24 | (8%) | 1 | (1%) | 0.003 |
| ACR70 | 7 | (3%) | 1 | (0%) | 0.11 |
| CDAI LDA | 25 | (9%) | 2 | (2%) | 0.013 |
| SJC 66 (0–66) | 11.3 | (9.6) | 13.3 | (9.7) | 0.05 |
| TJC 68 (0–68) | 20.1 | (15.9) | 25.5 | (17.1) | 0.004 |
| PGA (VAS 0–10) | 5.3 | (2.5) | 6.2 | (2.5) | <0.001 |
| EGA (VAS 0–10) | 4.8 | (2.6) | 5.9 | (2.3) | 0.002 |
| Pain (VAS 0–10) | 5.5 | (2.5) | 6.7 | (2.2) | <0.001 |
| HAQ-DI (0–3) | 1.6 | (0.6) | 1.7 | (0.7) | 0.07 |
| CRP (mg/dL) | 2 | (2.8) | 3.1 | (3.4) | 0.005 |
| CDAI | 29.3 | (15.5) | 35.2 | (14.8) | <0.001 |
| SDAI | 31.3 | (16.1) | 38.3 | (15.6) | <0.001 |

Data are shown as mean (SD) or n (%).
.ACR, American College of Rheumatology response; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; csDMARD, conventional synthetic disease modifying antirheumatic drug; EGA, Evaluator Global Assessment; GC, glucocorticoids; HAQ-DI, Health Assessment Questionnaire Disability Index; LDA, low disease activity (ie, CDAI ≤10); MTX, methotrexate; PGA, Patient Global Assessment; SJC, swollen joint count; TJC, tender joint count.

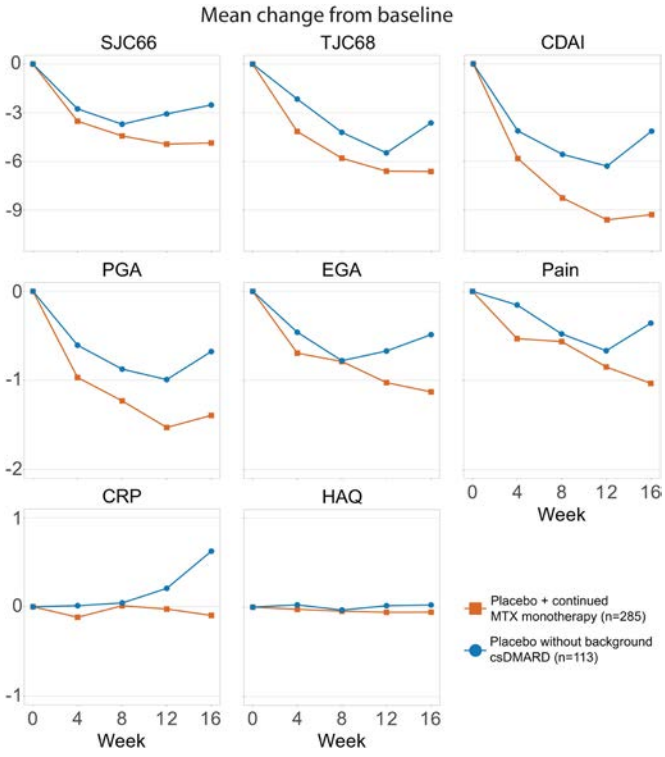


**Figure 2** Pooled analysis of mean change from baseline of core set parameters, SJC66, TJC68, PGA, EGA, Pain, HAQ, CRP, CDAI of patients randomised to the placebo arm of the GO-AFTER and SIRROUND-T study. Orange squares and lines show mean values of placebo-treated patients with concomitant methotrexate monotherapy. Patients receiving placebo without concomitant conventional synthetic disease modifying drug therapy are shown as blue circles/lines. CDAI, Clinical Disease Activity Index; CRP, C reactive protein; csDMARD, conventional synthetic disease modifying antirheumatic drug; EGA, Evaluator Global Assessment; HAQ, the Health Assessment Questionnaire; MTX, methotrexate; Pain, Patient Assessment of Pain; PGA, Patient Global Assessment; SJC66, swollen joint count 66; TJC68, tender joint count 68.

is still considered as an anchor drug for treating patients with RA, and should be part of the first treatment strategy.[2] Being in use for many decades, a balanced, well-established efficacy and safety profile as well as easy access and low costs make it the primary agent to choose when prescribing csDMARDs. In many clinical trial settings prior to study inclusion, to be classified as having insufficient response to MTX, patients must have been taking weekly MTX for more than 12 weeks with a stable dose of at least 8 weeks. However, compliance to this drug is hard to objectify as drug concentration levels are technically difficult and expensive to obtain.

Here, we hypothesised, that a suspected limited patient adherence to MTX in real life would potentially be improved after patients enrol into a tightly monitored trial setting. This could increase PBO responses in those on existing MTX therapy as opposed to those patients without prior therapy. To analyse this, data of patients with and without background therapy recruited into PBO arms of clinical trials are required. Only very few trials with these preconditions exist. The GO-AFTER and the SIRROUND-T trials provided the opportunity to analyse this question. In our analyses, we show that patients randomised to the PBO arms, who continued MTX monotherapy as background treatment during the trial, show higher response rates in major outcomes and also achieve good clinical states more frequently compared with PBO-treated patients without concomitant background csDMARD therapy. Although the original studies were not powered to show differences between strata of PBO-treated patients, we could demonstrate statistically significant differences in these exploratory efforts.

Limitations of this study are the small available number of trials investigating patients with and without ongoing csDMARD background therapy that would allow to investigate this question. Effects of other csDMARDs beyond MTX were not evaluated, due to a very low number of patients using sulfasalazine, leflunomide, hydroxychloroquine treatment or any combination of these therapies. Although adherence was not formally assessed in both trials, the observed difference in PBO rates between MTX treated and untreated patients in these randomised trials supports the hypothesis of a better adherence to pre-existing drugs in the setting of a clinical trial: trial settings allow for more frequent visits, closer management and specialised care, with no further costs for the patients than daily clinical practice. Real-world data reveal that patients frequently do not fill their prescriptions resulting in worse clinical outcomes across specialties ('adherence gap'),[15 16] while these drugs are provided in clinical trials and, therefore, adherence/persistence may be higher in trials.[17]

The results of our analyses may have to be considered in the future when planning and conducting clinical trials in RA, as every power calculation relies on sufficiently estimated PBO response rates in any population. Failing to estimate correct response rates may possibly lead to type II errors, as identification of a real difference in efficacy between active compound and PBO may be missed due to unexpectedly high PBO rates. Simply accepting higher PBO rates as a fact, however, may subsequently lead to recruitment of more patients into clinical trials than would otherwise be needed. The importance of our finding should help investigators and sponsors to plan and conduct clinical trials appropriately and avoid this important confounding

factor. Partly, this could be addressed by including a 'run-in' phase for a clinical trial by providing the background medication for a certain amount of time in a controlled setting—similar to the clinical trial itself—before starting the actual trial by administration of active therapy or PBO; most patients will still continue to be active, as seen here by the low ACR70 and LDA rates even in the background MTX group. Such an approach may not only help to reduce the numbers of patients needed to be recruited in a clinical trial, but also signifies the potential that lies in adequate MTX treatment in clinical care for patients with RA.

**Contributors** AK contributed to planning and conception of the study, interpretation of results, statistical analysis, figure development, manuscript draft and preparation. ZIR contributed to statistical analysis, manuscript draft and preparation, figure development. JSS contributed to planning and conception of the study, figure development, interpretation of results, manuscript preparation. DA contributed to planning of the study, interpretation of results, figure development, manuscript preparation. AK and DA had access to the data, controlled the decision to publish and accept full responsibility for the finished work of the study. DA is the guarantor of the study. All authors gave their final approval of the final document version to be published.

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**Patient and public involvement** Patients' representatives and the public were not involved in the planning and conduction of this study.

**Patient consent for publication** Not applicable.

**Ethics approval** This study involves human participants and was approved. This is a post hoc analysis of two randomised controlled trials. Both trials complied with the principles of the Declaration of Helsinki. All patients provided written informed consent, and the review board of each participating institution approved the protocol. Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data may be obtained from a third party and are not publicly available. This study, carried out under YODA Project # 2018-3704, used data obtained from the Yale University Open Data Access Project (https://yoda.yale.edu/), which has an agreement with Janssen Research & Development, LLC. The interpretation and reporting of research using this data are solely the responsibility of the authors and does not necessarily represent the official views of the Yale University Open Data Access Project or Janssen Research & Development, LLC.



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CLINICAL SCIENCE

# Standardisation of ACPA tests: evaluation of a new candidate reference preparation

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## ABSTRACT

**Introduction** Commercial assays measuring antibodies to citrullinated protein/peptide (ACPA) show poor quantitative agreement. The diagnostic industry has never adopted the International Union of Immunological Societies-Centers for Disease Control and Prevention (IUIS-CDC) ACPA reference standard. Recently, the National Institute for Biological Standards and Control (NIBSC) prepared a new candidate ACPA standard (18/204). We evaluated both reference materials using different commercially available ACPA assays.

**Materials and methods** This is an international study in which the NIBSC candidate ACPA standard and the IUIS-CDC ACPA reference material were analysed together with 398 diagnostic samples from individuals with rheumatoid arthritis (RA) and in 1073 individuals who did not have RA using nine commercial ACPA assays.

**Results** For both reference materials and samples from individuals with RA and individuals who did not have RA, there were large differences in quantitative ACPA results between assays. For most assays, values for the IUIS-CDC standard were lower than values for NIBSC 18/204 and the IUIS-CDC/NIBSC ratio was comparable for several, but not all assays. When NIBSC 18/204 was used as a calibrator, an improvement in alignment of ACPA results across several of the evaluated assays was obtained. Moreover, NIBSC 18/204 could align clinical interpretation for some but not all assays.

**Conclusion** Adoption of an international standard for ACPA determination is highly desirable. The candidate NIBSC 18/204 standard improved the standardisation and alignment of most ACPA assays and might therefore be recommended to be used as reference in commercial assays.

### WHAT IS ALREADY KNOWN ABOUT THE SUBJECT?

⇒ Results obtained with commercial antibodies to citrullinated protein/peptide (ACPA) assays show poor quantitative agreement.
⇒ Adoption of an international standard for ACPA by the diagnostic industry is highly desirable.

### WHAT DOES THIS STUDY ADD?

⇒ The candidate National Institute for Biological Standards and Control 18/204 standard improved alignment of most, but not all ACPA assays.

### HOW MIGHT THIS IMPACT ON CLINICAL PRACTICE?

⇒ Alignment of ACPA assays would be particularly important in the context of the American College of Rheumatology/European Alliance of Associations for Rheumatology 2010 rheumatoid arthritis (RA) classification criteria, where ACPA concentration has a high impact on rRA classification.

## INTRODUCTION

Antibodies to citrullinated protein/peptide (ACPA) are established biomarkers for diagnosis and classification of rheumatoid arthritis (RA).[1] Measurement of ACPA is widely used and several manual and (semi-)automated assays are commercially available. However, there is poor agreement among the currently available ACPA assays, which may have an impact on RA classification of a patient.[2 3]

An international ACPA reference preparation derived from a single patient donor has been prepared by the International Union for Immunological Societies (IUIS) and Centers for Disease Control and Prevention (CDC) and is available through the Autoantibody Standardisation Committee (www.AutoAb.org).[4] A preliminary evaluation of this preparation using 12 ACPA ELISAs and samples from 20 patients with RA and 50 healthy subjects concluded that it could be used as a reference standard.[5] However, this preparation has not been adopted by the in vitro diagnostic kit manufacturers as a reference standard for establishing calibration curves in the commercial assays.

Due to the role of ACPA quantification in classification, diagnosis,[6 7] risk stratification and prognosis

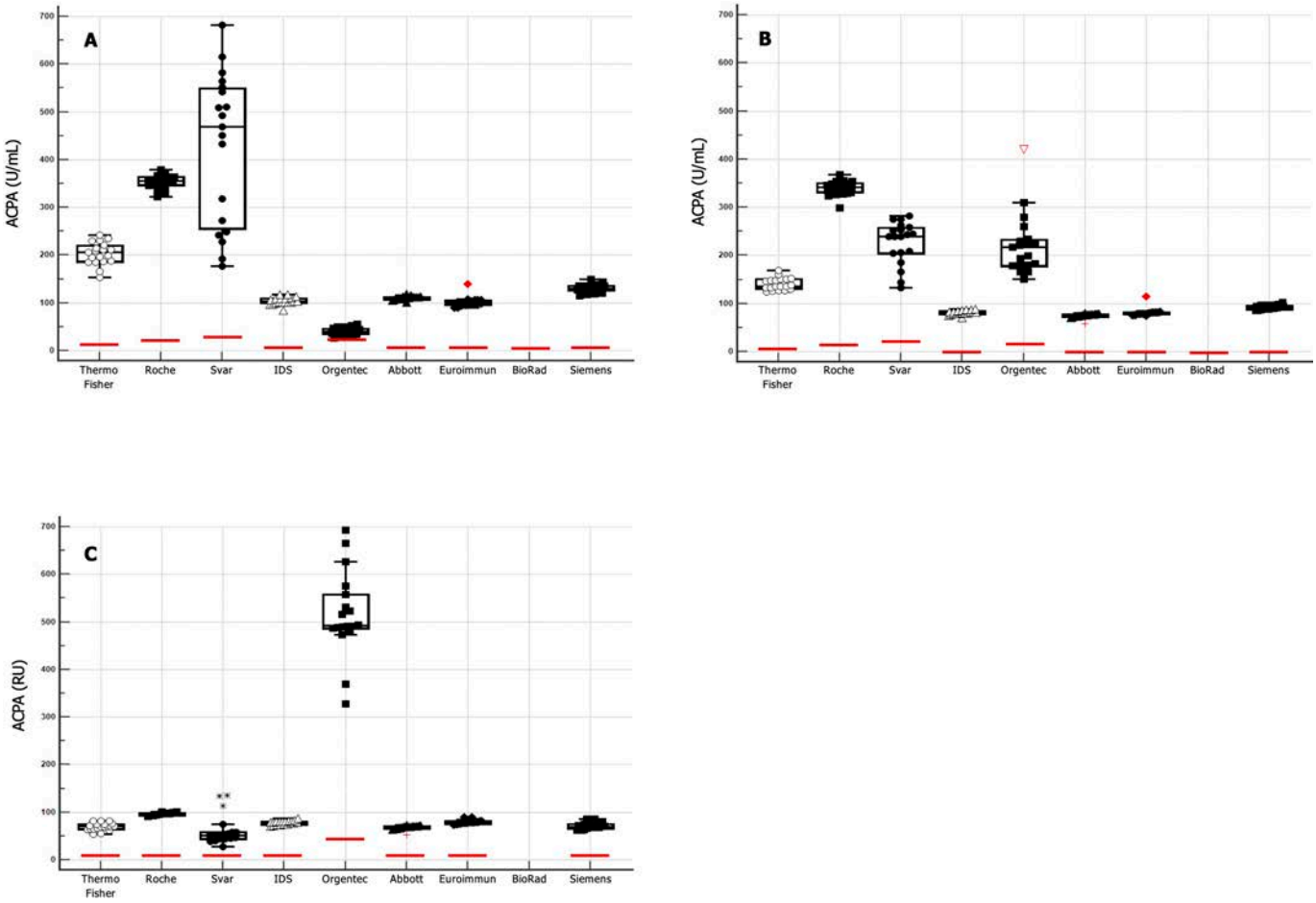



**Figure 1** Quantification of candidate NIBSC 18/204 ACPA reference material (A) and IUIS-CDC ACPA reference material (B). The reference materials were reconstituted according to the guidelines, aliquoted, stored frozen (–20°C) on analysis and tested in 19 different runs with every ACPA assay. (A, B) Box-whisker plots of the results obtained. Boxes represent median and IQR, whiskers represent lowest and highest measurement excluding 'outside' values (ie, larger than the upper quartile plus 1.5 times the IQR; highlighted in red). The manufacturer's cut-offs are marked as red bars. The y-axis represents the manufacturer-specific units. (C) Box-whisker plots of the CDC ACPA reference material recalculated taken the reactivity of the candidate NIBSC 18/204 ACPA standard arbitrarily as 100 units. ACPA, antibodies to citrullinated protein/peptide; CDC, Centers for Disease Control and Prevention; IUIS, International Union of Immunological Societies; NIBSC, National Institute for Biological Standards and Control.

of individuals with RA,[8 9] the International Working Group on the Harmonisation of Autoantibody tests of the International Federations of Clinical Chemistry and Laboratory Medicine listed ACPA as one of the antibodies for which the production of a commutable reference material is urged.[10] Moreover, traceability to a higher-order reference material (if available) is mandatory according to the In-Vitro Diagnostic Medical Devices Regulation (EU) 2017/746 (IVDR).[11]

Therefore, the National Institute for Biological Standards and Control (NIBSC) recently prepared a candidate ACPA standard named 18/204 which has been evaluated in a large international collaborative study; the results and conclusions of which will be presented to the WHO in Autumn 2022 as official candidate for the first WHO international ACPA standard (personal communication). The material consists of a serum pool of five individuals with RA and will be made available by NIBSC in due course. A reference material derived from a pool of 5 sera should more closely mimic the polyclonal response than a single donor-derived reference serum.

Here, independently of the NIBSC international study described above, we evaluated NIBSC 18/204 together with the IUIS-CDC ACPA reference material using different commercially available ACPA assays and sera from individuals with RA and individuals who did not have RA (either suffering from another (rheumatic) disease or healthy).

## MATERIALS AND METHODS

ACPA assays from nine different manufacturers (Thermo Fisher Scientific, Uppsala, Sweden; Roche Diagnostics, Mannheim, Germany; Svar Life Science, Malmö, Sweden; Immunodiagnostic Systems (IDS), Tyne and Wear, UK; Orgentec, Mainz, Germany; Abbott, Wiesbaden, Germany; Euroimmun, Lübeck, Germany; BioRad Laboratories, Hercules, California, USA; and Siemens Healthineers, Sudbury, UK) encompassing different technological platforms (ELISA, fluoroenzyme immunoassay, chemiluminescence assay and addressable laser bead assay) were included in the study. Details on the different assays are given in online supplemental table 1. The antigens used in all assays are cyclic citrullinated synthetic peptides (second generation) except for the Orgentec assay which uses cyclic citrullinated vimentin peptides.

The IUIS-CDC ACPA reference material was obtained from Plasma Services Group (Moorestown, New Jersey, USA).[4 5] The NIBSC 18/204 candidate standard was provided by NIBSC (see online supplemental data 'Description of NIBSC 18/204'

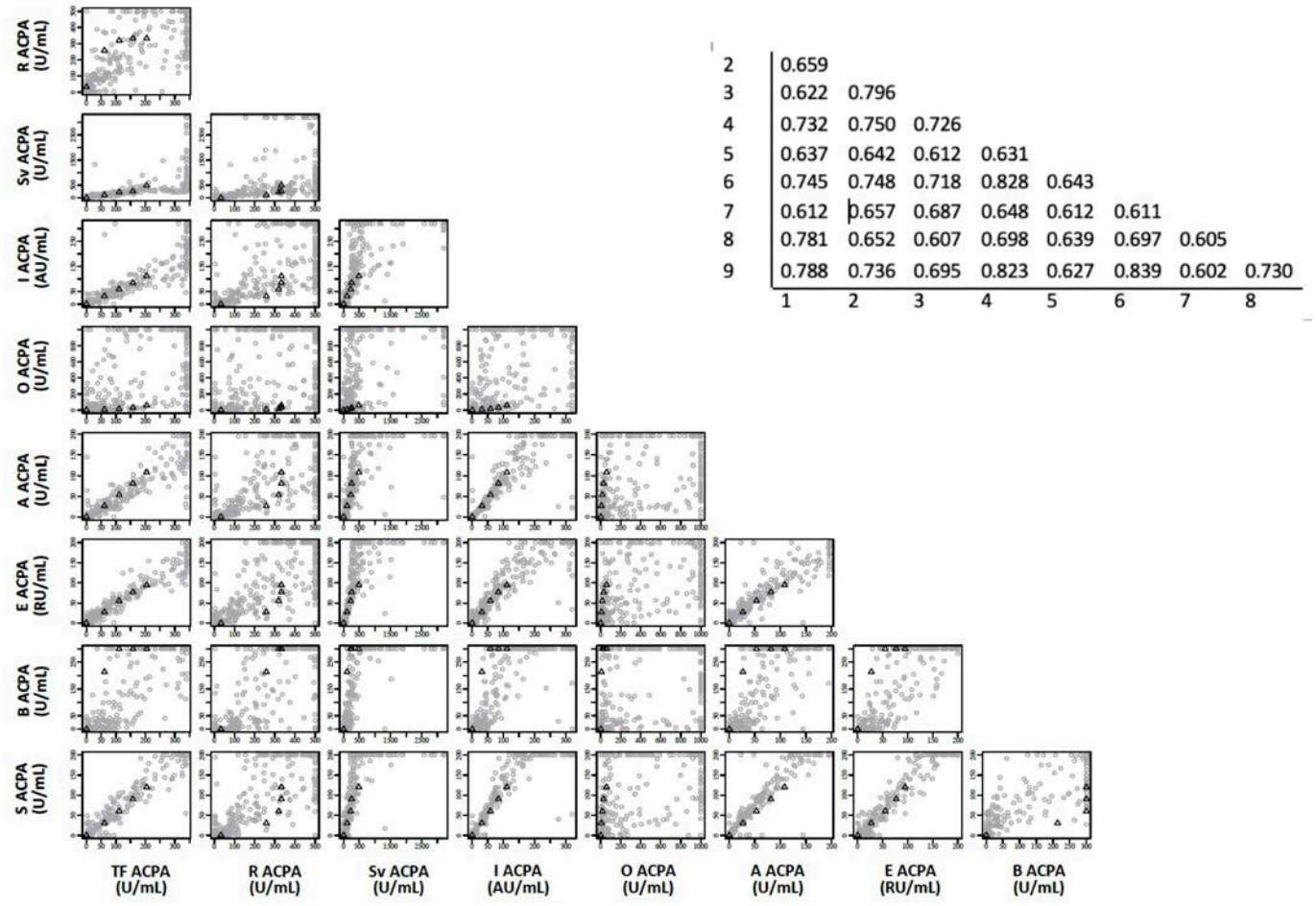



**Figure 2** Correlations of individual values of ACPA measured by nine different immunoassays. The immunoassays included were from Thermo Fisher (TF, 1), Roche (R, 2) Svar life science (SV, 3), IDS (I, 4), Orgentec (O, 5), Abbott (A, 6), Euroimmun (E, 7), BioRad (B, 8) and Siemens (S, 9). The samples were from patients with RA (n=398) and (disease) controls (n=1073) obtained in 11 European hospitals. The NIBSC 18/204 candidate ACPA reference preparation and dilutions thereof (0/4-1/4-2/4-3/4-4/4) are represented by triangles. Spearman's rank correlation coefficients (rs) are shown in the insert on the graph. Detailed statistical data on Spearman's correlation and Bland-Altman are given in online supplemental table 5. ACPA, antibodies to citrullinated protein/peptide; IDS, immunodiagnostic systems; NIBSC, National Institute for Biological Standards and Control.

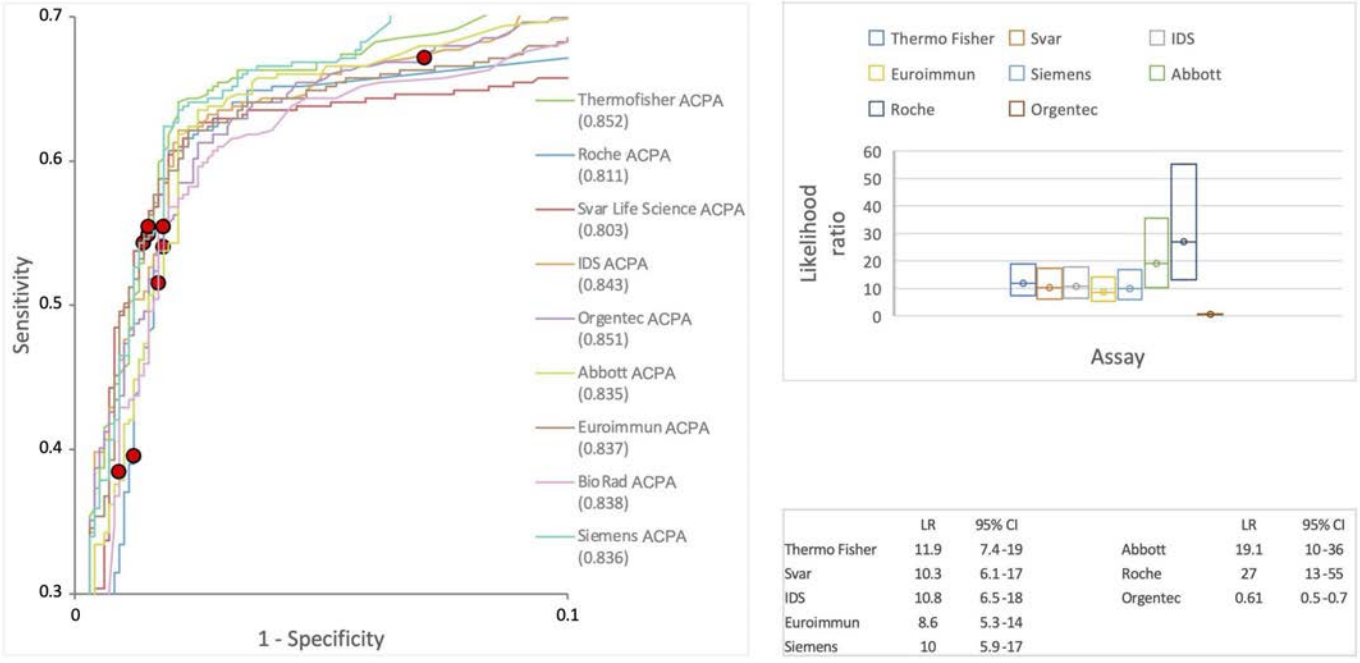
for details on the preparation and properties of the material). NIBSC 18/204 is intended as reference material for IgG ACPA antibodies, not for IgA ACPA antibodies (NIBSC, personal communication). Both materials were reconstituted according to the guidelines of the provider and aliquoted. Both reference materials were measured in 19 different runs.

Imprecision of all ACPA assays was determined using (1) manufacturer's internal quality control (iQC) materials and (2) patient serum samples with a low, medium and high ACPA concentration.[12] All iQC samples were measured before and after every run during 19 runs.

Linearity was assessed by diluting the IUIS-CDC ACPA and NIBSC 18/204 standards with increasing amounts of phosphate buffered saline. Every dilution was analysed three times in different runs.[13]

Serum samples from 398 individuals with RA and 1073 individuals who did not have RA were included. Serum samples were obtained from 11 European hospitals: Division of Rheumatology, Medical University of Vienna (Austria), University Hospital of Leuven (Belgium), University Hospital of Ghent (Belgium), OLV Hospital of Aalst (Belgium), National Institute of Rheumatology and Physiotherapy of Budapest (Hungary), Centre Hospitalier de Luxembourg (Luxembourg), University Medical Centre of Ljubljana (Slovenia), Sahlgrenska Academy Hospital of Gothenburg (Sweden), University Hospital of Linköping (Sweden), University Hospital of Basel (Switzerland), and Kantonsspital of Aarau (Switzerland).

The RA cohort (n=398) consisted of consecutive individuals with newly diagnosed RA. The individuals who did not have RA (n=1073) consisted of (1) a rheumatological disease control group (n=656) (ie, consecutive individuals consulting a rheumatology clinic for the first time but in whom RA was eventually excluded); (2) specific disease control cohorts (ie, individuals with established diagnoses of antineutrophil cytoplasmic antibody associated vasculitis with arthritis (n=24), osteoarthritis (n=25), psoriatic arthritis (n=25), reactive arthritis (n=20), spondyloarthritis (n=25), systemic lupus erythematosus (n=50) and primary Sjögren's syndrome (n=48)) and (3) and healthy individuals (n=200). Sample collection complied with the Word Medical Association Declaration of Helsinki (as revised in 2013). A detailed description of the study groups is provided in[14] and in online supplemental table 2 (individuals with RA) and online supplemental table 3 (individuals with no RA). The diagnostic performance of the ACPA assays included in this study based on the samples from individuals with RA and who did not have RA was published previously.[14] In short, when the manufacturer's cut-off was used, the sensitivity ranged from 57.8% to 64.6% and the specificity from 94.9% to 97.8%. When three times




| | LR | 95% CI | | LR | 95% CI |
|---|---|---|---|---|---|
| Thermo Fisher | 11.9 | 7.4-19 | Abbott | 19.1 | 10-36 |
| Svar | 10.3 | 6.1-17 | Roche | 27 | 13-55 |
| IDS | 10.8 | 6.5-18 | Orgentec | 0.61 | 0.5-0.7 |
| Euroimmun | 8.6 | 5.3-14 | | | |
| Siemens | 10 | 5.9-17 | | | |

**Figure 3** ROC curve analysis and likelihood ratios for NIBSC 18/204. Left hand pane: ROC for nine different ACPA assays with indication of the sensitivity and '1-specificity' of the result associated with a 1:4 dilution of NIBSC 18/204 (red filled circle surrounded by black line). Right handpanel: likelihood ratio of a test result interval with as centre the result of the candidate NIBSC standard. The interval was chosen such that the number of data points with results higher than the result of NIBSC 18/204 equaled the number of data points with results that were lower than the NIBSC 18/204. The intervals were as follows: thermo Fisher: 9–148 U/mL, Roche: 134–387 U/mL, Svar: 28.6–200 U/mL, IDS: 7.9–677 AU/mL, Orgentec: 3.2–16.3 U/mL, Abbott: 7.3–65.1 U/mL, Euroimmun: 9.7–59.9 U/mL, Siemens: 5.3–69.9 RU/mL.For BioRad, no likelihood ratio was calculated as many results had values exceeding the upper limit. ACPA, antibodies to citrullinated protein/peptide; CCP, cyclic citrullinated synthetic peptides; IDS, immunodiagnostic systems; NIBSC, National Institute for Biological Standards and Control; ROC, receiver operating characteristics.

the upper limit of normal was used as threshold, the sensitivity ranged from 50.8% to 60.1% and the specificity from 98.0% to 98.5%.[14]

ROC curves were generated with Analyse-it for Microsoft Excel.

## RESULTS

Data on imprecision using patient serum samples with a low, intermediate and high ACPA concentration are given in online supplemental table 4A. Imprecision data obtained with the two reference materials are given in online supplemental table 4B. The highest imprecision was found for ELISAs. Except for the assay from Roche, the CUSUM test for linearity did not reveal significant deviation from linearity (online supplemental figure 1 legend, online supplemental figure 1).

The candidate NIBSC 18/204 ACPA standard and the IUIS-CDC ACPA reference material were measured in 19 different runs. For both reference materials, there were (large) differences in quantitative ACPA results between assays (online supplemental table 4B, figure 1A). With BioRad, values exceeded the measuring range for both reference materials. All assays scored both reference materials as 'strongly positive' according to the 2010 American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) criteria.[1]

For NIBSC 18/204, results obtained with assays from IDS, Abbott and Euroimmun were similar with a median value of 103.7 AU/mL, 110.7 U/mL and 100.6 RU/mL, respectively. Somewhat higher values were obtained with the Siemens (median 130.1 U/mL) and Thermo Fisher (median 206.0 U/mL) assays. The highest values were obtained with the Roche (355.9 U/mL) and Svar Life Science (468.8 U/mL) assays. The lowest results were obtained with the Orgentec assay (40.6 U/mL).

For IUIS-CDC, a comparable spread of results across the different assays similar to that of NIBSC 18/204 was found, except for Orgentec which, in contrast, did not give the lowest result for IUIS-CDC (figure 1B). Results obtained with assays from Thermo Fisher, Abbott, Siemens, Euroimmun, and IDS amounted to 70.7%, 69.0%, 69.7%, 78.8% and 79%, respectively, of those obtained for NIBSC 18/204. Results obtained with assays from Roche, Svar Life Science and Orgentec amounted to 97.0%, 51.5% and 492.8%, respectively, of those obtained for NIBSC 18/204. Thus, for most assays, values for IUIS-CDC were lower than those for NIBSC 18/204 and the ratio of IUIS-CDC/NIBSC was comparable for several, but not all assays. In summary, when NIBSC 18/204 was used as a calibrator, an improvement in the alignment of ACPA results across several of the evaluated assays was obtained (figure 1C). Indeed, significant agreement was found for (1) Siemens, Thermo Fisher and Abbott, (2) Siemens, IDS and Abbott, (3) Euroimmun and IDS ($p>0.2$, Mann-Whitney U test), but not for Roche, Orgentec and Svar Life Science ($p<0.004$ for comparison to all other assays).

Figure 2 shows the correlation between different ACPA assays. Full details are shown in online supplemental table5. Results obtained with different dilutions of NIBSC 18/204 are also shown. The best correlations (Spearman's r 0.823–0.839) were found between IDS and Abbott, IDS and Siemens and Abbott and Siemens. There was a large dispersion of the results for comparisons with assays from Orgentec, Roche and BioRad. There was good commutability of NIBSC 18/204 with patient samples across Siemens, Thermo Fisher, Abbott, Euroimmun, IDS (and

Svar Life Science). There was lower commutability for BioRad, Roche and Orgentec. It should be noted that a substantial fraction of patients with RA (range 23.1% (Svar Life Science) – 53.0% (Orgentec); mean 35.8%) and controls (range 0.1% (Svar Life Science) – 1.8% (Orgentec); mean 0.6%) had ACPA values that exceeded the NIBSC 18/204 ACPA level. For comparison, the fraction of patients with RA and controls that had ACPA values that exceeded the measuring range was 8.3% (Svar Life Science) – 34.5% (BioRad) and 0.0% (Svar Life Science) – 0.7% (Roche and BioRad), respectively.

In order to explore whether the candidate NIBSC standard can be employed to align clinical interpretation we located the sensitivity and '1 – specificity' associated with a 1:4 dilution of NIBSC 18/204 on the Receiver Operating Characteristics (ROC) curves (generated with 398 individuals with RA and 1073 individuals with no RA) (figure 3). Strikingly, for five assays (Thermo Fisher, Svar Life Science, IDS, Euroimmune and Siemens) the sensitivity / '1-specificity' points almost coincided on the ROC curves. For the Abbott assay, the location of the sensitivity/'1-specificity' point was close to those of the 5 above-mentioned assays, whereas for the Roche, BioRad and Orgentec assays, the location was separate. This separate location relates to the non-commutability and/or non-linearity of NIBSC 18/204 with assays from Roche, Orgentec and BioRad (see above).

A similar location on the ROC curve suggests that the likelihood ratios associated with that particular test result are comparable. Next, we determined the likelihood ratio associated with a test result interval with as centre the result obtained with a 1:4 dilution of NIBSC 18/204. For Thermo Fisher, Svar Life Science, IDS, Euroimmune and Siemens, the likelihood ratio associated with such result interval was ~10. By contrast, it was 19, 27 and 0.61 for Abbott, Roche and Orgentec, respectively.

Taken together, the candidate NIBSC standard can be used to align clinical interpretation for five of the nine tested assays. In practical terms, ACPA test results obtained with assays from Thermo Fisher, Svar Life Science, IDS, Euroimmun and Siemens exceeding the result of a 1:4 dilution of NIBSC 18/204 will have an associated likelihood ratio of at least 10. This does not hold for assays from Roche, Abbott, Orgentec or BioRad. It may hold for other assays not included in this study under the condition that for these assays there is good commutability of the reference material with the assays from Thermo Fisher, Svar Life Science, IDS, Euroimmun and/or Siemens. It should be noted that for all assays included in this study, test result interval-specific likelihood ratios have been described.[14]

## CONCLUSION

NIBSC 18/204 was evaluated as a candidate reference material to standardise ACPA assays. This candidate standard improved the standardisation and alignment of most ACPA assays evaluated in this study. It may also help to align clinical interpretation of test results. However, differences in results between (some) assays still remain. As has been shown for anti-neutrophil cytoplasmic antibodies, using a common reference material does not assure a common clinical interpretation for all assays.[15] Factors that might contribute to the non-commutability across assays include non-linearity, difference in antigen recognition and assay configuration.[10 16 17] Adoption of an international standard for ACPA, as it has been defined for rheumatoid factor, is highly desirable and would facilitate comparison between ACPA assays of different manufacturers.[18 19] This would be particularly important in the context of the ACR/EULAR classification criteria, where ACPA concentration has a high impact on RA classification.[1] NIBSC 18/204 could be used as a calibrator by kit manufacturers or as a reference reagent by diagnostic laboratories to standardise the results and line up clinical interpretation.

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**Contributors** LVH, XB, BVC, DS, GS and PV contributed to the study conception and design. Patient Samples were selected by LVH, BVC, DS, DA, PV, CB, EN, RP, SC, CD, IH, LB, FB, CW, RM, DK, CS, AK, RJ, BJ, EK and PJ. Reference material was provided by PSG and by NIBSC (LS). Material preparation and data collection was performed by SVdB, LB, LVH, AVL, GV, JR; data analysis by LVH and XB; XB acts as guarantor. Manuscript was prepared by LVH, XB, LS, DS and GS and commented by all authors. The final manuscript was read and approved by all authors.

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**Competing interests** XB, LVH, GS and DS have received speaker fees from Thermo Fisher Scientific and have been a consultant for Thermo Fisher Scientific; BL and IH has received speaker fees from Thermo Fisher Scientific. All participating diagnostic companies in-kind provided the ACPA assays and provided technical training and support:Thermo Fisher Scientific, Uppsala, Sweden; Roche Diagnostics, Mannheim, Germany; Svar Life Science, Malmö, Sweden; Immunodiagnostic Systems (IDS), Tyne and Wear, United Kingdom; Orgentec, Mainz, Germany; Abbott, Wiesbaden, Germany; Euroimmun, Lübeck, Germany; Bio-Rad Laboratories, Hercules, California, USA; and Siemens Healthineers, Sudbury, United Kingdom.

**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 EC of University Hospital Leuven and OLV Hospital Aalst with Belgian registration number: B322201939877. Participants gave informed consent to participate in the study before taking part.

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CLINICAL SCIENCE

# Longitudinal trajectories of fatigue in early RA: the role of inflammation, perceived disease impact and early treatment response

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## ABSTRACT

**Objective** Fatigue is common in rheumatoid arthritis (RA). We aimed to explore its longitudinal course, predictors and association with disease activity in early RA.

**Methods** Data came from the 2-year treat-to-target trial CareRA (Care in early RA) and its 3-year extension. Fatigue was measured on Visual Analogue Scale, Multidimensional Fatigue Inventory and Short Form-36 (SF-36) vitality. Longitudinal fatigue trajectories were identified with multivariate growth mixture modelling. Early predictors of fatigue and the association of fatigue and its trajectories with disease activity and clinical/psychosocial outcomes were studied with linear mixed models and multilevel mediation.

**Results** We included 356 and 244 patients in the 2-year and 5-year analyses, respectively. Four fatigue trajectories were identified: rapid, gradual, transient improvement and early deterioration, including 10%, 14%, 56% and 20% of patients. Worse pain, mental health and emotional functioning were seen in the early deterioration group. Higher pain, patient global assessment (PGA) and disability (Health Assessment Questionnaire), lower SF-36 mental components, and fewer swollen joints at baseline predicted higher fatigue over 5 years, while early disease remission strongly improved 5-year fatigue. The association between Simple Disease Activity Index and fatigue was mediated by PGA, pain, mental health and sleep quality.

**Conclusions** Although fatigue evolves dynamically over time in early RA, most patients do not achieve sustained fatigue improvement despite intensive disease-modifying antirheumatic drug therapy. Higher 5-year fatigue levels were seen in patients with more perceived disease impact and fewer swollen joints at baseline. Conversely, early inflammatory disease control strongly improved long-term fatigue, pointing towards an early window of opportunity to prevent persistent fatigue.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Although patients with rheumatoid arthritis (RA) commonly experience fatigue as a complex unmet need, its long-term longitudinal evolution in early RA has rarely been described with multidimensional measures and it remains unclear if disease activity directly affects this evolution.

### WHAT THIS STUDY ADDS

⇒ This study shows that fatigue is a persistent symptom in RA despite intensive disease-modifying antirheumatic drug treatment, with only one in four patients making lasting improvements and 20% even experiencing worsening multidimensional fatigue.

⇒ While more perceived disease impact and fewer swollen joints at baseline predicted higher fatigue over up to 5 years of follow-up, improved long-term fatigue was particularly seen when disease remission was achieved early, even when relapses of disease activity occurred later on.

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

⇒ These findings support the existence of an early window of opportunity to prevent long-term fatigue in RA through prompt inflammatory disease control with pharmacological therapy.

⇒ Nonetheless, pain, sleep and psychosocial determinants seem to play an important mediating role in the experience of fatigue, and clinicians should reserve specific attention to these factors to timely consider additional non-pharmacological approaches.



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## INTRODUCTION

Fatigue is common in rheumatoid arthritis (RA) and is a major challenge in its management.[1] Fatigue is an experience of severe tiredness or exhaustion not clearly caused by excessive energy expenditure.[2] An estimated 10%–20% of the general population regularly report significant fatigue, attributable to both physical and psychosocial causes.[3–5] This burden is even more apparent in the rheumatic diseases, and 40%–80% of patients with RA are affected by severe fatigue.[6 7] Patients experience fatigue as overwhelming and unpredictable, inciting a vicious circle that strongly impacts quality of life.[8] Moreover, people suffering from RA consider fatigue a crucial disease outcome and consistently rate it among the primary treatment goals in both established and early disease.[9–11] Consequently, fatigue has long been recognised as an essential outcome to assess in RA-related trials.[12 13]

Although complementary care strategies such as nurse-led care,[14 15] peer mentoring[16] and cognitive–behavioural therapy[17] could be beneficial, assessing and managing RA-related fatigue remains challenging. Fatigue is a multidimensional symptom whose causes, experience and impact are unique to each individual.[18] Therefore, it is ideally assessed with multidimensional instruments.[19] However, most studies measure fatigue on a Visual Analogue Scale (VAS), which, despite being a reliable alternative,[20] might lack detail about the underlying mechanisms. To comprehensively assess RA-related fatigue, understanding its root causes is crucial. Although inflammation could be involved by influencing neurotransmitters,[21] the relationship between RA disease activity and fatigue is complex and confounded by cognitive and psychosocial aspects.[18] For instance, while fatigue can improve with disease-modifying antirheumatic drugs (DMARDs),[22] many patients still experience fatigue despite inflammatory disease control.[23–25] More insight into the contributors of persistent fatigue could highlight mechanisms other than inflammation and support management of this burden. Moreover, given its unpredictability, RA-related fatigue might evolve differently over time across specific patient subgroups.[26–28]

Studies on contributors of RA-related fatigue should therefore include multidimensional fatigue measures, assessed longitudinally, starting in early disease, and with multivariate methods that account for confounders.[29] Moreover, contributors should be differentiated into either predisposing factors to support early identification of at-risk patients or time-dependent associated factors, such as inflammation, that could be modifiable with interventions.[5] We aimed to identify predisposing and associated factors of RA-related fatigue by examining the longitudinal trajectory of multidimensional fatigue in early RA.

## METHODS

### Study design

Data were obtained from the Care in early RA (CareRA) trial and its extension CareRA-plus. CareRA was a 2-year, investigator-initiated, randomised controlled trial comparing several DMARD regimens with/without glucocorticoid-bridging in DMARD-naïve patients with early RA. CareRA-plus was its 3-year observational extension. Details on the trial design have been published elsewhere (also see online supplemental file 1).[30 31] All participants completing CareRA were eligible for CareRA-plus. All participants provided written informed consent.

### Patient and public involvement

Although patients were not actively involved in designing the trial, patient-reported outcomes (PROs) to collect were selected based on daily contacts between the investigators and patients with RA, and the relevance of these outcomes in early RA was confirmed in a qualitative study.[9]

### Outcomes

Assessment at screening included demographic characteristics, routine radiographs, rheumatoid factor and/or anticitrullinated peptide antibodies, and comorbidities scored on the Rheumatic Diseases Comorbidity Index (RDCI). Clinical assessments during follow-up included tender/swollen joint counts (TJC28/SJC28), patient/physician global assessment of disease activity (PGA/PhGA), C-reactive protein and erythrocyte sedimentation rate (CRP/ESR). The Simple Disease Activity Index (SDAI) was derived as the primary composite measure of disease activity.[32] In addition, participants completed the Health Assessment Questionnaire (HAQ) and VAS for pain and fatigue at every visit, and the Short Form-36 (SF-36), Revised Illness Perception Questionnaire (IPQ-R) and Pittsburgh Sleep Quality Index (PSQI) at baseline and at weeks 16, 52 and 104.[33–35]

Starting from year 2, participants were assessed 6-monthly until year 5. Assessments during this phase were identical to the first 2 years, but PROs were limited to the HAQ and VAS for pain and fatigue.

### Fatigue

Multiple measures of fatigue were collected, including VAS (0–100) at every visit. Additionally, SF-36 vitality (0–100, higher score implies less fatigue) and the Multidimensional Fatigue Inventory (MFI) were recorded at baseline and at weeks 16, 52 and 104.[36] The MFI is a 20-item questionnaire covering five dimensions of fatigue: general fatigue, physical fatigue, mental fatigue, reduced motivation and reduced activity. Higher scores (4–20) indicate higher fatigue.

### Statistical analysis

Based on conceptual knowledge and data exploration, missingness (15% total) was assumed to be at random and handled with multiple imputation (m=20). The results were pooled using Rubin's rules wherever possible.[37] Descriptive statistics were reported as mean (SD) or proportions, and measures of fatigue at baseline were compared with Spearman correlation.

Before investigating distinct fatigue trajectories, we first studied the group-level evolution of fatigue (VAS) over time with linear mixed models (LMMs) including only participants with available 5-year data (online supplemental file 2A).

Based on these models, and because multidimensional fatigue measures were available only during this timeframe, the first 2 years were chosen to study longitudinal fatigue trajectories. We constructed a multivariate growth mixture model (GMM) following a three-step approach,[38] with participant-specific random intercepts, and including as dependent variables the five dimensions of the MFI, SF-36 vitality and fatigue VAS. GMMs attempt to identify distinct classes of individuals with similar evolutions of one or more outcomes over time, while including random effects accounting for interindividual, within-trajectory variance.[39] Models were constructed for two to five trajectories with linear, quadratic, cubic and spline functions to model time. The optimal model was selected based on model fit statistics,[40] and models deriving classes of <10% of participants were excluded to avoid overfitting (online supplemental file 3).

Second, after identifying trajectories, the longitudinal association of trajectory membership with clinical/psychosocial outcomes was studied with LMMs adjusted for age, gender, treatment type, autoantibodies, RDCI and time-varying SDAI. CIs were derived through bootstrapping (5000 iterations of random sampling with replacement).

Third, baseline predictors of fatigue (VAS) over 2 and 5 years were studied with multivariable LMMs including participant-specific random intercepts and adjusted for age, gender, treatment type and time. This method was chosen over predicting trajectory membership because it allowed prediction of fatigue over the full 5-year follow-up. First, all candidate predictors were included simultaneously in an initial multivariable model and subsequently excluded through a backwards-stepwise procedure based on predictors' statistical significance, model fit statistics and conceptual reasons. Baseline PGA, PhGA, pain and HAQ were studied in separate models due to collinearity (Spearman r>0.60). Additionally, early treatment response was studied as a candidate predictor, defined as 'early remission with

sustained control', 'secondary relapse', 'delayed remission' or 'non-remission', based on whether remission (SDAI ≤3.3) was achieved by week 16 with/without relapse before year 2. Considering treat-to-target recommendations, relapse was defined for this purpose as loss of low disease activity (SDAI >11).[41] Finally, the time-varying association of fatigue with clinical/psychosocial variables was studied in similar LMMs, adjusting for age, gender, treatment type and time. The time-varying association between SDAI and fatigue (VAS) was then studied in more detail with a multilevel mediation analysis, including these clinical/psychosocial variables as candidate mediators and clustering within participants.

P-values were adjusted for multiple comparisons with Bonferroni-Holm correction where applicable, and p-values <0.05 were considered statistically significant. All analyses were carried out in R V.4.0.3 (packages: *mice*, *lcmm*, *lme4*, *lavaan* and *lavaanPlot*).

## RESULTS

In total, 379 patients were included in CareRA, of whom 23 were excluded from this analysis because they did not complete the baseline MFI (online supplemental file 4). All remaining 356 participants were included in the 2-year analyses after imputation. Of these, 244 entered CareRA-plus and were included in the 5-year analyses. The baseline characteristics of the 2-year and 5-year study populations were similar (table 1).

### Baseline fatigue

On average, participants reported moderate levels of fatigue at baseline, with a mean of 48 out of 100 on both VAS and SF-36 vitality, and scores of 10–14 out of 20 on the different MFI subscales. VAS fatigue was moderately to strongly correlated with more complex measures of fatigue (online supplemental file 5). However, it correlated less convincingly with measures of more cognitive fatigue, such as the MFI subscales mental fatigue (r=0.33), reduced motivation (r=0.36) and reduced activity (r=0.39).

### Group-level fatigue evolution over 5 years

On average, fatigue (VAS) improved rapidly during the first 16 weeks, before reaching seemingly stable values (online supplemental file 2B). However, there continued to be significant changes over time at both the group and interindividual level until year 2 (online supplemental file 2C). Between years 2 and 5, fatigue no longer changed significantly, implying that 5-year fatigue outcomes were mainly determined during the first 2 years of the trial.

### Longitudinal fatigue trajectories and associated factors

Growth mixture analysis identified four latent trajectory classes for the evolution of multidimensional fatigue during the first 2 years (figure 1). The rapid improvement group (n=37/356, 10%) showed a vast improvement in all fatigue measures over the first 16 weeks, before reaching stable values around week 52. In the gradual improvement trajectory (n=50/356, 14%), all measures of fatigue improved more steadily until week 104. Most participants (n=198/356, 56%) were characterised by a transient improvement in fatigue, where fatigue decreased over the first months, but any net improvement was lost by week 52. Finally, 20% of participants showed an early deterioration (n=71/356) of fatigue over the first 16 weeks, before reaching stable scores at higher levels than baseline.

**Table 1** Baseline characteristics of participants included in analyses

| | 2-year data available (n=356) | 5-year data available (n=244) |
|---|---|---|
| Age, years | 52 (13) | 53 (13) |
| Women, n (%) | 243 (68) | 164 (67) |
| BMI, kg/m$^2$ | 26 (4) | 27 (4) |
| RF-positive, n (%) | 241 (66) | 169 (69) |
| ACPA-positive, n (%) | 237 (65) | 176 (72) |
| Erosive disease, n (%) | 95 (27) | 67 (27) |
| RDCI (0–9) | 0.8 (1.1) | 0.9 (1.1) |
| Symptom duration, months | 8 (12) | 8 (13) |
| **Fatigue** | | |
| VAS, mm (0–100) | 48 (24) | 47 (24) |
| MFI general fatigue (0–20) | 14 (4) | 14 (4) |
| MFI physical fatigue (0–20) | 14 (4) | 14 (4) |
| MFI mental fatigue (0–20) | 10 (4) | 10 (4) |
| MFI reduced activity (0–20) | 13 (4) | 13 (4) |
| MFI reduced motivation (0–20) | 11 (4) | 11 (4) |
| SF-36 vitality (0–100) | 48 (20) | 48 (20) |
| **Clinical variables** | | |
| SDAI | 37 (32) | 38 (33) |
| DAS28-CRP | 4.5 (1.3) | 4.5 (1.3) |
| TJC28 (0–28) | 9 (6) | 9 (6) |
| SJC28 (0–28) | 7 (5) | 7 (5) |
| PGA, mm (0–100) | 55 (24) | 55 (23) |
| Pain, mm (0–100) | 56 (24) | 55 (23) |
| PhGA, mm (0–100) | 52 (19) | 50 (18) |
| CRP, mg/L | 10 (24) | 12 (26) |
| ESR, mm/hour | 30 (23) | 31 (24) |
| HAQ (0–3) | 1.0 (0.7) | 1.0 (0.7) |
| SF-36 PCS (0–100) | 33 (10) | 34 (10) |
| SF-36 MCS (0–100) | 47 (12) | 47 (12) |

Results are reported as mean (SD) unless otherwise specified.
ACPA, anticitrullinated peptide antibody; BMI, body mass index; CRP, C-reactive protein; DAS28-CRP, Disease Activity Score in 28 joints with C-reactive protein; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; MCS, mental component score; MFI, Multidimensional Fatigue Inventory; PCS, physical component score; PGA, patient global assessment of disease activity; PhGA, physician global assessment of disease activity; RDCI, Rheumatic Diseases Comorbidity Index; RF, rheumatoid factor; SDAI, Simple Disease Activity Index; SF-36, Short Form-36; SJC28, swollen joint count in 28 joints; TJC28, tender joint count in 28 joints; VAS, Visual Analogue Scale.

Compared with the rapid improvement group, participants with an early deterioration trajectory reported higher pain (VAS) over both 2 and 5 years of follow-up and had significantly lower scores on SF-36 mental health and emotional role functioning after adjusting for confounders such as SDAI and comorbidities (table 2). Similarly, the gradual improvement group scored worse than rapid improvers on SF-36 mental health and social functioning over 2 years. Similar trends suggested worse outcomes for transient improvers, although these differences were not significant after adjusting for multiple comparisons. No differences were found between trajectories for SDAI, HAQ or PSQI.

### Predictors and associated factors of long-term fatigue

During variable selection, no predisposing effects on fatigue over both 2 and 5 years were found for autoantibodies, RDCI, symptom duration, erosive disease or body mass index (online supplemental file 6). Similarly, age, gender and treatment type did not predict long-term fatigue but were kept in the final models as covariates.

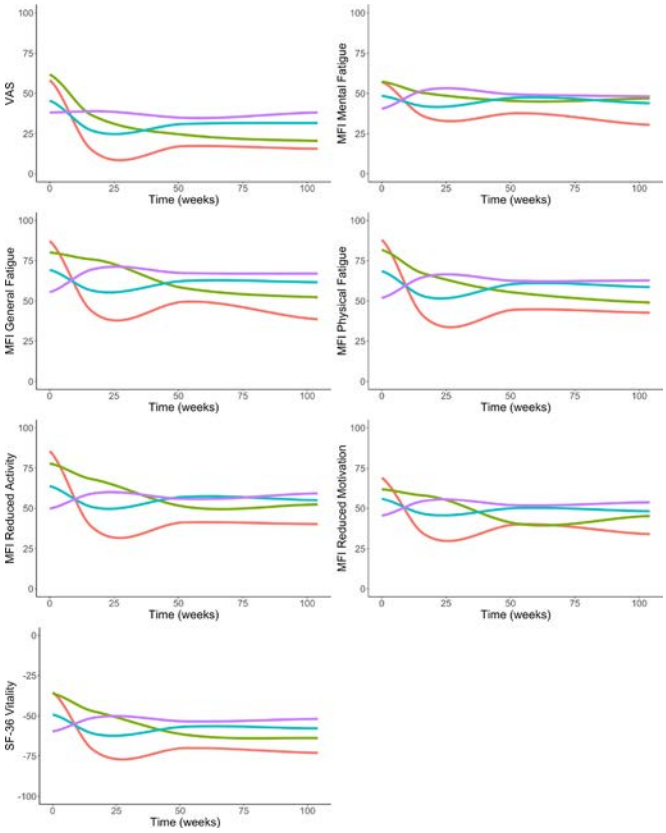


**Figure 1** Latent trajectories of fatigue evolution over the first 2 years in CareRA (Care in early RA) (n=356). Red: rapid improvement (10%); green: gradual improvement (14%); blue: transient improvement (56%); purple: early deterioration (20%). Trajectories were derived through multivariate growth mixture modelling with participant-specific random intercepts and including as dependent variables the five dimensions of the MFI, SF-36 vitality and VAS fatigue. All fatigue outcomes were standardised (0–100) for comparability. MFI, Multidimensional Fatigue Inventory; SF-36, Short Form-36; VAS, Visual Analogue Scale.

In these final models, higher PGA, pain and HAQ, and lower SF-36 MCS at baseline were associated with higher fatigue over both 2 and 5 years of follow-up (table 3). Furthermore, higher 2-year and 5-year fatigue levels were seen in patients with a lower baseline SJC28 and in patients with delayed remission (n=98) or non-remission (n=121) rather than early remission with sustained control (n=85). Among patients with early remission, no difference in 2-year or 5-year fatigue was found for sustained control compared with secondary relapse (n=52).

In the time-varying LMMs, only pain, mental health and sleep quality were independently associated with fatigue over time (online supplemental file 7). Adjusted for other associated factors and multiple comparisons, SDAI, HAQ, IPQ-R and the remaining SF-36 psychosocial dimensions were not associated with fatigue over time.

Moreover, the association between SDAI and fatigue (VAS) was fully mediated by PGA, pain, mental health and sleep quality (figure 2). Specifically, although there was a significant positive association between SDAI and VAS (standardised ß=0.39; 95% CI 0.31 to 0.46), this association was fully explained by PGA (ß=0.19; 95% CI 0.10 to 0.28) and pain (ß=0.18; 95% CI 0.11 to 0.26), and to a lesser extent by SF-36 mental health (ß=0.04; 95% CI 0.02 to 0.06) and PSQI global score (ß=0.02; 95% CI 0.00 to 0.04) (online supplemental file 8).

## DISCUSSION

To our knowledge, this study is the first to describe in detail the longitudinal course of fatigue in early RA with rigorous, multivariate growth modelling methods and based on multidimensional measures of fatigue. Our results suggest that fatigue evolves dynamically during the first treatment months but often remains a persistent symptom, with less than 25% of patients experiencing lasting improvements despite intensive DMARD treatment. Remarkably, one in five patients in our study even experienced worsening fatigue during early treatment and these patients also reported more pain and impaired mental health over time. Moreover, higher scores on pain, disability, PGA and impaired mental health at baseline were associated with persistently higher fatigue over 5 years of follow-up. However, the strongest predictor of long-term fatigue in our study was early achievement of disease remission, even when disease activity later relapsed. Despite this, mediation analysis suggested that the relationship between disease activity and fatigue is complex and fully mediated by PGA, pain, mental health and sleep quality, implying a mainly indirect relation between fatigue and inflammation.

Several studies in early RA cohorts have suggested that the first treatment months are the most influential to determining long-term fatigue. Although long-term follow-up studies have identified improvements in group-level fatigue during the first year of treatment, fatigue remained largely unchanged during subsequent years.[42 43] In a recent publication from the Canadian Early Arthritis Cohort (CATCH), the average improvement in fatigue was most pronounced during the first 3 months of treatment.[44] Similarly, in our study, group-level fatigue improved predominantly during the first 4 months, whereas no significant changes were apparent between year 2 and year 5.

However, we found important interindividual variation in fatigue evolution over time, characterised by either rapid, gradual or transient improvement, or by early deterioration. These longitudinal trajectories depict fatigue as a persistent symptom, with most patients experiencing only temporary improvement. This is in line with previous longitudinal studies on RA-related fatigue which have either reported stable trajectories over time[27 28] or found that only up to a third of patients experienced an improving trajectory.[26 43] However, the trajectories we identified seem more dynamic, possibly because fatigue was assessed in detail during early disease, with both short assessment intervals and multidimensional instruments. For instance, 20% of our participants not only made no sustained improvement in fatigue, but even experienced worsening fatigue during the first treatment months. To our knowledge, only one study has reported a similar deterioration of fatigue during the early course of RA, although this did not result in a distinct trajectory.[43] Our findings contribute to the awareness of a crucial unmet need for patients with RA, particularly since worsening fatigue was associated with more pain and impaired mental health irrespective of disease activity. Furthermore, ample research has shown that fatigue often persists despite improved treatment and even when achieving disease remission.[23 25 42 45]

Stated differently, the association between disease activity and fatigue appears more complex than one might assume. We found that disease activity was indeed positively correlated with fatigue over time, but this association was fully mediated by PGA and pain, and to a lesser extent by mental health and sleep quality. Conversely, joint counts and classic inflammatory markers played no apparent role in this association. These findings add to several studies reporting that fatigue is predominantly associated

**Table 2** Association of fatigue trajectory with outcomes over (A) 2 years and (B) 5 years

| (A) | Gradual improvement (n=50/356, 14%) | | Transient improvement (n=198/356, 56%) | | Early deterioration (n=71/356, 20%) | |
|---|---|---|---|---|---|---|
| **Outcome** | **ß (95% CI)** | **P value (*)** | **ß (95% CI)** | **P value (*)** | **ß (95% CI)** | **P value (*)** |
| SDAI | 0.66 (–2.61 to 3.93) | 0.69 (0.96) | –0.10 (–0.07 to 0.04) | 0.94 (0.94) | 0.42 (–0.36 to 1.20) | 0.79 (0.79) |
| Pain (VAS) | 3.01 (–2.93 to 8.95) | 0.32 (0.96) | 6.57 (1.69 to 11.45) | ***0.008 (0.06)*** | 9.82 (4.19 to 15.45) | **<0.001 (0.004)** |
| HAQ | 0.12 (–0.04 to 0.28) | 0.14 (0.58) | 0.08 (–0.06 to 0.22) | 0.26 (0.53) | 0.08 (–0.08 to 0.24) | 0.27 (0.55) |
| SF-36 MH | –8.98 (–14.92 to –3.04) | **0.003 (0.018)** | –5.94 (–10.76 to –1.12) | ***0.016 (0.10)*** | –9.42 (–14.93 to –3.91) | **<0.001 (0.005)** |
| SF-36 SF | –12.09 (–19.40 to –4.78) | **0.001 (0.007)** | –5.01 (–11.09 to 1.07) | 0.11 (0.53) | –8.56 (–15.48 to –1.64) | ***0.015 (0.06)*** |
| SF-36 RE | –12.44 (–24.08 to –0.80) | 0.04 (0.18) | –7.77 (–17.45 to 1.91) | 0.12 (0.53) | –14.87 (–25.89 to –3.85) | **0.008 (0.04)** |
| PSQI global | 0.66 (–0.69 to 2.01) | 0.33 (0.96) | 0.82 (–0.28 to 1.92) | 0.15 (0.53) | 1.10 (–0.15 to 2.35) | 0.086 (0.26) |
| **(B)** | **Gradual improvement (n=40/244, 16%)** | | **Transient improvement (n=131/244, 54%)** | | **Early deterioration (n=52/244, 21%)** | |
| **Outcome** | **ß (95% CI)** | **P value** | **ß (95% CI)** | **P value** | **ß (95% CI)** | **P value** |
| SDAI | –0.12 (–3.47 to 3.23) | 0.95 (1.00) | –0.53 (–3.45 to 2.39) | 0.72 (0.72) | 0.64 (–2.61 to 3.89) | 0.70 (0.70) |
| Pain (VAS) | 1.87 (–5.48 to 9.22) | 0.62 (1.00) | 5.10 (–4.94 to 15.14) | 0.12 (0.35) | 9.77 (2.64 to 16.90) | **0.007 (0.021)** |
| HAQ | 0.08 (–0.14 to 0.29) | 0.47 (1.00) | 0.11 (–0.09 to 0.31) | 0.26 (0.52) | 0.13 (–0.09 to 0.35) | 0.25 (0.50) |

Results were obtained from multivariate linear mixed models with the reported outcome as the dependent variable and fatigue trajectory as the predictor (rapid improvement trajectory as the reference class). The SF-36 vitality dimension was not studied as an outcome since it was included as a determinant of the fatigue trajectories.
All models were adjusted for age, gender, treatment arm, autoantibody status, SDAI and RDCI as possible confounders.
CIs were derived through bootstrapping (5000 iterations of random sampling with replacement).
*P-value adjusted for multiple comparisons with Bonferroni-Holm correction. Since correction was applied separately for each trajectory and for 2-year and 5-year outcomes, up to seven p-values were considered in these adjustments. P-values were presented in bold when significant and in italics when no longer significant after adjustment.
HAQ, Health Assessment Questionnaire; MH, mental health; PSQI, Pittsburgh Sleep Quality Index; RDCI, Rheumatic Diseases Comorbidity Index; RE, emotional role functioning; SDAI, Simple Disease Activity Index; SF, social functioning; SF-36, Short Form-36; VAS, Visual Analogue Scale.

with pain, sleep and psychological aspects such as mood and self-efficacy, whereas inflammatory markers seem to contribute little to this association directly.[46–48] Similarly, it has been suggested that improvements in fatigue with DMARDs are largely due to improved pain.[7]

Because of these associations between fatigue and other PROs, it is unsurprising that most studies have identified PGA, pain and mental health as baseline predictors of fatigue.[26 43 49] Our findings confirm this, with higher baseline pain, PGA and HAQ, and lower SF-36 MCS associated with consistently higher

**Table 3** Baseline and early predictors of fatigue (VAS) levels over time

| | Baseline–year 2 (n=356) | | Baseline–year 5 (n=244) | |
|---|---|---|---|---|
| **Baseline predictors** | **ß (95% CI)** | **P value (*)** | **ß (95% CI)** | **P value (*)** |
| BMI (kg/m$^2$) | 0.43 (0.04 to 0.82) | ***0.036 (0.14)*** | – | – |
| PGA (0–100) | 0.22 (0.14 to 0.30) | **<0.001 (<0.001)** | 0.15 (0.05 to 0.25) | **0.002 (0.01)** |
| PhGA (0–100) | 0.21 (0.09 to 0.33) | **0.001 (0.007)** | 0.10 (–0.05 to 0.25) | 0.20 (0.40) |
| SJC28 (0–28) | –0.64 (–1.13 to –0.15) | **0.010 (0.05)** | –0.91 (–1.56 to –0.26) | **0.006 (0.04)** |
| TJC28 (0–28) | 0.18 (–0.25 to 0.61) | 0.42 (0.84) | 0.59 (0.04 to 1.14) | 0.038 (0.15) |
| CRP (mg/L) | 0.02 (–0.06 to 0.10) | 0.56 (0.84) | 0.00 (–0.08 to 0.09) | 0.92 (0.92) |
| Pain (0–100) | 0.21 (0.13 to 0.29) | **<0.001 (<0.001)** | 0.17 (0.08 to 0.26) | **<0.001 (0.004)** |
| HAQ (0–3) | 5.22 (2.18 to 8.26) | **0.001 (0.007)** | 4.67 (1.08 to 8.26) | **0.011 (0.05)** |
| SF-36 MCS (0–100) | –0.36 (–0.52 to –0.20) | **<0.001 (<0.001)** | –0.42 (–0.60 to –0.24) | **<0.001 (<0.001)** |
| Treatment response† | | | | |
| Secondary relapse (n=52/356) | 5.24 (–0.39 to 10.87) | 0.07 (0.21) | 5.37 (–1.04 to 11.78) | 0.10 (0.30) |
| Delayed remission (n=98/356) | 9.87 (4.91 to 14.83) | **<0.001 (<0.001)** | 10.15 (4.39 to 15.91) | **<0.001 (0.005)** |
| Non-remission (n=121/356) | 21.66 (17.15 to 26.17) | **<0.001 (<0.001)** | 20.89 (15.48 to 26.30) | **<0.001 (<0.001)** |

Results were obtained from multivariable linear mixed models with fatigue (VAS) as the dependent variable and a participant-specific random intercept. Fatigue (VAS) and SF-36 vitality were not studied as baseline predictors since these models were intended to study average fatigue levels over time rather than fatigue evolution relative to baseline.
PGA, PhGA, pain and HAQ were included in separate models due to collinearity (Spearman r>0.60).
All models were adjusted for age, gender, treatment type and time as confounders.
P-value adjusted for multiple comparisons with Bonferroni-Holm correction. Since correction was applied separately for 2-year and 5-year outcomes, up to 12 p-values were considered in these adjustments. P-values were presented in bold when significant and in italics when no longer significant after adjustment.
*P-value adjusted for multiple comparisons with Bonferroni-Holm correction. Since correction was applied separately for 2-year and 5-year outcomes, up to 12 p-values were considered in these adjustments. P-values were presented in bold when significant and in italics when no longer significant after adjustment.
†Early remission with sustained control as reference category (n=85/356). This model was adjusted for age, gender, treatment type, time and baseline SDAI.
BMI, body mass index; CRP, C-reactive protein; HAQ, Health Assessment Questionnaire; MCS, mental component score; PGA, patient global assessment of disease activity; PhGA, physician global assessment of disease activity; SDAI, Simple Disease Activity Index; SF-36, Short Form-36; SJC28, swollen joint count in 28 joints; TJC28, tender joint count in 28 joints; VAS, Visual Analogue Scale.

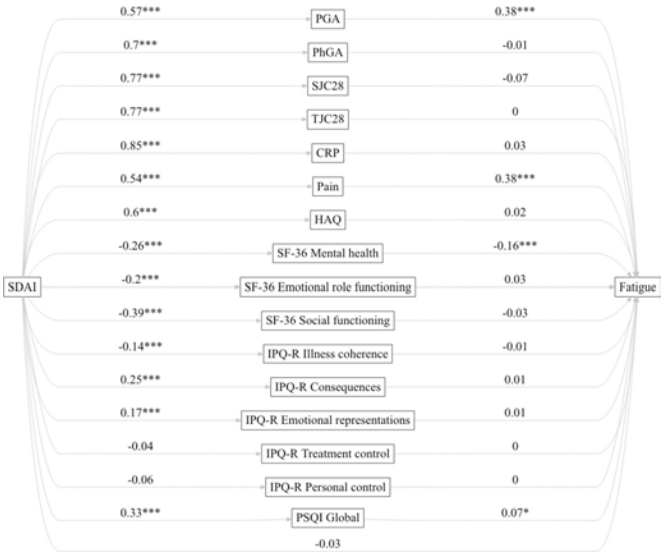


**Figure 2** Mediation analysis of the association between SDAI and fatigue (VAS) over time. Results were obtained from multilevel mediation analysis studying the association between SDAI and fatigue (VAS) across baseline and weeks 16, 52 and 104, with participant-specific random intercepts (n=356). Reported are standardised regression coefficients with indicators of significance (*p<0.05, **p<0.01, ***p<0.001). CRP, C-reactive protein; HAQ, Health Assessment Questionnaire; IPQ-R, Revised Illness Perception Questionnaire; PGA, patient global assessment of disease activity; PhGA, physician global assessment of disease activity; PSQI, Pittsburgh Sleep Quality Index; SDAI, Simple Disease Activity Index; SF-36, Short Form-36; SJC28, swollen joint count in 28 joints; TJC28, tender joint count in 28 joints; VAS, Visual Analogue Scale.

levels of fatigue over 5 years of follow-up. Strikingly though, a higher baseline swollen joint count predicted lower long-term fatigue. These findings add to the recent results of the ARCTIC trial (Aiming for Remission in rheumatoid arthritis: a randomised trial examining the benefit of ultrasound in a Clinical TIght Control regimen), in which more swollen joints and higher ultrasound inflammation at baseline were associated with less fatigue at year 2, while a predisposing effect was seen for PGA.[50] Together, the results of both trials could indicate that RA-related fatigue is a composite of inflammation-driven fatigue and fatigue with a stronger psychosocial background. Nevertheless, to improve long-term fatigue outcomes, it appears particularly important to achieve disease control early, likely through positive effects on both these pathways.[51] For instance, both our study and the ARCTIC trial identified early remission as the strongest predictor of long-term fatigue. In our study, this effect was evident from early on to even 5 years of follow-up. Moreover, our results add the crucial insight that these beneficial effects of early remission on long-term fatigue remain even when relapses of disease activity later occur, pointing towards an early window of opportunity to prevent long-term fatigue in RA.

Our study has some limitations. Whereas during the first 2 trial years fatigue was measured frequently with multiple instruments, fatigue assessments during CareRA-plus were limited to a 6-monthly VAS. Consequently, our finding of a more stable fatigue course during this timeframe might be influenced by study design.

However, several strengths add credibility to our findings. Most studies assess fatigue on VAS or Numeric Rating Scale. While our results showed that VAS correlates well with measures of general and physical fatigue, it seemed to capture aspects related to mental fatigue and motivation less convincingly. We assessed fatigue not only through several multidimensional instruments, but also longitudinally for up to 5 years in a pragmatic clinical trial representative of a population of patients with early RA. Moreover, fatigue was measured frequently during the first treatment months and studied with rigorous statistical methods, providing a uniquely detailed picture of its complexity in early RA.

## CONCLUSION

We showed that fatigue is a dynamic but persistent symptom in early RA, with less than 25% of patients making lasting improvements despite intensive DMARD treatment and one in five patients even experiencing worsening fatigue during the first months. However, achieving early disease remission strongly improved fatigue over up to 5 years of follow-up, even when disease activity later relapsed. Thus, the first step to managing fatigue in early RA should be to seize this window of opportunity for prompt inflammatory disease control. Nonetheless, the association between disease activity and fatigue seems to be mainly explained by pain, mental health and sleep quality. Moreover, higher fatigue over time was seen in patients who at baseline had more perceived disease impact and fewer swollen joints. Clinicians should thus reserve specific attention to the psychosocial determinants of fatigue and timely consider additional non-pharmacological approaches, particularly when no rapid improvement is made with pharmacotherapy.

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**Contributors** PV, JJ and RW designed the study protocol in collaboration with the CareRA study group. Investigators of the CareRA study group, including PV and RW, recruited and enrolled patients and were responsible for daily patient management. PV and JJ were responsible for coordination of the trial and collection of data. MD analysed the data and drafted the article. All authors contributed to interpretation of the data and revised the article critically for content. All authors gave final approval of the manuscript to be published. PV and MD are the guarantors.

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CLINICAL SCIENCE

# Treat-to-target dose reduction and withdrawal strategy of TNF inhibitors in psoriatic arthritis and axial spondyloarthritis: a randomised controlled non-inferiority trial

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## ABSTRACT

**Objectives** Tumour necrosis factor inhibitors (TNFi) are effective in psoriatic arthritis (PsA) and axial spondyloarthritis (axSpA), but are associated with a small (0.6%) increase in serious infection risk, patient burden due to need for self-injection and high costs. Treat-to-target (T2T) tapering might ameliorate these drawbacks, but high-quality evidence on T2T tapering strategies is lacking in PsA and axSpA.

**Methods** We performed a pragmatic open-label, monocentre, randomised controlled non-inferiority (NI) trial on T2T tapering of TNFi. Patients with PsA and axSpA using a TNFi with ≥6 months stable low disease activity (LDA) were included. Patients were randomised 2:1 to disease activity-guided T2T with or without tapering until withdrawal and followed-up to 12 months. Primary endpoint was the difference in proportion of patients having LDA at 12 months between groups, compared with a prespecified NI margin of 20%, estimated using a Bayesian prior.

**Results** 122 patients (64 PsA and 58 axSpA) were randomised to a T2T strategy with (N=81) or without tapering (N=41). The proportion of patients in LDA at 12 months was 69% for the tapering and 73% for the no-tapering group: adjusted difference 5% (Bayesian 95% credible interval: −10% to 19%) which confirms NI considering the NI margin of 20%. The mean percentage of daily defined dose was 53% for the tapering and 91% for the no-tapering group at month 12.

**Conclusions** A T2T TNFi strategy with tapering attempt is non-inferior to a T2T strategy without tapering with regard to the proportion of patients still in LDA at 12 months, and results in a substantial reduction of TNFi use.

**Trial registration number** NL 6771.

### WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

⇒ Fixed tumour necrosis factor inhibitor (TNFi) dose reduction strategies seem feasible in psoriatic arthritis (PsA) and axial spondyloarthritis (axSpA), whereas discontinuation warrants caution due to risk of flares.

⇒ Current evidence on (stepwise) treat-to-target (T2T) tapering strategies is limited and inconsistent in PsA and axSpA.

### WHAT DOES THIS STUDY ADD?

⇒ This first randomised controlled trial on disease activity-guided stepwise T2T tapering strategies demonstrates non-inferiority with regard to the proportion of patients in low disease activity accompanied by a substantial reduction in TNFi use in both PsA and axSpA.

### HOW MIGHT THIS IMPACT ON CLINICAL PRACTICE OR FUTURE DEVELOPMENTS?

⇒ Implementing T2T tapering strategies into practice will reduce TNFi use, and thereby patient burden, risk for adverse events and costs, while maintaining disease control.

## INTRODUCTION

Psoriatic arthritis (PsA) and axial spondyloarthritis (axSpA) are pathophysiologically and clinically related inflammatory rheumatic diseases. PsA is characterised by asymmetrical peripheral arthritis associated with psoriasis. AxSpA is predominantly identified by axial inflammation resulting in inflammatory back pain. Biological disease-modifying antirheumatic drug (bDMARDs), especially tumour necrosis factor inhibitors (TNFi), are widely used in both PsA and axSpA, and have proven to be safe and effective.[1 2] However, these drugs have drawbacks such as a small increased risk of infection, injection site reactions and relatively high costs,[3–7] which adds to the financial burden of healthcare. Treat-to-target (T2T) tapering until complete withdrawal or flare might reduce these disadvantages,[4] and has shown to be safe and (cost-)effective in rheumatoid arthritis (RA) trials.[8 9] However, although this strategy is already being recommended for PsA and axSpA, high quality evidence for this recommendation is lacking.

Current recommendations on dose tapering are based on fixed dose reduction or discontinuation studies, and data on stepwise T2T tapering strategies for PsA and axSpA is lacking. In PsA, one randomised controlled trial (RCT) showed that continuation of ixekizumab was superior to

discontinuation, but the majority of patients with loss of efficacy after discontinuation regained low disease activity (LDA) after reinstatement.[10] In axSpA, six RCTs studied fixed dose reduction or discontinuation using different TNFi.[11–16] The majority of tapered patients in these studies maintained clinical remission or LDA; or regained it quickly after therapy reinstatement, whereas discontinuation was discouraged due to the risk of flares.

We therefore performed an RCT to investigate whether a T2T strategy with tapering is non-inferior to a T2T strategy without tapering.

## METHODS

### Trial design and patients

We performed a pragmatic, open-label, monocentre, randomised controlled, non-inferiority (NI) trial, to compare the effect of a stepwise T2T tapering strategy (intervention) with a T2T strategy without tapering (control) regarding disease activity, (concomitant) medication use, physical function, quality of life and joint damage (for PsA).

Patients, ≥16 years of age, had to have stable LDA at least 6 months prior to inclusion. For PsA, LDA was defined as Psoriatic Arthritis Disease Activity Score (PASDAS) ≤3.2 and modified body surface area (mBSA) involvement ≤3% (as used in the minimal disease activity (MDA) status for PsA). For axSpA, LDA was defined as Ankylosing Spondylitis Disease Activity Score (ASDAS) <2.1 for axSpA and/or according to the treating rheumatologist and patient). The study rationale and design were extensively described before[17] and are further explained in online supplemental appendix 1.

The study has been registered in the Dutch Trial Register. The trial was conducted according to the principles of the Declaration of Helsinki and the International Conference on Harmonisation guideline on Good Clinical Practice. Written informed consent of all eligible patients was received at trial procedure commencement. Patients were enrolled between 9 January 2019 and 16 July 2020 at the rheumatology departments of the Sint Maartenskliniek, located in Nijmegen and Woerden, the Netherlands. A data safety monitoring board with members independent of the study met every 4 months and looked at recruitment, efficacy (mean PASDAS for PsA and ASDAS for axSpA), number of flares and (serious) adverse events per group.

### Randomisation

Patients were allocated to a T2T strategy using TNFi with or without tapering attempt in a ratio of 2:1 using varying block sizes of three or six, stratified for diagnosis (PsA or axSpA) and concomitant conventional synthetic DMARD (csDMARD) use (yes or no). In total, there are four strata (2×2), with every stratum having its own randomisation list. Randomisation sequences for each of the four strata were generated online by an independent researcher at the Sint Maartenskliniek (LMV) and were concealed during the study period, with the researcher (LMV) sealing them into sequentially numbered opaque envelopes. The allocation in these envelopes were revealed to the patients and physician after inclusion. Patients visited the outpatient clinic every 3 months and were followed for 12 months.

### T2T strategy with and without tapering

Patients in both groups were treated according to the prespecified protocol regarding dose tapering, co-medication and treatment of flares, from which the rheumatologist could deviate in shared decision-making with the patient. Patients randomised to the tapering group were tapered stepwise starting at baseline, from 100% to 66% and 50% until discontinuation (table 1) during each visit where low disease activity was maintained. Patients who were using <100% of the authorised TNFi dose stepped in at the nearest dosing interval, for example, patients using adalimumab one time every 3 weeks (66%), stepped in at an every 4-week interval (50%). Patients randomised to the no-tapering group continued their original TNFi dose or interval. Concomitant csDMARDs were not tapered during the study. At each visit, the treating rheumatologist was advised by the researcher, guided by the PASDAS and mBSA for PsA and the ASDAS for axSpA. Patients visited the outpatient clinic every 3 months and in case of flares. At every visit, disease activity state, (concomitant) medication use, (serious) adverse events, function and quality of life was determined. In case of a (suspected) flare patients were assessed at the outpatient clinic, where concomitant treatment as non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids could be added to the current treatment. After this, patients were re-evaluated 4 weeks later: in case of a persistent flare (>4 weeks), treatment was intensified, in case the flare was adequately addressed by glucorticoid or NSAID bridging, no further treatment changes were made. The dose was adjusted to the last effective interval or dosage which was maintained throughout the study period. When already using full TNFi dose or if dose adjustment did not suffice, patients were switched to another b/targeted synthetic (ts)DMARD. Since treatment changes were based on shared decision-making between patient and physician, treatment could also be intensified if the proposed flare criteria were not met.

**Table 1** Stepwise tapering protocol for patients with PsA and axSpA in the T2T strategy group with tapering steps at baseline, 3 months and 6 months. Introduction of first tapering step at baseline visit, assuming the use of the authorised TNFi dose

| TNFi | 100%* | 66% | 50% | 0% |
|---|---|---|---|---|
| Adalimumab/ certolizumab pegol | 40 mg 2-week interval | 40 mg 3-week interval | 40 mg 4-week interval | Stop TNFi |
| Etanercept | 50 mg 1-week interval | 50 mg 10-day interval | 50 mg 2-week interval | Stop TNFi |
| Golimumab | 50 mg 1-month interval | 50 mg 1.5-month interval | 50 mg 2-month interval | Stop TNFi |
| Infliximab† | 3 mg/kg 8-week interval | 2.25 mg/kg 8-week interval | 1.5 mg/kg 8-week interval | Stop TNFi |

*Full authorised TNFi dose, used before baseline: adalimumab/certolizumab pegol 40 mg/200 mg every other week; etanercept 50 mg every week; golimumab 50 mg every month; infliximab 3 mg/kg every 8 weeks.
†In our local protocol, in line with rheumatoid arthritis, standard infliximab dose is started at 3 mg/kg every 8 weeks for PsA and axSpA, instead of the registered 5 mg/kg every 6 weeks (for axSpA).
axSpA, axial spondyloarthritis; PsA, psoriatic arthritis; TNFi, tumour necrosis factor inhibitors; T2T, treat-to-target.

### Flare definition

Flare was defined for PsA by a current PASDAS >3.2 or increase of ≥0.8[17], and for axSpA as a current ASDAS≥2.1 or increase of ≥0.9 points.[18] For both diseases, a flare was also noted when an important worsening of mBSA or active extra-musculoskeletal symptoms (as judged by the treating rheumatologist) occurred. Clear cut-off values for important worsening are lacking for mBSA and treatment was adjusted as judged by the treating rheumatologist and patient in clinical practice.

### Assessments

Disease activity was measured at every visit by PASDAS (0 to ≈10) for PsA and ASDAS (0.6–6.3) for axSpA. Adverse events

(AEs) and serious AEs (SAEs) were recorded and graded according to the Common Terminology Criteria for Adverse Events V.5.0. For function the health assessment questionnaire disability index (0–3) and for axSpA the Bath Ankylosing Spondylitis Functional Index (0–10) was used, with higher scores indicating greater disability. Quality of life was measured by using the EuroQol five-dimension scale with three levels (0–1) and the Short Form Health Survey 12 (SF-12) (0–100 for each component score) which consist of a physical and mental component score (0–100), with higher scores indicating better quality of life. For axSpA specifically, quality of life was also scored by the Assessment of SpondyloArthritis international Society Health Index (ASAS-HI) (0–17). For PsA, radiographs of hands and feet were taken at baseline and 12 months. Progression of joint damage was assessed by using the Short Erosion Narrowing Score (SENS) (0–86), with a higher score indicating more joint damage. Sets of radiographs were scored independently and without blinding for allocation by two out of three readers each, with known sequence. For axSpA, sacroiliitis was assessed by radiography of sacroiliac (SI) joints at baseline and scored by using the modified New York criteria (0–4 for each joint), with a higher score depicting more damage. Radiographs of the SI-joints were graded in known sequence by two rheumatologists and dependent on this grading sacroiliitis was diagnosed (yes or no). Any disagreements were resolved by consensus. In axSpA it is predominantly of importance to assess sacroiliitis for the fulfilment of the supporting ASAS classification criteria. We decided not to assess radiographic progression as a secondary outcome because of limited effect of TNFi on this outcome in axSpA especially within our follow-up period, since an extensive review demonstrated that radiographic changes only occur after 2 years of follow-up.[19]

### Outcomes

The primary outcome of this study was the difference in proportion of patients in LDA (PASDAS ≤3.2 and BSA ≤3% of the skin (PsA), ASDAS <2.1 (axSpA) and an absence of active extra-musculoskeletal symptoms) between the tapering and no-tapering group at 12 months follow-up, compared with the prespecified NI margin of 0.2 (20%). Secondary outcomes at 3, 6, 9 and 12 months were differences in the TNFi use between both groups, by calculating the mean percentage of daily defined dose (%DDD); efficacy measured by change in the mean PASDAS for PsA and ASDAS for axSpA between both groups; start or escalation of concomitant csDMARDs, oral or intra-articular/intramuscular glucocorticoids and NSAIDs; flares and infections; functioning; and quality of life. At 12 months, differences were assessed in bDMARD drug retention between both groups; the percentage of patients in the tapering group still on a tapered dose and the percentage who had discontinued their TNFi altogether. Additionally, progression of joint damage was assessed at 12 months between both groups (PsA only).

### Statistical analyses

The sample size and choice for NI margin have been extensively discussed in a previous article.[17] The sample size was based on a Bayesian analysis where NI would be claimed if the lower limit of the Bayesian 95% credibility interval of the difference lies above 20%. A minimum of 95 patients was needed to have 80% power to claim NI, taking dropout into account, for further details see online supplemental appendix 2. Our primary Bayesian analyses were done per-protocol (PP) and in addition on an intention-to-treat (ITT) basis. For PP analyses, we included all patients in the tapering group that attempted at least one dose optimisation step and all patients in the no-tapering group who did not attempt dose optimisation, unless when medically required such as in the case of adverse events or contraindications. Descriptive statistics included mean and SD, median (p25–p75) or frequencies/percentages depending on the type of distribution of the data. Continuous data and categorical data were compared between arms using an unpaired t-test or Mann-Whitney U test and $\chi^2$ test (cumulative incidences). Differences in (serious) AEs were presented by 95% CIs and Poisson regression (incidence densities) was used. Analysis of variance was used for representation of radiographic results such as the smallest detectable difference and smallest detectable change (SDC).[20] For exclusion and dropout, numbers and reasons were reported to ensure internal validity. All data were registered in patients' electronic health record and entered anonymously in an electronic database (Castor EDC) and subsequently exported to Stata (V.13.1) for statistical analyses.

## RESULTS

### Patients

We enrolled 122 patients, who were allocated to the tapering (N=81 (PsA, N=42; axSpA, N=39)) or no-tapering group (N=41 (PsA, N=22; axSpA, N=19)). Baseline characteristics were similar between both groups (table 2), except for csDMARD use, sex and extend of joint involvement in PsA (see online supplemental table 3). Medication use was similar between both groups with adalimumab being the most frequently used TNFi. One visit at 9 months was missing, with no missing values influencing the primary outcome and missings for other outcomes <5%, therefore all analyses were performed on a complete-case basis.

### Disease activity and medication use (efficacy)

All patients adhered to the prespecified treatment protocol and according to our definitions, the PP population was therefore the same as the ITT population (figure 1). Our primary Bayesian analysis showed that the proportion of patients in LDA at 12 months was 69% for the tapering and 73% for the no-tapering group: adjusted difference 5% (Bayesian 95% credible interval (CI): −10% to 19%) confirming NI (figure 2). See online supplemental tables 2 and 3 for the Bayesian sensitivity analyses of proportion of LDA for diseases separately and for baseline imbalances. The mean %DDD was 53% (95% CI (44% to 63%)) for the tapering and 91% (95% CI (85% to 97%)) for the no-tapering group at month 12. Mean disease activity and mean percentage of the TNFi dose during each timepoint (3, 6, 9 and 12 months) are shown in figure 3 and online supplemental tables 4-6. The percentage of patients with PsA meeting MDA during each time point is shown in online supplemental table 7. The cumulative incidence of start or escalation of concomitant medication was higher in the tapering group, and significantly so for NSAID use: csDMARDs (only for PsA): 1 (2%) versus 1 (5%) (p=0.64); NSAIDs: 44 (54%) versus 10 (24%) (p=0.002); glucocorticoids intramuscular: 24 (30%) versus 7 (17%) (p=0.15); glucocorticoids intra-articular: 12 (15%) versus 3 (7%) (p=0.66); glucocorticoids oral: 3 (4%) versus 2 (5%) (p=0.29) (see online supplemental table 8 for additional information). Additional sensitivity analyses per diagnosis showed slightly more NSAIDs use in the tapering group compared with the no-tapering group: 21 (50%) versus 5 (23%) (p=0.035) for PsA and 23 (59%) versus 5 (26%) (p=0.019) for axSpA. For glucocorticoid use was this respectively: 12 (29%) versus

**Table 2** Baseline characteristics of T2T strategy treated patients with PsA and axSpA with or without tapering

| Characteristic | T2T with tapering (N=81) | T2T without tapering (N=41) |
|---|---|---|
| Diagnosis, n (%) | | |
| PsA | 42 (52) | 22 (54) |
| axSpA | 39 (48) | 19 (46) |
| Female, n (%) | 28 (35) | 20 (49) |
| Age in years at inclusion, mean (SD) | 50 (14) | 52 (15) |
| Disease duration at inclusion, years, median (IQR) | 11 (5–21) | 12 (5–21) |
| Rheumatoid factor positivity, n (%) - (64/64 PsA) | 3 (7) | 1 (5) |
| Anti-CCP positivity, n (%) - (64/64 PsA) | 0 (0) | 1 (5) |
| HLA-B27 positivity, n (%) - (58/58 axSpA) | 34 (87) | 18 (95) |
| CASPAR criteria, n (%) | 34 (81) | 17 (77) |
| ASAS criteria, n (%) | 35 (90) | 17 (89) |
| Concomitant psoriasis, n (%) | 39 (48) | 18 (44) |
| Concomitant IBD, n (%) | 4 (5) | 2 (5) |
| BMI (kg/m$^2$), mean (SD) - (121/122) | 27 (4) | 26 (4) |
| Monoarticular/oligoarticular as PsA type, n (%) - (64/64 PsA) | 27 (64) | 7 (32) |
| Erosive disease, n (%) - (64/64 PsA) | 13 (31) | 8 (36) |
| Sacroiliitis on radiographic imaging, n (%) - (58/58 axSpA) | 25 (64) | 11 (58) |
| Disease activity, mean (SD) | | |
| PASDAS - (64/64 PsA) | 1.60 (1.26) | 1.63 (0.98) |
| ASDAS - (57/58 axSpA) | 1.34 (0.87) | 1.21 (0.61) |
| Number of previous bDMARD, n (%) | | |
| 0 | 61 (75) | 26 (63) |
| 1 | 14 (17) | 13 (32) |
| ≥2 | 6 (7) | 2 (5) |
| Duration of current bDMARD use, years, median (IQR) | 2 (1–6) | 2 (2–7) |
| Current bDMARD use, n (%) | | |
| Adalimumab | 62 (77) | 28 (68) |
| Etanercept | 10 (12) | 6 (15) |
| Certolizumab pegol | 2 (2) | 1 (2) |
| Golimumab | 2 (2) | 1 (2) |
| Infliximab | 5 (6) | 5 (12) |
| Current csDMARD use, n (%) | | |
| None | 63 (78) | 31 (76) |
| Methotrexate | 9 (11) | 6 (15) |
| Hydroxychloroquine | 0 (0) | 1 (2) |
| Leflunomide | 6 (7) | 3 (7) |
| Sulfasalazine | 2 (2) | 0 (0) |
| Azathioprine | 1 (1) | 0 (0) |
| Current NSAID use, n (%) | 26 (32) | 14 (34) |

Anti-CCP, anti–cyclic citrullinated peptide; ASAS, Assessment of SpondyloArthritis international Society; ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial spondyloarthritis; bDMARD, biological disease modifying anti-rheumatic drug; BMI, body mass index; CASPAR, Classification Criteria for Psoriatic Arthritis; csDMARD, conventional synthetic DMARD; HLA-B27, human leukocyte antigen B27 ; IBD, inflammatory bowel disease; NSAID, non-steroidal anti-inflammatory drug; PASDAS, Psoriatic Arthritis Disease Activity Score; PsA, psoriatic arthritis; T2T, treat-to-target.

4 (18%) (p=0.13) (intramuscular); 10 (24%) versus 2 (9%) (p=0.28) (intra-articular); 2 (5%) versus 2 (9%) (p=0.38) (oral) for PsA and 12 (31%) versus 3 (16%) (p=0.34) (intramuscular); 2 (5%) versus 1 (5%) (p=0.69) (intra-articular); 1 (3%) versus

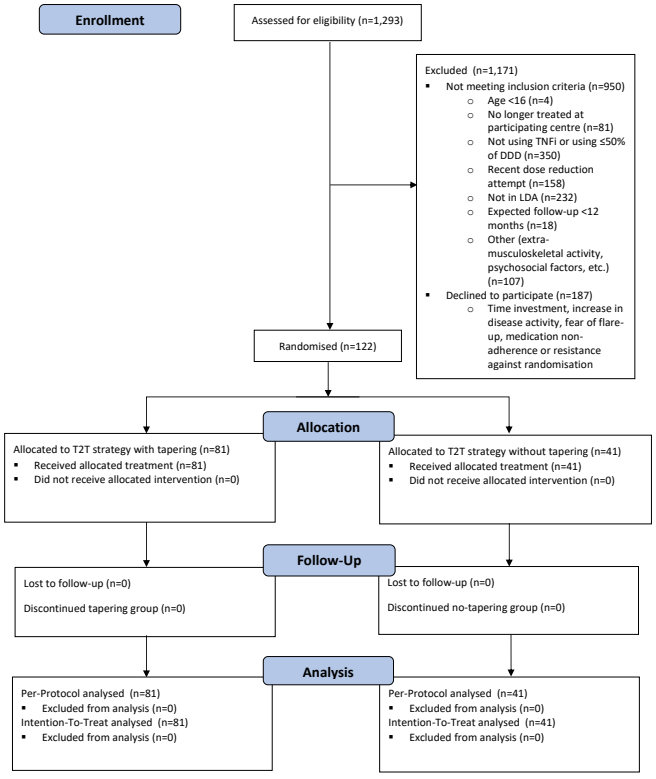


**Figure 1** Flow diagram regarding enrolment, randomisation to a T2T strategy with or without tapering, follow-up and per-protocol and intention-to-treat analyses of patients with PsA and axSpA in the DRESS-PS study. axSpA, axial spondyloarthritis; DDD, daily defined dose; DRESS-PS, Dose REduction Strategy Study in Psoriatic arthritis and axial Spondylartritis; LDA, low disease activity; PsA, psoriatic arthritis; TNFi, tumour necrosis factor inhibitors; T2T, treat-to-target.

0 (0%) (p=0.48) (oral) for axSpA. The cumulative incidence of flare was 85% in the tapering and 78% in the no-tapering group (p=0.32). At 12 months, of the patients in the tapering group, 58/81 (72%) patients remained tapered, of whom 23/58 (28%

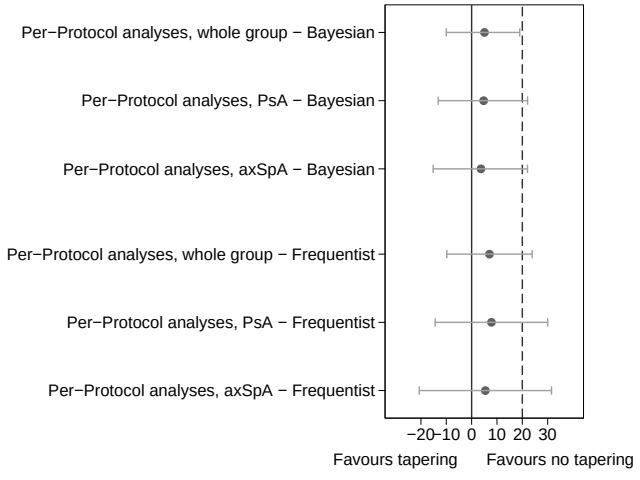


**Figure 2** Difference in proportion of LDA according to Bayesian and frequentist per-protocol analyses with a non-inferiority margin of 20%. Differences in proportion of LDA are reported with point estimates and the corresponding 95% CIs. The dotted line represents the non-inferiority margin of 20% (see online supplemental table 1). axSpA, axial spondyloarthritis; LDA, low disease activity; PsA, psoriatic arthritis.

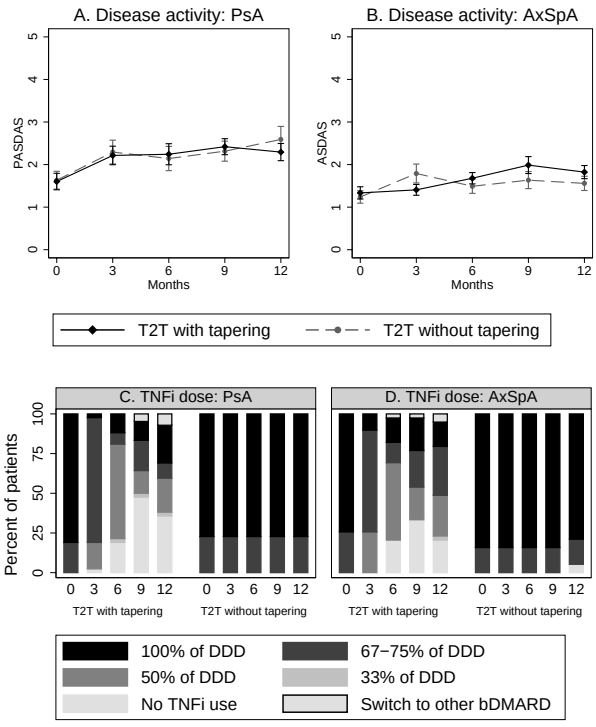


**Figure 3** Mean disease activity and %DDD of T2T strategy treated patients with PsA (A and C) and axSpA (B and D) with or without tapering at baseline, 3, 6, 9 and 12 months (per-protocol/intention-to-treat population). Disease activity was measured by the PASDAS for PsA and ASDAS for axSpA. The disease activity is displayed as a mean with their corresponding 95% CI. Both the disease activity and percent of patients with their corresponding %DDD are displayed at each time point. ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial spondyloarthritis; bDMARD, biological disease modifying anti-rheumatic drug; %DDD, percentage of daily defined dose; PASDAS, Psoriatic Arthritis Disease Activity Score; PsA, psoriatic arthritis; TNFi, tumour necrosis factor inhibitors; T2T, treat-to-target.

of the total group) were able to discontinue their TNFi. Another 23/81 (28%) of the patients could not taper of whom 18/23 (22% of the total group) were reinstalled on 100% of their TNFi dose and 5/23 (6% of the total group) patients switched their TNFi to another bDMARD due to AEs (N=1) or loss of LDA (N=4). In the no-tapering group, one patient discontinued TNFi therapy due to adverse events and did not switch to another bDMARD.

### Safety

For SAEs similar results were seen between both groups, with the occurrence of nine SAEs in total (table 3 and online supplemental tables 9 and 10) and no deaths.

### Function, quality of life and radiographic outcomes

Mean function and quality of life did not differ significantly between both groups at any time point (table 4 and online supplemental table 11 for diseases separately). In PsA, for the tapering group the median SENS was 4 (IQR, 0.75–11) at baseline and 4.25 (IQR, 1.25–13) at follow-up. For the no-tapering group this was respectively, 7.25 (IQR, 2.25–16.25) and 8 (IQR, 2.25–16.75). For the median erosion score and joint narrowing between both groups, see table 5. The SDC was 1.5. The distribution of progression was similar in both groups apart from a few very slightly higher progressors in the tapering group (table 5 and online supplemental figure 1).

## DISCUSSION

Our results indicate that a T2T tapering strategy is an effective and safe alternative to a T2T full dose continuation strategy in patients with PsA and axSpA with stable LDA using TNFi. The strategy resulted in non-inferior disease control, and a sizeable reduction in TNFi use.

Our findings seem to be in line with other studies on T2T tapering strategies with biologicals in different diseases, although outcomes vary, depending on the level of T2T execution and the primary outcome. In the DRESS study in RA, NI was shown for occurrence of major flare and disease activity in patients with RA,[9] although in the smallerSTRASS study tapering showed to be somewhat inferior, possibly due to suboptimal T2T execution.[21] In the psoriasis CONDOR study, NI was demonstrated numerically for the secondary outcome Dermatology Life Quality Index score, but not for the primary outcome Psoriasis Area and Severity Index score.[22] The NI margin for the latter outcomes might well have been too stringent, emphasising the importance for the correct choice of NI margin.

Although the treatments for several inflammatory diseases are similar, differences in ease of monitoring or consequences of flaring influence the feasibility of the T2T strategies. A T2T tapering strategy in psoriasis is conceptually easiest to monitor, assess and treat with visible improvement after treatment adaptation and without risk of damage from this non-scarring disease. T2T tapering strategies in PsA and axSpA seems likewise relatively safe and easy to monitor. In comparison, in IBD these strategies may be much more challenging as monitoring disease activity is harder and consequences of flare may be more severe, potentially causing complications such as fistulas and even bowel surgery.[23]

Strengths of our study include the high internal validity due to our randomised design, inclusion of the intended number of participants with nearly 40% of eligible patients participating in our trial, and good data integrity with no missing data for our primary outcome. Protocol adherence was high, shown by all patients in the tapering group and no patients in the no-tapering group initiating tapering. This also illustrates the acceptability of the treatment strategy for patients and their care providers. The choice for a Bayesian instead of a frequentist approach has had the advantage that adequate precision could be attained with less patients in a smaller time frame, because priors could be based on knowledge from earlier studies in a comparable disease. Frequentist sensitivity analyses showed that the prior did not impact the point-estimate. Lastly, generalisability seems good, as we used broad inclusion criteria, and implemented T2T using readily available measures.[24]

Potential limitations of our study are; first, the open-label nature, potentially causing nocebo effects and incorrect attribution resulting in a perception of a higher disease activity status and flares because of tapering. We expect this should have led to a bias in the conservative direction (towards inferiority), but cannot exclude a bias towards the desired outcome (towards non-inferiority). However, the open nature of our trial is more generalisable, as the communication to patients is more akin to tapering in clinical care. Furthermore, we combined both subtypes of spondyloarthritis, with the risk that the effect of tapering may differ between patients with PsA and axSpA, but sensitivity analyses showed that the effect did not differ between both diseases. Of note, the outcome of NI of the T2T tapering

**Table 3** Occurence of (serious) adverse events with adjusted difference in T2T strategy treated patients with PsA and axSpA with or without tapering

| | T2T strategy with tapering (N=81) | T2T strategy without tapering (N=41) | Incidence rate ratio (IRR) or relative risk (RR) |
|---|---|---|---|
| Any adverse event | | | |
| Number of events: | 176 | 86 | |
| Incidence rate (events/patient-year) (95% CI), IRR | 2.18 (1.88 to 2.53) | 2.09 (1.69 to 2.58) | 1.04 (0.80 to 1.35) |
| Cumulative incidence of adverse events: | 75 | 31 | |
| Number of patients<br>Proportion (95% CI), RR | 0.93 (0.84 to 0.97) | 0.76 (0.60 to 0.87) | 1.22 (1.01 to 1.48) |
| **Serious adverse events** | | | |
| Any serious adverse event | | | |
| Number of events: | 6 | 3 | |
| Incidence rate (events/patient-year) (95% CI), IRR | 0.07 (0.03 to 0.17) | 0.07 (0.02 to 0.23) | 1.02 (0.26 to 4.09) |
| Cumulative incidence of serious adverse events: | 6 | 3 | |
| Number of patients<br>Proportion (95% CI), RR | 0.07 (0.03 to 0.16) | 0.07 (0.02 to 0.21) | 1.02 (0.27 to 3.90) |
| **Adverse events of interest** | | | |
| Any infection | | | |
| Number of events: | 85 | 38 | |
| Incidence rate of any infection (events/patient-year) (95% CI), IRR | 1.05 (0.85 to 1.30) | 0.92 (0.67 to 1.27) | 1.14 (0.78 to 1.67) |
| Cumulative incidence of infections: | 49 | 24 | |
| Number of patients<br>Proportion (95% CI), RR | 0.60 (0.49 to 0.71) | 0.59 (0.42 to 0.73) | 1.04 (0.77 to 1.41) |
| Cumulative incidence of infections (grade ≥2): | 26 | 14 | |
| Number of patients<br>Proportion (95% CI), RR | 0.32 (0.23 to 0.43) | 0.34 (0.21 to 0.50) | 0.93 (0.55 to 1.58) |
| Cumulative incidence of infections (grade 3/4): | 1 | 1 | |
| Number of patients<br>Proportion (95% CI), RR | 0.01 (0.00 to 0.09) | 0.02 (0.00 to 0.17) | 0.54 (0.04 to 7.96) |
| Any injection reaction | | | |
| Number of events: | 9 | 6 | |
| Incidence rate of any injection reaction (events/patient-year) (95% CI), IRR | 0.11 (0.06 to 0.21) | 0.15 (0.07 to 0.32) | 0.77 (0.27 to 2.16) |
| Cumulative incidence of injection reactions: | 9 | 6 | |
| Number of patients<br>Proportion (95% CI), RR | 0.11 (0.06 to 0.20) | 0.15 (0.06 to 0.30) | 0.77 (0.30 to 2.00) |

Comparison of intervention group to control group. Of the total 122 patients, 16 patients did not experience an adverse event from any cause during the study period (intervention: 6 and control: 10). No grade 4 or 5 adverse events or deaths unrelated to adverse events occurred during the study period.
axSpA, axial spondyloarthritis; PsA, psoriatic arthritis; T2T, treat-to-target.

strategy is not only dependent on the percentage of patients that can taper or stop, but mostly on the implementation of the T2T strategy and the effectiveness of increased or restarted dosing on disease activity. We did not anticipate effect modification between the two closely related diseases and this was confirmed in the analyses stratified by disease. The use of SENS, which is intended for RA instead of PsA, also limits the strength of our conclusions of radiographic progression. Another potential limitation is the fact that we based our T2T on a flare definition that has not been formally validated, as validated flare criteria are absent for PsA and axSpA. However, we did use validated disease activity measures to base the flare criteria on. Also, for axSpA we used the previously determined minimally clinically important worsening[18] and interestingly, our 'guesstimated' minimally clinically important worsening for the PASDAS in PsA of 0.9 turned out to be not that far from the recently determined formally minimal important worsening of 0.7.[25]

A final potential limitation would be suboptimal execution of the T2T tapering or continuation strategy which could jeopardise the study conceptually in three ways. First of all, tapering could have been executed too reluctantly, resulting in a NI outcome, but no to low bDMARD dose reduction. The study would then in fact infer true and valid NI, but the tapering strategy would not provide any other benefits, so this NI would be a moot point. In light of the approximately 40% DDD reduction difference between the strategies this is clearly not the case. It remains possible that a more protocolised T2T tapering strategy would have achieved an even higher reduction of TNFi, although then it also might not have reached NI regarding disease activity. Second, tapering could have been executed well, but T2T could have been done suboptimally. This would have resulted in differences in proportion of patients in LDA between the groups, and the strategy would then not be non-inferior. This was however not seen in our data. Third, tapering and T2T could have been done optimally, but result in the exchange of bDMARDs for other medication such as NSAIDs, glucocorticoids or other DMARDs. This would result in a correct claim of NI, but without the associated benefits in medication use. No relevant increase in use of other DMARDS and glucocorticoids were seen in our data. In

**Table 4** Questionnaires of function and health status at baseline, 3, 6, 9 and 12 months in T2T strategy treated patients with PsA and axSpA with (intervention) or without (control) tapering (per-protocol/intention-to-treat population)

| | Baseline | | 3 months | | | 6 months | | | 9 months | | | 12 months | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Function** | **Intervention** | **Control** | **Intervention** | **Control** | **95% CI difference** | **Intervention** | **Control** | **95% CI difference** | **Intervention** | **Control** | **95% CI difference** | **Intervention** | **Control** | **95% CI difference** |
| HAQ-DI | 0.35 (0.47) | 0.39 (0.51) | 0.38 (0.47) | 0.45 (0.54) | (−0.11 to 0.26) | 0.32 (0.44) | 0.47 (0.53) | (−0.03 to 0.33) | 0.42 (0.55) | 0.46 (0.52) | (−0.17 to 0.25) | 0.39 (0.51) | 0.53 (0.58) | (−0.07 to 0.34) |
| BASFI | 2.59 (2.32) | 2.03 (1.86) | 2.61 (2.31) | 2.48 (1.93) | (−1.37 to 1.11) | 2.76 (2.29) | 2.50 (2.05) | (−1.50 to 0.98) | 3.15 (2.58) | 2.58 (2.08) | (−1.93 to 0.79) | 3.01 (2.38) | 2.17 (2.04) | (−2.11 to 0.44) |
| **Health status** | **Intervention** | **Control** | **Intervention** | **Control** | **95% CI difference** | **Intervention** | **Control** | **95% CI difference** | **Intervention** | **Control** | **95% CI difference** | **Intervention** | **Control** | **95% CI difference** |
| EQ-5D-3L | 0.81 (0.13) | 0.81 (0.15) | 0.80 (0.17) | 0.80 (0.16) | (−0.07 to 0.06) | 0.81 (0.14) | 0.80 (0.18) | (−0.07 to 0.05) | 0.81 (0.17) | 0.78 (0.22) | (−0.10 to 0.04) | 0.79 (0.19) | 0.78 (0.19) | (−0.08 to 0.06) |
| SF-12 PCS | 44 (7.5) | 44 (6.6) | 43 (7.1) | 43 (6.7) | (−2.90 to 2.41) | 44 (6.8) | 42 (7.2) | (−4.61 to 0.66) | 44 (7.4) | 42 (6.6) | (−4.03 to 1.47) | 44 (7.0) | 43 (7.0) | (−3.82 to 1.49) |
| SF-12 MCS | 53 (10) | 55 (7.8) | 52 (10) | 52 (9.8) | (−4.15 to 3.39) | 52 (11) | 54 (9.4) | (−2.47 to 5.42) | 53 (9.7) | 53 (8.8) | (−3.74 to 3.46) | 52 (11) | 53 (8.8) | (−3.68 to 4.06) |
| ASAS-HI | 4.70 (3.27) | 3.50 (2.79) | 3.82 (3.02) | 4.56 (2.86) | (−0.96 to 2.46) | 4.72 (3.81) | 5.12 (3.71) | (−1.80 to 2.60) | 4.57 (3.20) | 4.67 (4.19) | (−1.95 to 2.14) | 4.35 (3.47) | 4.83 (3.71) | (−1.54 to 2.51) |

All values are expressed as a mean (SD); Health Assessment Questionnaire-Disability Index (HAQ-DI), ranges from 0 to 3, with higher scores indicating lower functioning. Not all patients fulfilled the HAQ-DI score (T0:1, T9:1, T12:1 missing); Bath Ankylosing Spondylitis Functional Index (BASFI), range 0–10 with a higher score indicating a higher degree of functional limitations. The BASFI was only assessed for patients with axSpA (intervention: 39 and control: 19), not all patients fulfilled the BASFI (T0:1, T3:2 missing); EuroQol five-dimension scale with three levels (EQ-5D-3L) ranges from 0 to 1, with a with higher scores indicating a better health status. Of the EQ-5D-3L score, not all patients fulfilled the EQ-5D-3L score (T0:1, T9:1 missing); Short Form Health Survey 12 (SF-12) consist of a physical and mental component score (PCS and MCS), range 0–100 for each component score with a higher score indicating better health status. Not all patients completed the SF-12 (T0:2, T9:1 missing); Assessment of SpondyloArthritis International Society-Health Index (ASAS-HI) ranges from 0 to 17, with a higher score indicating lower health status. The ASAS-HI was only assessed for patients with axSpA (intervention: 39 and control: 19), of the in total 58 patients with axSpA, not all patients fulfilled the ASAS-HI (T0:2, T3:3, T6:2, T9:3, T12: 1 missing).
axSpA, axial SpondyloArthritis; PsA, Psoriatic Arthritis; T2T, Treat-to-Target.

**Table 5** Radiographic outcomes in T2T strategy treated patients with PsA with or without tapering

| | T2T with tapering (N=42) | T2T without tapering (N=22) | P value |
|---|---|---|---|
| Progression >SDC (1.54), n (%) | 5 (13) | 2 (10) | 0.78 |
| Progression >0.5, n (%) | 17 (43) | 7 (35) | 0.58 |
| Mean progression, mean (SD) | 0.8 (1.4) | 0.52 (0.82) | 0.33* |
| Median progression, median (IQR) | 0.5 (0–1) | 0.5 (0–1) | 0.77† |

Not all patients had complete radiographs (intervention: 2 and control: 2 missing at 12 months).
*Welch T-test.
†Wilcoxon rank-sum test.
PsA, psoriatic arthritis; SDC, smallest detectable change; T2T, treat-to-target.

addition, NSAID increase was much lower than the bDMARD decrease and often temporary.

We chose the PASDAS as our disease activity measurement tool for PsA because first it is a continuous composite disease index with parametric distribution that best fitted our study design. Also, it contains almost all domains necessary, and has sufficiently been validated. It has the advantage over, for example, MDA criteria that it is a continuous outcome, and that different thresholds can be used. However, this measure has some drawbacks such as the inclusion of the functional (dis)ability domain (SF-12) which is different from the construct of actual disease activity.[26] This makes it prone to overestimating disease activity, since functional ability can also be affected by many other factors. In addition, the SF-12 requires an annual license fee, which makes it less suited to use in clinical practice. Finally, the calculation of the PASDAS is quite cumbersome, which could be more problematic for usage in clinical practice where parametric distribution is less important. Indeed, other composite indices than the PASDAS are available, such as the Disease Activity in Psoriatic Arthritis, MDA criteria, the Composite Psoriatic Disease Activity Index, the Arithmetic Mean of the Desirability Function and the GRAppa Composite scorE project, but they have their specific drawbacks also. However, all things considered, the required variables for all composite disease indices are largely comparable, therefore no major difference in workload is to be expected and so far no other studies compared the validity of T2T for any proposed composite disease indices in PsA, in an RCT or clinical care. The PASDAS has proven to be feasible both as T2T instrument as well as primary trial outcome. A final study limitation could be the limited follow-up period. We do think 12 months follow-up is sufficient to capture (primary and second order) effects of tapering, however, we anticipate an observational extension study to provide more insights in the long-term effects of this T2T strategy.

## CONCLUSION

In conclusion, our study shows that a stepwise T2T strategy with tapering is non-inferior to a T2T strategy without tapering with regard to maintenance of LDA at 12 months in PsA and axSpA. Furthermore, TNFi use was strongly reduced, as the majority of patients were able to maintain LDA with a lower dose, and about a quarter were able to discontinue their TNFi. Implementing T2T tapering strategies into practice will reduce TNFi use, and thereby potentially AEs, patient burden, and costs.

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**Competing interests** DvdH has received consulting fees from AbbVie, Bayer, BMS, Cyxone, Eisai, Galapagos, Gilead, GlaxoSmithKline, Janssen, Lilly, Novartis, Pfizer and UCB Pharma and is director of Imaging Rheumatology. AAdB has received research grants (to the institution) from AbbVie, Lilly, Novartis, Sanofi, Galapagos and Gilead. The remaining authors have declared no conflicts of interest.

**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 Commissie Mensgebonden Onderzoek Regio Arnhem-Nijmegen. File number: 2018-4538. NL-number: NL66181.091.18. Participants gave informed consent to participate in the study before taking part.

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TRANSLATIONAL SCIENCE

# Keratinocyte-derived S100A9 modulates neutrophil infiltration and affects psoriasis-like skin and joint disease

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## ABSTRACT

**Objectives** S100A9, an alarmin that can form calprotectin (CP) heterodimers with S100A8, is mainly produced by keratinocytes and innate immune cells. The contribution of keratinocyte-derived S100A9 to psoriasis (Ps) and psoriatic arthritis (PsA) was evaluated using mouse models, and the potential usefulness of S100A9 as a Ps/PsA biomarker was assessed in patient samples.

**Methods** Conditional S100A9 mice were crossed with DKO* mice, an established psoriasis-like mouse model based on inducible epidermal deletion of c-Jun and JunB to achieve additional epidermal deletion of S100A9 (TKO* mice). Psoriatic skin and joint disease were evaluated in DKO* and TKO* by histology, microCT, RNA and proteomic analyses. Furthermore, S100A9 expression was analysed in skin, serum and synovial fluid samples of patients with Ps and PsA.

**Results** Compared with DKO* littermates, TKO* mice displayed enhanced skin disease severity, PsA incidence and neutrophil infiltration. Altered epidermal expression of selective pro-inflammatory genes and pathways, increased epidermal phosphorylation of STAT3 and higher circulating TNFα were observed in TKO* mice. In humans, synovial S100A9 levels were higher than the respective serum levels. Importantly, patients with PsA had significantly higher serum concentrations of S100A9, CP, VEGF, IL-6 and TNFα compared with patients with only Ps, but only S100A9 and CP could efficiently discriminate healthy individuals, patients with Ps and patients with PsA.

**Conclusions** Keratinocyte-derived S100A9 plays a regulatory role in psoriatic skin and joint disease. In humans, S100A9/CP is a promising marker that could help in identifying patients with Ps at risk of developing PsA.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ S100A9 is an alarmin that forms calprotectin (CP) heterodimers with S100A8, which is highly expressed in inflammatory skin diseases, such as psoriasis (Ps), but how S100A9-expressing keratinocytes contribute to Ps, and whether these affect psoriatic arthritis (PsA), is still unknown.

### WHAT THIS STUDY ADDS

⇒ First-time investigation of the role of epidermal-derived S100A9 in vivo using genetically modified mouse models, providing a better appreciation of S100A9 complexes in cells, tissues and the whole organism.

⇒ Demonstration that epidermal-specific inactivation of S100A9 increases Ps-like severity and PsA incidence, suggesting that keratinocyte-derived S100A9 is protective in chronic skin and joint inflammation.

⇒ Side-by-side evaluation of circulating S100A8, S100A9 and CP (S100A8/S100A9) in patients with Ps and PsA.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study indicates that targeting S100 proteins might help treat skin and joint inflammation. Testing whether circulating S100A9/CP could help in identifying patients with Ps at risk of developing PsA in larger and appropriately designed studies is crucial to improve disease outcomes, prevent disability and reduce healthcare and societal costs.

## INTRODUCTION

Psoriatic disease develops from a complex cross-talk between proliferating keratinocytes and infiltrating immune cells that leads to secretion of various cytokines and chemokines including IL-17, IL-21, IL-22, IL-6, IL-1β, TNFα and CXCL1/3/5, which initiate a pro-inflammatory systemic response.[1] In 30% to 40% of patients with psoriasis (Ps), the disease is complicated by psoriatic arthritis (PsA).[2] In 75% of these cases, skin involvement precedes joint inflammation, usually with a gap of 5–10 years between the appearance of psoriatic plaques and the first signs of arthritis.[2 3] Early PsA diagnosis and intervention are critical to avoid joint damage that leads to impaired physical function, fatigue, depression and poor quality of life.[4–6] The most common therapies for psoriatic disease target several cytokines such as TNFα, IL-12/23- and IL-17A, with usually better efficacy on the skin than on the joint manifestations.[7] Little is known about the mediators involved in PsA development. Therefore, there is unmet need for novel diagnostic markers unique to PsA and for identifying the molecular determinants of skin–joint crosstalk.[6 8]



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Genetically engineered mouse models (GEMMs) with inducible epidermal deletion of c-Jun and JunB (DKO*) revealed the function and/or therapeutic potential of distinct Ps/PsA mediators, such as TNFR signalling,[9] S100A9,[10] VEGF[11] and thymic stromal lymphopoietin[12] as well as microRNAs[13] and amygdalin analogues.[14] These DKO* mice present a psoriasis-like inflammatory skin disease with articular changes strongly resembling PsA.[9] Thus, skin-specific genetic interventions can trigger PsA-like disease.

S100A8 and S100A9 are two calcium binding proteins upregulated in inflammatory conditions that form homodimers or S100A8/A9 heterodimers termed calprotectin (CP).[10 15–17] During skin inflammation, the main S100A9 expressing cells are keratinocytes, neutrophils and macrophages.[18 19] S100A9 accounts for up to 40% of cytosolic proteins in neutrophils,[20] acts intracellularly by modulating the cytoskeleton, and extracellularly by recruiting other immune cells to inflammation sites and upregulating pro-inflammatory cytokines.[19 21] CP has important anti-microbial properties[22] and faecal CP is a validated clinical biomarker for gut inflammation.[23]

We previously reported that global S100A9 inactivation alleviated skin and joint inflammation in the DKO* mouse model.[10] How S100A9-expressing keratinocytes contribute to Ps, and whether these affect PsA development, is still unknown. Here, we crossed a newly generated S100A9 floxed allele into the DKO* model to inactivate S100A9 only in epidermal cells. We tested whether and how epidermal expression of S100A9 influences Ps-like and PsA-like disease and whether circulating S100A9, S100A8 and CP could be used as markers for PsA.

## METHODS

Materials and methods are described in the online supplemental file.

## RESULTS

### Role for epidermal S100A9 in psoriasis-like disease

DKO* mice were crossed with S100A9 floxed mice to generate a new GEMM with inducible triple epidermal deletion of c-Jun, JunB and S100A9 (**TKO***). Deletion of the floxed alleles in keratin 5–positive basal epidermal cells of adult DKO* and TKO* mice was achieved by intraperitoneal tamoxifen injections (online supplemental figure S1A). Ps-like and PsA-like disease developed in both DKO* and TKO* mice within 2 weeks after the last injection (online supplemental figures S1B and S2A). S100A9 deletion in TKO* was first assessed by immunofluorescence (figure 1A). Ear sections from DKO*-S100A9$^{-/-}$ mice[10] were used to confirm antibody specificity and JunB immunofluorescence included for comparison. As previously reported,[10 12] JunB expression in DKO* epidermis is patchy, while S100A9 is readily detectable in all lesional keratinocytes and in dermis-infiltrating immune cells. In TKO* mice, S100A9 expression appeared similar to that of JunB with a mosaic staining pattern, while dermis-infiltrating cells still expressed S100A9 (figure 1A). In TKO* mice, S100A9 was expressed in less than 18% of lesional epidermis in ears (figure 1B).

### Absence of epidermal S100A9 leads to more severe psoriatic skin disease

A macroscopic Ps-like classification was established as a function of ear inflammation/plaques and ventral skin inflammation (online supplemental figure S1B). In DKO* mice, the extent of weight loss and serum IL-17A and S100A9 levels correlated with skin disease severity (online supplemental figure S1C-E). Importantly, the skin of TKO* mice was overall more severely affected than DKO* (figure 1C), suggesting that keratinocyte-derived S100A9 inhibits severe skin inflammation.

While weight loss was similar between the two groups (figure 1D), DKO* and TKO* mice with moderate to severe skin phenotype had higher circulating S100A8 and S100A9 and similar CP (S100A8/A9), when compared with wild-type littermates (figure 1E–G). However, there was no difference between the two groups indicating that keratinocytes are not the major contributor to serum S100A9-containing dimers (figure 1E–G). Psoriasis-associated cytokines IL-17A, IL-6 and TNFα were also elevated in DKO* and TKO* sera compared with controls, but only TNFα was higher in TKO* (figure 1H–J). These data suggest that simultaneous inactivation of c-Jun, JunB and S100A9 in epithelial cells leads to an increase in Ps-like skin disease severity.

### Absence of epidermal S100A9 leads to more severe psoriatic arthritis

Next, DKO* and TKO* mice with severe skin phenotype were macroscopically scored for signs of nail and entheseal involvement resembling PsA. While macroscopic swelling in the distal interphalangeal (DIP) joints appeared comparable between DKO* and TKO* (online supplemental figure S2A), a significant increase in PsA prevalence was observed in TKO* mice (figure 2A). Cartilage degradation was assessed by toluidine blue staining in the third DIP joint of the right hind limb. Compared with controls, decreased proteoglycan was observed in the articular region, but the extent of proteoglycan loss was comparable between DKO* and TKO* (figure 2B,C). Histological evaluation revealed extensive nail disease (figure 2D), enthesitis in the distal phalanx (figure 2E) and bone marrow osteitis in the distal phalanx (figure 2F) in both DKO* and TKO* mice. As PsA is associated with bone loss, we next quantified bone in the hind limbs using radiography and micro-CT (online supplemental figure S2B–I). Bone loss was apparent in both DKO* and TKO* mice, but not in controls, and was consistent with increased in serum IL-17.[24] However, bone loss was not different in DKO* and TKO* mice (online supplemental figure S2B–I). Overall, these data indicate that keratinocyte-derived S100A9 decreases the incidence of PsA in the context of severe Ps-like skin disease, but does not affect the severity of PsA once it develops.

### Epidermal S100A9 modulates neutrophil recruitment to inflammatory sites

Immunofluorescence co-staining of S100A9 and Ly6B, a surface marker expressed by neutrophils, inflammatory monocytes and some activated macrophages, was performed on whole ear sections from WT, DKO* and TKO* mice (figure 3A). Computer-assisted quantification was performed after digital removal of autofluorescent cartilage areas and neutrophil-rich, but difficult to quantify Munro micro-abscesses (online supplemental figure S3A). Ly6B+ cells were increased in DKO* skin sections compared with wild-type littermates and further significantly increased in TKO* skin sections (figure 3B). Around 50% of Ly6B+ cells also expressed S100A9 and a similar difference of 2–3 folds in absolute numbers was observed between DKO* and TKO* when considering Ly6B/S100A9 double-positive cells (figure 3C). Abundant S100A9+Ly6B+ myeloid cells were also observed in the inflamed joints of DKO* and TKO* mice (figure 3D–F). While synovial neutrophils were similarly increased in DKO* and TKO*, S100A9-positive neutrophils were more abundant in TKO* than DKO* mice (figure 3E–F). Interestingly, both Ly6B-positive and Ly6B/S100A9-double positive cells were increased in the bone marrow of TKO* relative to DKO* (online supplemental figure S3B–D), pointing to a possible contribution of

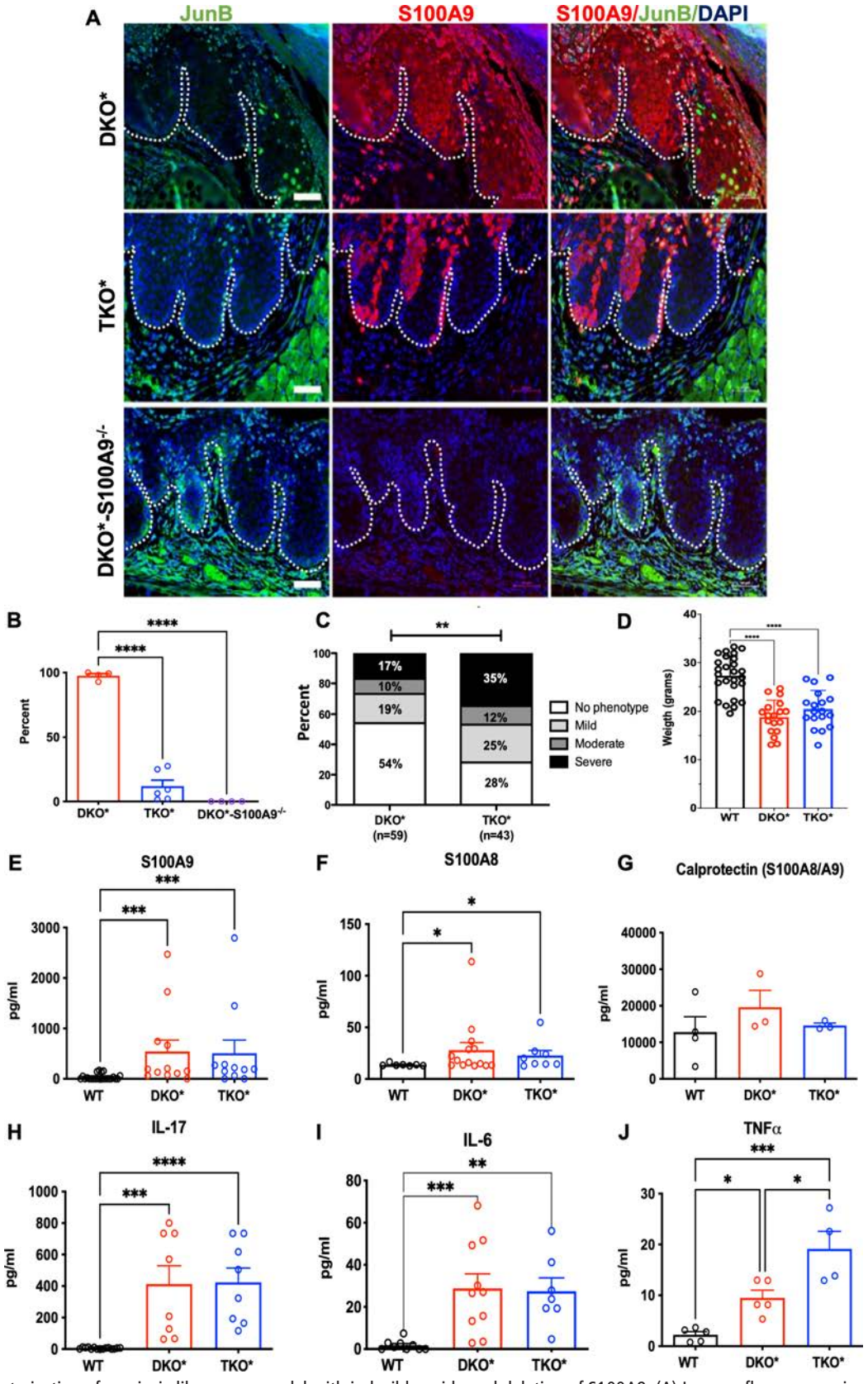


**Figure 1** Characterisation of psoriasis-like mouse model with inducible epidermal deletion of S100A9. (A) Immunofluorescence images of the ear skin of mice with inducible dual epidermal deletion of c-Jun and junB (DKO*), inducible triple epidermal deletion of c-Jun, JunB and S100A9 (TKO*) and DKO* mice with total deletion of S100A9 (DKO*-S100A9$^{-/-}$) 23 days after first tamoxifen (TAM) injection (red: S100A9, green: JunB, scale bar=50 µm). (B) Quantification of S100A9-positive epidermal cells in the ear of DKO*, TKO* and DKO*-S100A9$^{-/-}$ mice (n=4–6). (C) Skin disease severity scoring in DKO* and TKO* mice. (D) Weight of control wild-type (WT), DKO* and TKO* with moderate/severe skin phenotype 23 days after first TAM injection (n=18–26). (E–J) S100A9 (E), S100A8 (F), calprotectin (G), IL-17A (H), IL-6 (I) and TNFα (J) concentrations in the sera of WT, DKO* and TKO* mice with moderate-severe psoriasis-like phenotype (n=4–13).

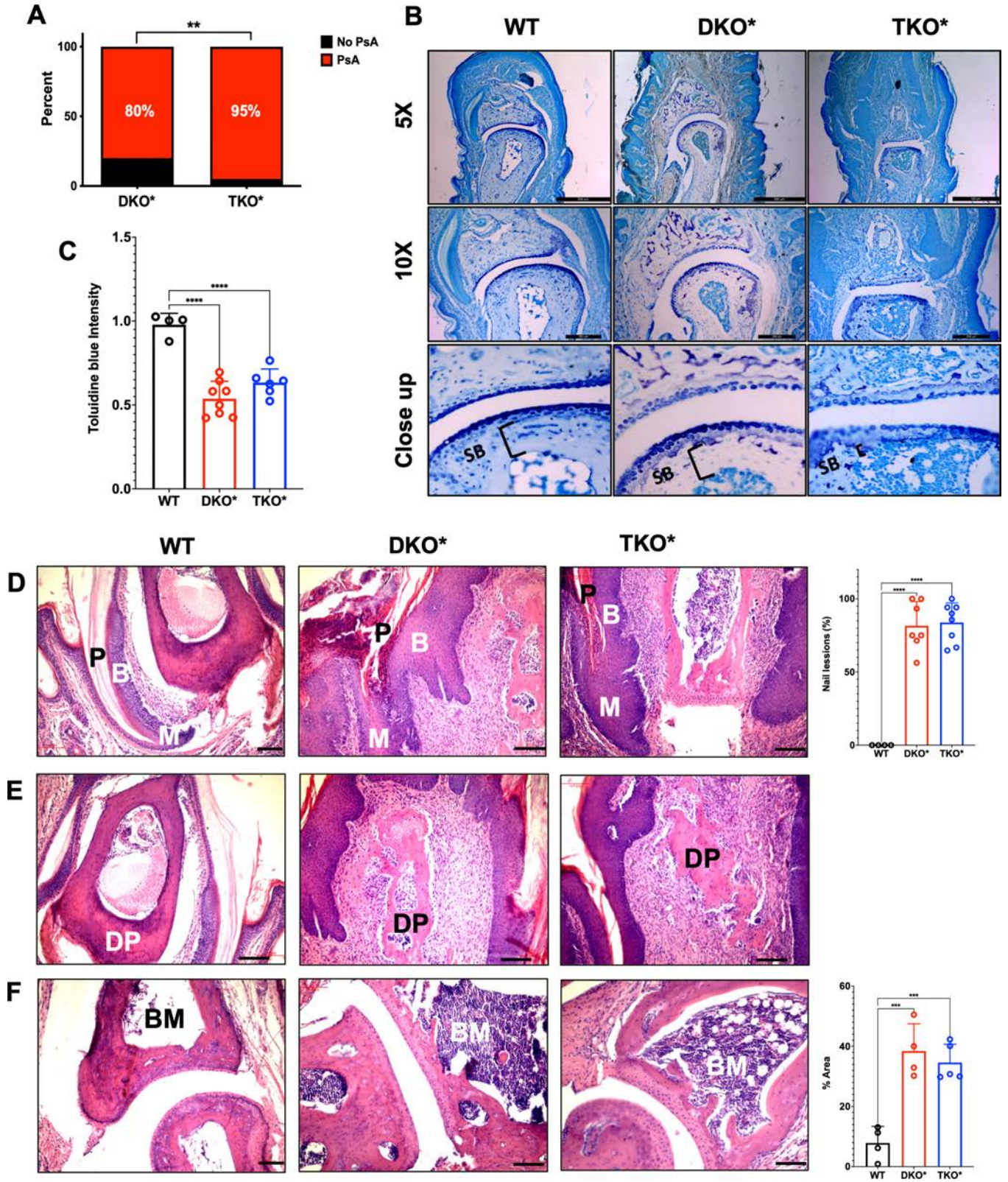


**Figure 2** Psoriatic-arthritic-like (PsA) phenotype in mice with severe psoriasis-like disease. (A) Prevalence of psoriatic arthritis (PsA) in mice with inducible dual epidermal deletion of c-Jun and junB (DKO*) and triple epidermal deletion of c-Jun, JunB and S100A9 (TKO*) with severe psoriasis-like disease (n=20 per group). (B) Toluidine blue staining of the distal interphalangeal (DIP) joint of control wild-type (WT), DKO* and TKO* mice with PsA (scale bar=200 µm). (C) Quantification of toluidine blue staining intensity of articular cartilage in WT, DKO* and TKO* mice (WT n=4; DKO* n=8; TKO* n=6; each point represents the median of several joints measured per sample). (D) H&E-stained histological images showing psoriatic nail involvement with changes in the nail plate (P), nail matrix (M) and nail bed (B) of DKO* and TKO* mice and quantification of nail lesions (scale bar=100 µm). (E) H&E histological images of the distal phalanx (DP) showing enthesitis with high immune infiltration in the areas around the bone (scale bar=100 µm). (F) H&E histological images showing osteitis of the bone marrow (BM) of the distal phalanx (DP) in DKO* and TKO* mice (BM=bone marrow) with quantification of per cent area of bone marrow covered by inflammation (scale bar=100 µm).

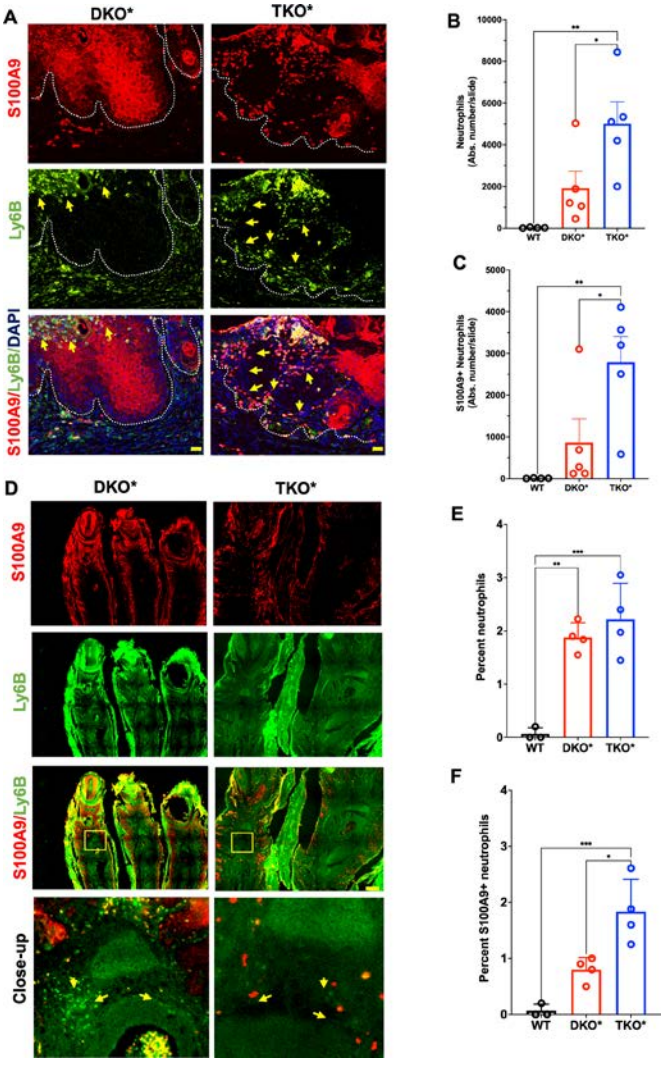


**Figure 3** Neutrophil infiltration in mice with severe psoriasis-like disease. (A) S100A9 (red) and Ly6B (neutrophils; green) immunostaining of lesional ears in mice with inducible dual epidermal deletion of c-Jun and junB (DKO*) and triple epidermal deletion of c-Jun, JunB and S100A9 (TKO*) (dotted lines represent the basal membrane, yellow arrows point to Ly6B/S100A9-double positive cells, scale bar=20 µm). (B–C) Confocal microscopy–based quantification of absolute number of neutrophils (Ly6B-positive) (B) and S100A9-positive neutrophils (C) in the whole ear sections of DKO* and TKO* mice and wild-type (WT) littermates (n=4–5 mice). (D) S100A9 (red) and Ly6B (green) immunostaining of lesional psoriatic arthritis (PsA)–like paws (scale bar=200 µm). Yellow arrows point to infiltrating cells. (E–F) Confocal microscopy–based quantification of neutrophil (Ly6B-positive) (E) and S100A9-positive neutrophil (F) in the distal interphalangeal joints of DKO* and TKO* mice and WT littermates (average of 3–5 regions per paw, n=4 mice).

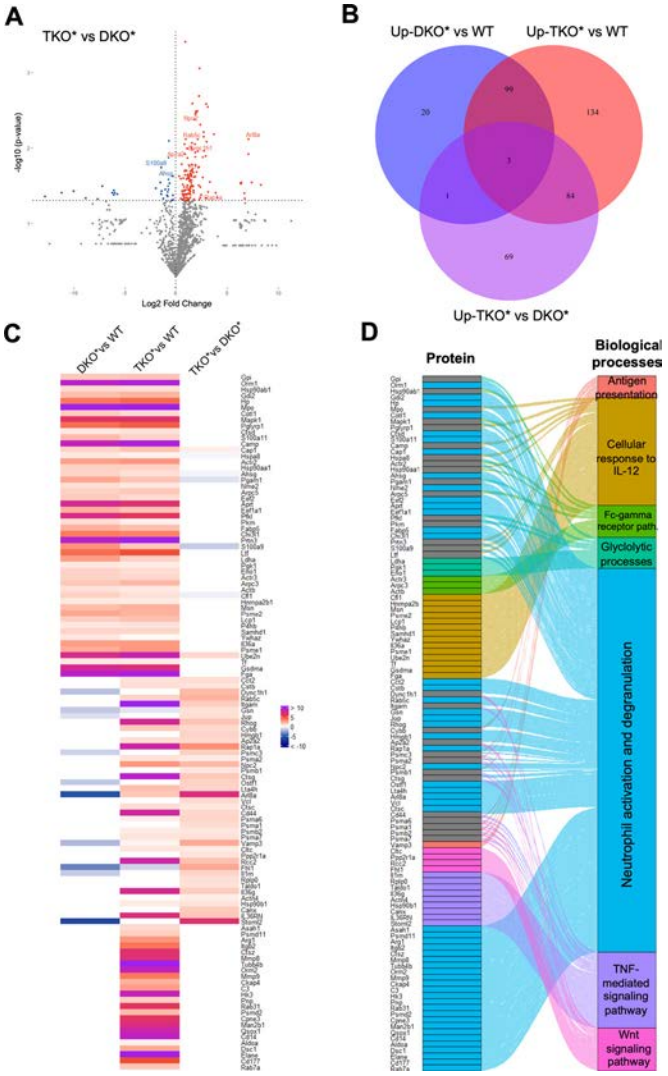


**Figure 4** Proteomic analyses in whole ear extracts of DKO* and TKO* mice. (A) Volcano plot showing upregulated (red) and downregulated (blue) proteins (n=3–5 per condition; p<0.05); proteins in grey are below statistical significance. (B) Venn diagram depicting statistically significant upregulated proteins in (I) mice with inducible dual epidermal deletion of c-Jun and junB (DKO*) compared towild-type (WT) controls, (II) mice with triple epidermal deletion of c-Jun, JunB and S100A9 (TKO*) compared toWT mice and (III) TKO* compared to DKO* mice (n=3–5 per condition; p<0.05). (C) Heat map of significantly upregulated proteins (left) and Alluvial plot (right) of enriched biological processes associated to each protein.

these cells to increased PsA incidence in TKO*. These data suggest that S100A9-expressing neutrophils infiltrate skin and joints in DKO* and TKO* mice and that increased neutrophils might potentiate skin and joint disease in TKO* mice.

### Epidermal S100A9 affects neutrophil-related proteins and pathways in skin

Mass spectrometry–based proteomics of whole ear lysates was next performed and volcano plots identified statistically significant proteins upregulated and downregulated in TKO* and DKO* mice, when compared with each other (figure 4A) or to control mice (online supplemental figure S4A,B). Venn diagrams identifiedproteins uniquely upregulated in each comparison as well as shared proteins (figure 4B). Enrichment analyses identified the most relevant pathways in each comparison and peptides statistically significant in at least one comparison were displayed in a heatmap, grouped by Gene Ontology terms and connected to their respective biological processes in an Alluvial plot (figure 4C). All these analyses revealed a largely predominant neutrophil activation signature in TKO* mice, consistent with our histological observations. TNF and Wnt signalling, which are involved in inflammation and aberrant bone formation, respectively, were also enhanced in TKO* mice, while other biological processes such as cellular response to IL-12 were similarly enriched in DKO* and TKO*. A connectivity network further confirmed the relevance of neutrophil granulation, innate immunity

and TNF-mediated signalling in TKO* skin proteome with 76, 22 and 16 nodes, respectively (online supplemental figure S4C).

### Epidermal S100A9 modulates cytokine and chemokine expression during skin inflammation

Ear epidermis was isolated from DKO* and TKO* mice and littermate controls, dissociated and subjected to FACS analysis and sorting (online supplemental figure S5A). A similar increase in CD45+ immune cells was observed in DKO* and TKO* epidermal samples (online supplemental figure S5B,C) when compared with controls. Ly6G/CD11b double-positive neutrophils were higher in TKO* than DKO*, although not reaching statistical significance (online supplemental figure S5B-D). This finding indicates that when including Munro's microabscesses, the overall number of immune cells and neutrophils in the epidermal area is comparable between the two genotypes. FACS-sorted neutrophils (figure 5A–F) and keratinocytes (figure 5G–L) were next analysed for cytokine and chemokine expression. A similar increase in *s100a9* and *s100a8* mRNA was observed in neutrophils isolated from DKO* and TKO* mice, compared with controls (figure 5A,B). *il-1b*, *il-6* and *tnf-a* mRNA

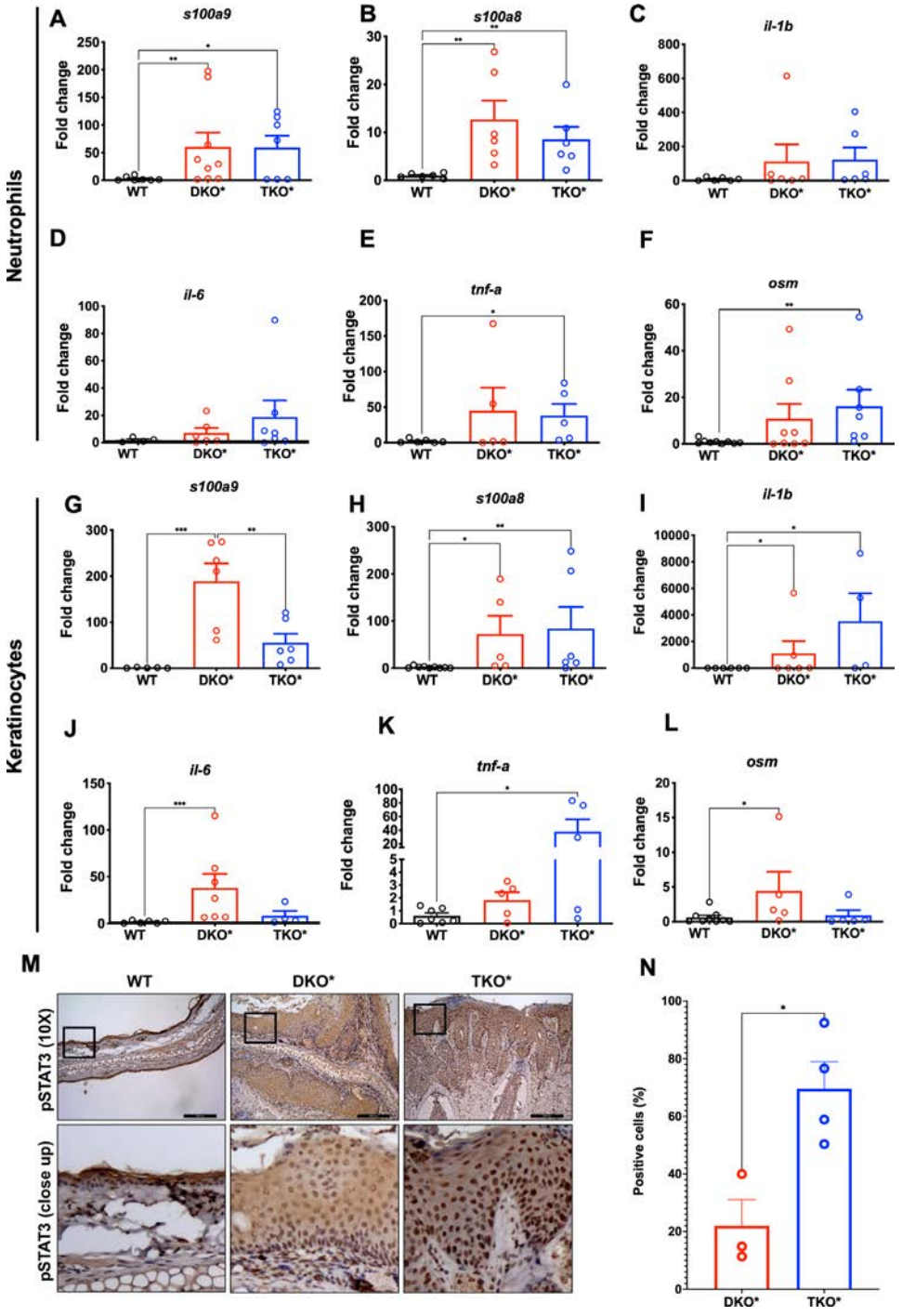


**Figure 5** Gene expression in FACS-sorted neutrophils and keratinocytes qRT-PCR analysis in (A–F) neutrophils and in (G–L) keratinocytes from the ears of mice with inducible dual epidermal deletion of c-Jun and junB (DKO*) and triple epidermal deletion of c-Jun, JunB and S100A9 (TKO*) with severe psoriasis-like phenotype (n=4–9). (M) pSTAT3 immunohistochemistry in the ears of DKO* and TKO* mice with severe psoriasis-like phenotype (scale bar=100 µm). (N) Quantification of positive pSTAT3 cells in the epidermis (n=3–4, 4–5 fields per slide).

were similarly increased in neutrophils isolated from DKO* and TKO* mice, although most changes were not statistically significant (figure 5C–E). Interestingly, mRNA expression of *osm*, encoding the IL-6 family cytokine Oncostatin-M that induces psoriasis-like lesions in mice,[25] appeared increased in neutrophils isolated from TKO* mice (figure 5F). Overall, neutrophils isolated from DKO* and TKO* epidermis display a similar pro-inflammatory mRNA expression profile.

In contrast, *s100a9* mRNA was significantly less induced in keratinocytes isolated from TKO* compared with DKO* (figure 5G), while the increase in *s100a8* was comparable (figure 5H). Interestingly, while *il-1b* was similarly increased in DKO* and TKO* keratinocytes, *il-6* and *osm* mRNA in TKO* were comparable with controls and *tnf-a* higher (figure 5I–L), suggesting that keratinocyte-derived *s100a9* affects epidermal expression of cytokines, such as *il-6* and *tnf-a*, during skin inflammation. STAT3 is an important transcription factor downstream of IL-6 and Oncostatin-M, involved in Ps and other inflammatory diseases.[26–28] DKO* and TKO* lesional skin displayed nuclear phosphorylated STAT3 expression, indicating activated JAK/STAT signalling (figure 5M). Importantly, pSTAT3 was higher in TKO* epidermis compared with DKO*, consistent with more severe skin phenotype (figure 5N). Altogether, keratinocyte-derived S100A9 likely modulates epidermal expression of genes potentiating JAK/STAT signalling and skin inflammation, while it reduces local and systemic TNFα production important for joint inflammation.

### S100A9 and CP but not S100A8 are potential Ps and PsA markers in humans

To translate these findings to human disease, we assessed the expression of S100A9 and S100A8 in lesional skin of patients with Ps and measured serum and synovial fluid levels of S100A9, S100A8, CP, IL-17, TNFα, IL-6, VEGF and LCN2 in patients with Ps, patients with PsA and healthy controls (HC). Consistent with increased CP in Ps lesional skin, S100A8 and S100A9 were elevated in hyperproliferating keratinocytes and infiltrating immune cells of psoriatic plaques, while both proteins were low to undetectable in skin from healthy individuals (figure 6A).

Analysis of matched serum and synovial fluid samples from patients with PsA showed overall comparable concentrations between the two biological samples, except for S100A9, IL-17, IL-6 and VEGF that were higher in synovial fluid (figure 6B, online supplemental figure S6A), possibly due to accumulation of immune cells, such as S100A9-expressing neutrophils in the joints. ELISA analyses of sera from a larger cohort of HC, patients with Ps and those with PsA revealed a reasonable correlation between circulating CP or S100A9 homodimers and disease activity scores in patients with Ps and PsA (online supplemental figure S6B). Importantly, S100A9 homodimers and CP were significantly increased in patients with PsA, when compared with Ps and to HC (figure 6C). Furthermore, levels of S100A9 homodimers and CP were increased in patients with Ps compared with HC while no significant differences between groups were observed for S100A8 homodimers (figure 6C). In comparison, while serum IL-17 and LCN2 were higher in patients with Ps and PsA, these could not discriminate between the two groups and high TNFα, IL-6 and VEGF would identify PsA but not Ps (figure 6C, online supplemental figure S6CS6C). These data suggest that S100A9 is a critical mediator in psoriatic skin and joint disease and that S100A9/CP may serve as markers to identify patients with Ps who develop PsA.

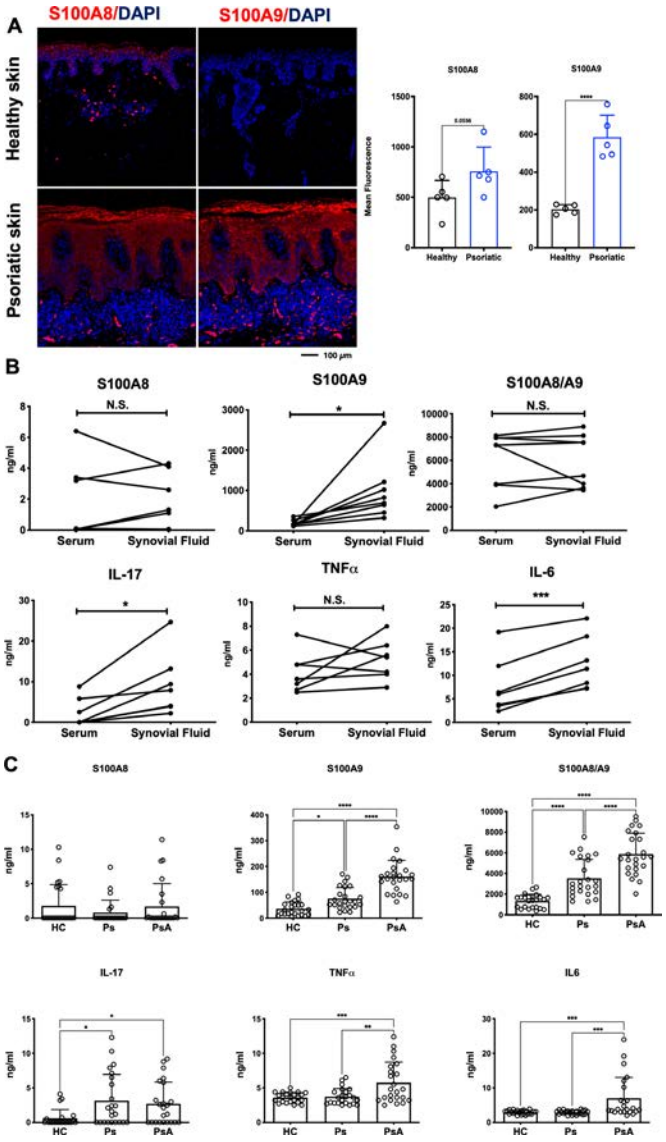


**Figure 6** Analyses in psoriatic patient samples. (A) S100A8 and S100A9 (red) immunofluorescence staining and quantification in human healthy skin and psoriatic skin (n=5, scale bar=100 µm). (B) S100A8, S100A9, S100A8/A9 (Calprotectin), Interleukin (IL)–17, Tumour necrosis factor alpha (TNF-α) and Interleukin (IL)–6 levels in the serum and synovial fluid of patients with psoriatic arthritis (PsA; n=8, ns=non-significant). (C) S100A8, S100A9, S100A8/A9 (Calprotectin), IL-17, TNF-α and IL-6 serum levels of healthy controls (HC), patients with psoriasis (Ps) or patients with PsA (each group n=24).

## DISCUSSION

Epithelial homeostasis is critical for the regulation of inflammation. Here, we show that epithelial expression of the S100A9 alarmin plays a critical role in the severity of psoriatic skin disease and its spreading to the joints. Several studies investigated the role the CP-forming alarmins S100A8 and S100A9 in Ps models. Both proteins are upregulated in the imiquimod (IMQ) model of psoriasiform skin inflammation[15 29] and in GEMMs for psoriasis-like disease, such as the DKO*,[9] CARD14$^{E138A}$ knock-in,[30] K14-Vegfa,[31] K14-IL-17A,[32] K14-IL-23[33] and K5-Stat3C[34] bi-transgenics, but functional studies where S100A8 or S100A9 are inactivated in Ps models are still scarce. While one study reported enhanced IMQ-induced skin hyperplasia in S100A8$^{-/-}$ and to a lesser extent in S100A9$^{-/-}$ mice,[15] we observed decreased skin thickening in IMQ-treated S100a9$^{-/-}$ mice that was consistent with ameliorated skin and joint disease on global inactivation of S100A9 in the DKO* genetic model.[10] While the contradictory results using IMQ could

be attributed to different genetic backgrounds and time points of analyses, the complex function of S100 proteins emphasises the need to evaluate cell-specific roles of S100A8, S100A9 and their complexes and to extend the analyses beyond the skin.[23]

In skin inflammation, S100A9-expressing cells are keratinocytes, neutrophils and macrophages. DKO* mice reconstituted with S100A9$^{-/-}$ bone marrow displayed reduced skin thickening, while transplanting S100A9-proficent bone marrow into DKO*-S100A9$^{-/-}$ mice did not worsen the disease,[10] indicating that keratinocytes and immune cells contribute together to Ps-like disease. Evaluating the role of neutrophil-expressed S100A9 in Ps and PsA genetically without resorting to bone marrow chimaeras is difficult since all available GEMMs for Ps and PsA rely on the Cre-lox system. The current study is the first to use a new S100A9 floxed allele to investigate the role of epidermal-derived S100A9 in vivo. One of the most striking observations from the analysis of TKO* mice is that the epidermis contributes very little to circulating S100A9 in psoriatic mice. Hence, there was no difference between DKO* and TKO* when measuring CP or S100A9 homodimers in serum, although S100A9 protein was greatly reduced in TKO* epidermis. This is in stark contrast with DKO*-S100A9$^{-/-}$ mice, where only S100A8 dimers are detected in the serum[10] (data not shown). Nevertheless, epidermal-specific inactivation of S100A9 enhanced skin inflammation and increased PsA incidence in TKO*, indicating a regulatory role of epidermal S100A9 in psoriasis-like disease. Expression of S100A9 in keratinocytes seems to modulate the number and activity of skin-infiltrating neutrophils. An increase of 2–3 folds of Ly6B+ myeloid cells and Ly6B-S100A9 double positive cells, most likely neutrophils, was observed in TKO* skin sections, while whole ear proteomics revealed neutrophil activation and degranulation signatures. Interestingly, our previous iTRAQ proteomic comparison of DKO* and DKO*-S100A9$^{-/-}$ epidermis identified immune cell trafficking and activation as one of the most altered pathways.[10] Our data thus indicate that neutrophils are likely more abundant and/or more active in the skin of TKO* mice and contribute to more severe local inflammation. How *s100a9* gene inactivation in keratinocytes leads to increased immune cell infiltration remains to be clarified, but altered expression of cytokines, such as IL-6 and TNFα, and increased JAK/STAT signalling in S100A9-deficient keratinocytes could provide a first hint.

The DKO* mouse is one of the few psoriasis models displaying features of PsA.[35] In contrast to the situation in DKO*-S100A9$^{-/-}$ mice,[10] epidermal inactivation of S100A9 led to increased incidence of PsA. PsA severity was, however, comparable between DKO* and TKO*, consistent with comparable levels of circulating S100A9, IL-17A and IL-6. As TNF signalling is essential for joint disease in DKO* mice,[9] we postulate that the modest increment in circulating TNFα in TKO* mice, likely originating from increased epidermal *tnf-a* expression, is one of the factors enhancing PsA incidence. IL-17, IL-6 and S100A9-containing complexes, which are not affected by epidermal inactivation of S100A9, together with S100A9-expressing myeloid cells infiltrating the joints, additionally contribute to bone[24] and proteoglycan loss in DKO* and TKO* mice.

In an experimental model for rheumatoid arthritis, S100A9/CP neutralising antibodies had beneficial effects comparable with anti-TNFα,[36] the most potent PsA inhibitors in the clinic.[37–39] In light of the mouse data, topical therapies aiming at inhibiting S100A9 in the skin might be counterproductive, while systemic inhibition of S100A9/CP with drugs or neutralising antibodies is worth evaluating in GEMMs with Ps and PsA, as a possible complement to anti-TNFα therapies.

Increased CP in serum[16 40–42] in skin biopsies[10 43 44] and more recently in the *stratum corneum*[45] has been correlated with disease activity in patients with Ps. We observed that S100A8 dimers were not increased in the serum of patients with Ps and those with PsA, thus S100A8 dimers likely play a minor role. In contrast, circulating CP and S100A9 homodimers were elevated in patients with Ps and even more in patients with PsA. The synovial concentrations of these species were either comparable (CP) or higher (S100A9) than in serum, which would support an active local role of S100A9-containing complexes and S100A9-producing cells in the joints. While serum CP has previously been correlated with PsA severity,[8 46] this is the first time that S100A8 and S100A9 dimers are measured along with S100A8/S100A9 CP complexes. We found that S100A9 and CP efficiently discriminated healthy, patients with Ps and patients with PsA. Serum S100A9 and/or CP could therefore help identifying patients with Ps developing PsA. Given that CP is already a validated clinical parameter in inflammatory bowel disease,[23] longitudinal assessment in larger patient cohorts with Ps is feasible. Early identification of patients at risk of developing PsA will certainly allow the implementation of better therapies and will advance our understanding of Ps.

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TRANSLATIONAL SCIENCE

# Cross-species transcriptome analysis for early detection and specific therapeutic targeting of human lupus nephritis

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## ABSTRACT

**Objectives** Patients with lupus nephritis (LN) are in urgent need for early diagnosis and therapeutic interventions targeting aberrant molecular pathways enriched in affected kidneys.

**Methods** We used mRNA-sequencing in effector (spleen) and target (kidneys, brain) tissues from lupus and control mice at sequential time points, and in the blood from 367 individuals (261 systemic lupus erythematosus (SLE) patients and 106 healthy individuals). Comparative cross-tissue and cross-species analyses were performed. The human dataset was split into training and validation sets and machine learning was applied to build LN predictive models.

**Results** In murine SLE, we defined a kidney-specific molecular signature, as well as a molecular signature that underlies transition from preclinical to overt disease and encompasses pathways linked to metabolism, innate immune system and neutrophil degranulation. The murine kidney transcriptome partially mirrors the blood transcriptome of patients with LN with 11 key transcription factors regulating the cross-species active LN molecular signature. Integrated protein-to-protein interaction and drug prediction analyses identified the kinases TRRAP, AKT2, CDK16 and SCYL1 as putative targets of these factors and capable of reversing the LN signature. Using murine kidney-specific genes as disease predictors and machine-learning training of the human RNA-sequencing dataset, we developed and validated a peripheral blood-based algorithm that discriminates LN patients from normal individuals (based on 18 genes) and non-LN SLE patients (based on 20 genes) with excellent sensitivity and specificity (area under the curve range from 0.80 to 0.99).

**Conclusions** Machine-learning analysis of a large whole blood RNA-sequencing dataset of SLE patients using human orthologs of mouse kidney-specific genes can be used for early, non-invasive diagnosis and therapeutic targeting of LN. The kidney-specific gene predictors may facilitate prevention and early intervention trials.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Prediction of patients with systemic lupus erythematosus (SLE) that will develop nephritis and early diagnosis represents an unmet need because of the limited value of known predictors and the invasiveness of kidney biopsy.
⇒ Even with best treatment up to 40% of patients fail to reach a complete renal response suggesting that early diagnosis and prompt treatment including targeting of renal specific pathways is needed.

### WHAT THIS STUDY ADDS

⇒ Distinct, renal-specific molecular pathways are associated with the development of nephritis and its progression from subclinical to full blown disease in murine SLE.
⇒ The mouse kidney transcriptome mirrors the human whole-blood transcriptome in lupus nephritis (LN).
⇒ Upstream and downstream regulators of the cross-species (murine and human) kidney-specific gene signatures have been identified as putative targets in LN and novel cross-species drug signatures for kidney disease in lupus.
⇒ Using the mouse kidney-specific transcriptome and through training by machine-learning techniques of a large whole-blood RNA-sequencing dataset of SLE patients, we developed and validated an algorithm that predicts patients that will develop LN based on a small number (no more than 20) of genes.

## INTRODUCTION

In lupus nephritis (LN), current therapy fails to induce remission in more than 50% of patients. Even in cases with clinical remission, repeat kidney biopsies often exhibit residual inflammation and increased fibrosis, with 15%–20% of patients eventually developing end-stage kidney disease.[1–3] Importantly, several clinical trials have failed to meet their primary endpoint[4 5] with only two new treatments approved for LN.[6–9] Accordingly, there is urgent need for therapeutic interventions targeting aberrant molecular pathways enriched within the kidneys, to maximise drug efficacy.

Subclinical (silent) LN represents an early stage in the natural history of the disease[10–12] prior to

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

⇒ Common cross-species (murine and human) genes could be prioritised as potential therapeutic targets for LN or tested as an alternative, non-invasive 'liquid biopsy' marker of kidney disease in patients with SLE.

⇒ The mouse kidney-specific set of gene predictors may be used towards monitoring human kidney disease in SLE patients and enrolment in LN prevention and early treatment studies.

full-blown disease.[13 14] Notably, genetic and immunological interventions in lupus models have underscored the potential to avert autoantibody deposition and ensuing immune responses within the kidneys,[15–19] suggesting that preemptive therapy might represent a valid therapeutic concept.[15 19] However, the mechanisms underlying the progression to clinical LN are not clearly understood and kidney biopsies at the preclinical stage are not performed.

In this paper, we performed sequential mRNA-sequencing studies in effector (spleen) and target tissues (kidneys, brain) from lupus and healthy mice, as well as in the whole blood of patients with systemic lupus erythematosus (SLE) (including patients with active or responding LN or neuropsychiatric lupus) and healthy individuals. Comparative cross-tissue and cross-species analyses yielded common, cross-species, nephritis-specific genes that could be prioritised as potential therapeutic targets. Using machine-learning algorithms, we constructed a clinical-transcriptome predictive model that can be tested as a non-invasive 'liquid biopsy' marker of kidney disease in patients with SLE, to be used for monitoring of kidney disease in SLE, as well as enrollment in LN prevention and early treatment studies.

## METHODS

### Patients and healthy individuals

Patients with SLE (n=261) who met the SLICC 2012 or EULAR/ACR 2019 classification criteria and age-matched and sex-matched healthy individuals (n=106) were recruited from the Departments of Rheumatology and Nephrology at the University Hospitals of Heraklio, 'Attikon' University Hospital and the respective Blood Transfusion Units. Active LN was defined by the presence of proteinuria more than 0.5 g/day and active urine sediment. A kidney biopsy was performed in all patients with evidence of active kidney disease. Patients either developed active LN de novo or had had a history of LN and were flaring at the time of sampling. Responding LN was defined by preservation or improvement of kidney function with reduction of proteinuria to less than 50% after 6 months of therapy or less than 0.5–0.7 g/day by 12 months.[20 21] Following informed consent, whole blood was sampled, and RNA was extracted from all participants.

### Animals

NZB/W-F1 mice were sacrificed at the prepuberty (1 month old), preautoimmunity (3 months old) and nephritic (6 months old with proteinuria more than 200 mg/dL for three consecutive days) stage of SLE. Age-matched C57BL/6 mice were used as controls. Spleen, kidneys and brain were removed for RNA extraction.

### RNA-sequencing

RNA libraries were prepared using the Illumina Truseq kit. Paired-end 37 bp (for mouse) and 67 bp (for human) mRNA-sequencing was performed on the Illumina HiSeq2000 and HiSeq4000, respectively, at the University of Geneva Medical School.[22] FastQC software assessed quality.[23] Raw reads were aligned to the mouse (mm10 version) and human (hg38 version) genome using STAR V.2.6 algorithm.[24] Gene quantification was performed using HTSeq.[25] Differential expression analysis of mouse and human data was conducted using DESeq2[26] and edgeR,[27] respectively. Enrichment and network analyses were performed using gProfiler[28] and GeneMANIA.[29] The Expression2Kinases (X2K)[30] was used to yield transcription factors (TFs), kinases and protein-to-protein interaction (PPI) networks. Prediction of drugs was performed with L1000CDS$^2$ search engine.[31] Statistical significance was set at 5% false discovery rate (Benjamini-Hochberg).

### Machine learning

The human mRNA-sequencing dataset was randomly split into training (70%) and validation (30%) sets. Using the training set and feature selection algorithms, the smallest set of human orthologs that most accurately predicted the outcome of interest was selected. Using these orthologs as predictors, models were fit and compared for their ability to predict human disease. To improve performance, clinical predictors (not included in the definition of active or responding LN) were added to the final model. Accuracy, sensitivity, specificity and area under (AUC) the receiver operating curve (ROC) were determined in the validation set.

Detailed information for all methods can be found in online supplemental material. Scripts used and online supplemental table can be found at https://1drv.ms/u/s!Au_gakpSntTbrGO3-3RQ39ByOId1?e=MLF007.

## RESULTS

### Molecular signatures associated with murine LN and transition from preclinical to clinical disease

Patients with SLE are in urgent need for therapeutic interventions targeting molecular pathways enriched within individual tissues to treat their disease effectively and safely. To decipher aberrant molecular pathways enriched uniquely within the kidneys in SLE, we profiled gene expression at the spleen (an effector peripheral lymphoid organ), kidneys and brain (major end-organ tissues) from NZB/W-F1 lupus mice and age-matched C57BL/6 healthy counterparts. Tissues were collected at the clinical (nephritic) stage of the disease when nervous system involvement also occurs. Differentially expressed genes (DEGs) in lupus versus healthy mice tissues were analysed. Using genes differentially expressed within kidneys of the NZB/W-F1 lupus mice but not in other tissues studied, we defined a 'kidney-specific signature' comprising 726 DEGs (425 upregulated, 301 downregulated) (online supplemental figure S1A,B, table S1A). Enriched functions within this signature included pathways linked to cell metabolism, innate immune system and neutrophil degranulation (online supplemental figure S1C, table S1B), reiterating the role of neutrophils in lupus kidney injury.[32] By representing the signature DEGs as a gene network, we found several hub genes with high-degree nodes of the network corresponding to human lupus-susceptibility loci[33–35] such as *FCGR2B, PTPRC, ITGAM, NCF1* and *RASGRP1* (online supplemental figure S1D, table S1C).

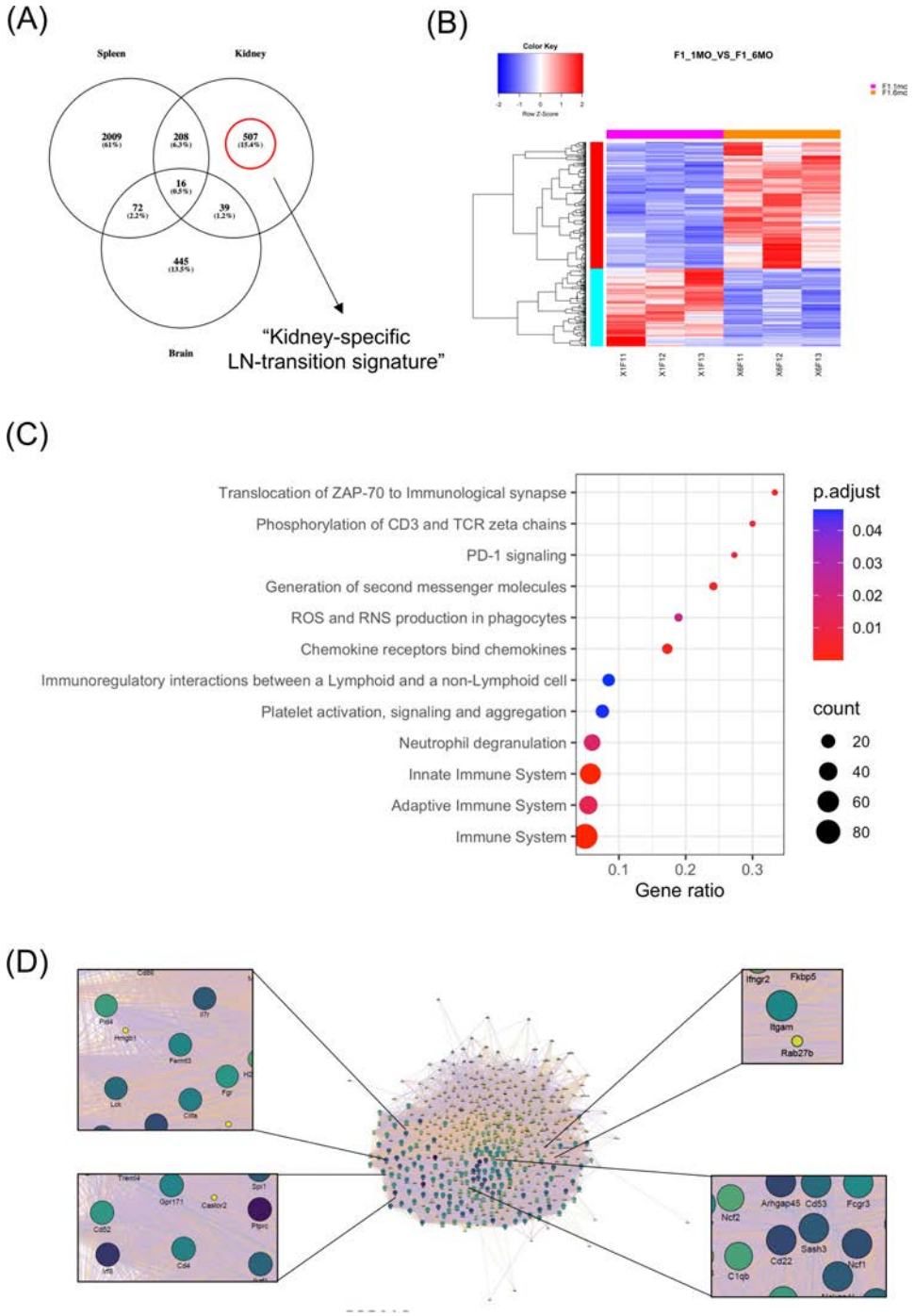


**Figure 1** Mouse kidney-specific transcriptome of lupus mice between the clinical (nephritic) and the preclinical (prepuberty) stage of the lupus. (A) Venn diagram demonstrating the comparison between differentially expressed genes (DEGs) within the spleen, the kidneys and the brain from NZB/W-F1 lupus mice at the clinical (nephritic) versus the preclinical (prepuberty) stage of lupus. The kidney-specific gene signature is defined by 507 genes that are differentially expressed only within kidneys but not in other tissues, (B) Heatmap of the 507 kidney-specific DEGs (316 upregulated, 191 downregulated), (C) Dot-plot diagram demonstrating functionally enriched REACTOME pathways of the 507 kidney-specific DEGs, (D) gene network representation of the 507 kidney-specific DEGs. Hub genes that correspond to lupus risk loci are depicted by larger size fonds. ROS, reactive oxygen species; TCR, T cell receptor.

Next, we examined the molecular events underlying transition from the preclinical to clinical stage of lupus kidney disease by comparing DEGs between the tissues from lupus mice probed at the prepuberty versus the nephritic stage. Genes that were differentially expressed uniquely within kidneys of the NZB/W-FI lupus mice but not in other tissues studied defined the 'kidney-specific LN-transition signature' comprising 507 DEGs (316 upregulated, 191 downregulated) (figure 1A,B, online supplemental table S2A) that were enriched in innate and adaptive immune system pathways. The former were linked to neutrophil degranulation and reactive oxygen species production in phagocytes, whereas the latter included T cell receptor signalling, signal transduction by G-protein coupled receptors (in particular, chemokine receptors) and costimulation through programmed cell death protein 1 (PD-1) signalling. In addition, pathways involved in platelet activation, signalling and

aggregation were identified (figure 1C, online supplemental table S2B). Of note, the lupus-susceptibility risk loci *PTPRC*, *NCF1* and *ITGAM* genes, as well as the *IRF8*,[33–35] emerged as hub network genes, suggesting a pathogenic role during evolution from preclinical to clinical LN (figure 1D, online supplemental table S2C).

To analyse the sequential molecular events underlying the evolution towards LN, we identified DEGs in tissues from lupus vs healthy mice demonstrating a strain-specific effect in a time-series analysis. DEGs within kidneys demonstrating the lupus-specific pattern were combined with genes within kidneys that were differentially expressed across all stages of the disease. Combined signatures were compared across tissues and genes that were differentially expressed uniquely within kidneys—but not in other tissues—defined the 'sequential kidney-specific signature', composed of 1668 genes (online supplemental table S3A). Functional interpretation of the result revealed enrichment in the establishment of sister chromatid cohesion pathway (online supplemental table S3B). Kidney-specific DEGs in lupus versus healthy mice at the preautoimmunity stage, kidney-specific DEGs from lupus mice at the preautoimmunity versus the prepuberty stage and the respective functional enrichment analyses are presented in online supplemental tables S3C–F. DEGs within kidneys demonstrating the strain-specific pattern in the time-series analysis are presented in online supplemental figure S2.

### The human peripheral blood and the murine kidney transcriptome share common kidney-specific signatures and associated hub genes

Kidney biopsy, an invasive procedure linked to increased risk for adverse events, is currently essential to confirm diagnosis and guide therapeutic decisions in LN; however, it is still an imperfect predictor of response to treatment. Previous studies have reported shared molecular signatures within LN kidneys of mice and humans,[36] as well as between kidney and non-kidney (eg, skin) tissues of patients with LN.[37 38] Recent evidence suggests that neutrophils from ultraviolet skin reach the kidney and cause inflammation in murine models; it is conceivable that these circulating neutrophils prior to their homing to the kidneys may be captured in the blood.[39] To this end, we next asked whether the kidney-specific signatures in murine lupus may exist also in patients with LN using blood as an easily accessible, minimally invasive tissue. Specifically, we investigated whether the mouse kidney could serve as non-invasive (not-requiring biopsy in humans) marker of kidney disease in human SLE. To address this, we performed whole-blood mRNA-sequencing in 141 SLE patients and 48 healthy counterparts. Data were combined with our previously analysed cohort,[22] thus yielding a dataset of 367 individuals (including 261 SLE patients and 106 healthy individuals) (online supplemental table S4A). We found extensive transcriptome perturbations with 10 672 DEGs between active LN patients and healthy individuals (online supplemental figure S3A, table S4B) and 4119 DEGs between active LN and SLE patients without history of kidney disease (non-LN patients) (figure 2A, online supplemental table S4C).

Next, we examined whether the human peripheral blood from patients with LN shares common gene expression aberrations with the mouse kidney-specific gene signatures. Using the human orthologous genes of the mouse genome, we examined if the mouse 'kidney-specific signature' is present in the blood of patients with active LN as compared with healthy individuals. A total 272 genes (193 upregulated and 79 downregulated) were common between the two datasets (online supplemental figure S3B,C, table S5A), referred to as 'shared active LN signature'. Neutrophil degranulation was the most significantly enriched pathway in this signature (online supplemental figure S3D, table S5B), whereas gene network analysis revealed that the lupus-susceptibility risk loci *NCF2*, *ITGAM, NCF1, RASGRP1* and *FCGR2A*[33–35] were high-degree hub genes, suggesting their central pathogenic role in LN (online supplemental figure S3E, table S5C).

A similar cross-species analysis was performed to determine whether the mouse 'kidney-specific LN-transition signature' intersects with the human blood transcriptome of patients with active LN versus non-LN patients. Ninety-seven common genes (67 upregulated and 30 downregulated) were identified (figure 2B,C, online supplemental table S6A), comprising the 'shared active LN-transition signature'. Functional enrichment analysis revealed pathways linked to hematopoietic cell lineage, B-cell receptor signalling and immunoregulatory interactions between lymphoid and non-lymphoid cell (figure 2D, online supplemental table S6B). *CD53*, *ITGB2* and *LAPTM5* were the highest-degree hub genes, underscoring their role in evolution of LN. The risk locus *ITGAX* was also identified, further supporting its pathogenic role[33] and its gene expression deregulation within kidneys during lupus progression (figure 2E, online supplemental table S6C).

To characterise the 'sequential kidney-specific signature' in the context of human LN, we compared the human orthologous genes of the mouse signature with the DEGs between active LN patients and healthy individuals and revealed 609 common genes that defined the 'shared sequential kidney-specific signature' (online supplemental table S7A). These genes were functionally enriched in pathways linked to selenocysteine synthesis and non-sense mediated decay independent of the exon junction complex (online supplemental table S7B).

In silico analysis of upstream regulators, downstream kinases and drug signatures for the identification of novel therapeutic targets in LN: Kinases TRRAP, AKT2, CDK16 and SCYL1 as putative targets for reversing the LN signature

Genetic association studies have identified TFs to play a major pathogenic role in SLE.[40] Taking advantage of our study design, we performed TF enrichment analysis[30] in the cross-species gene signatures and found a total of 11 TFs (including E2F4, FOXM1, SPI1 and SIN3A) and 6 TFs (including SPI1, IRF8, RUNX1 and VDR), which were predicted to regulate the 'shared active LN signature' (figure 3A, online supplemental table S8A) and the 'shared active LN-transition signature' (figure 3B, online supplemental table S9A), respectively.

To decipher downstream kinases of the shared gene signatures that might serve as druggable targets, the aforementioned lists of enriched TFs were expanded by identifying proteins previously shown to physically interact with them, followed by construction of PPI subnetworks (online supplemental table S8B, table S9B). Based on the overlap between known kinase–substrate phosphorylation interactions and the proteins in the subnetworks, we found kinases that phosphorylate the proteins interacting with the TFs. The kinase TRRAP was predicted to phosphorylate the NCOR2 and HCFC1 (hypergeometric p=0.0004799) that interact with the enriched TFs that regulate the 'shared active LN signature' (online supplemental table S8C); and the AKT2, CDK16 and SCYL1 kinases were predicted to phosphorylate ACTN4 and AES or SMARCA4 or AES (hypergeometric p=0.005443), respectively, that interact with the enriched TFs that regulate the 'shared active LN-transition signature' (online supplemental table S9C), suggesting they could represent

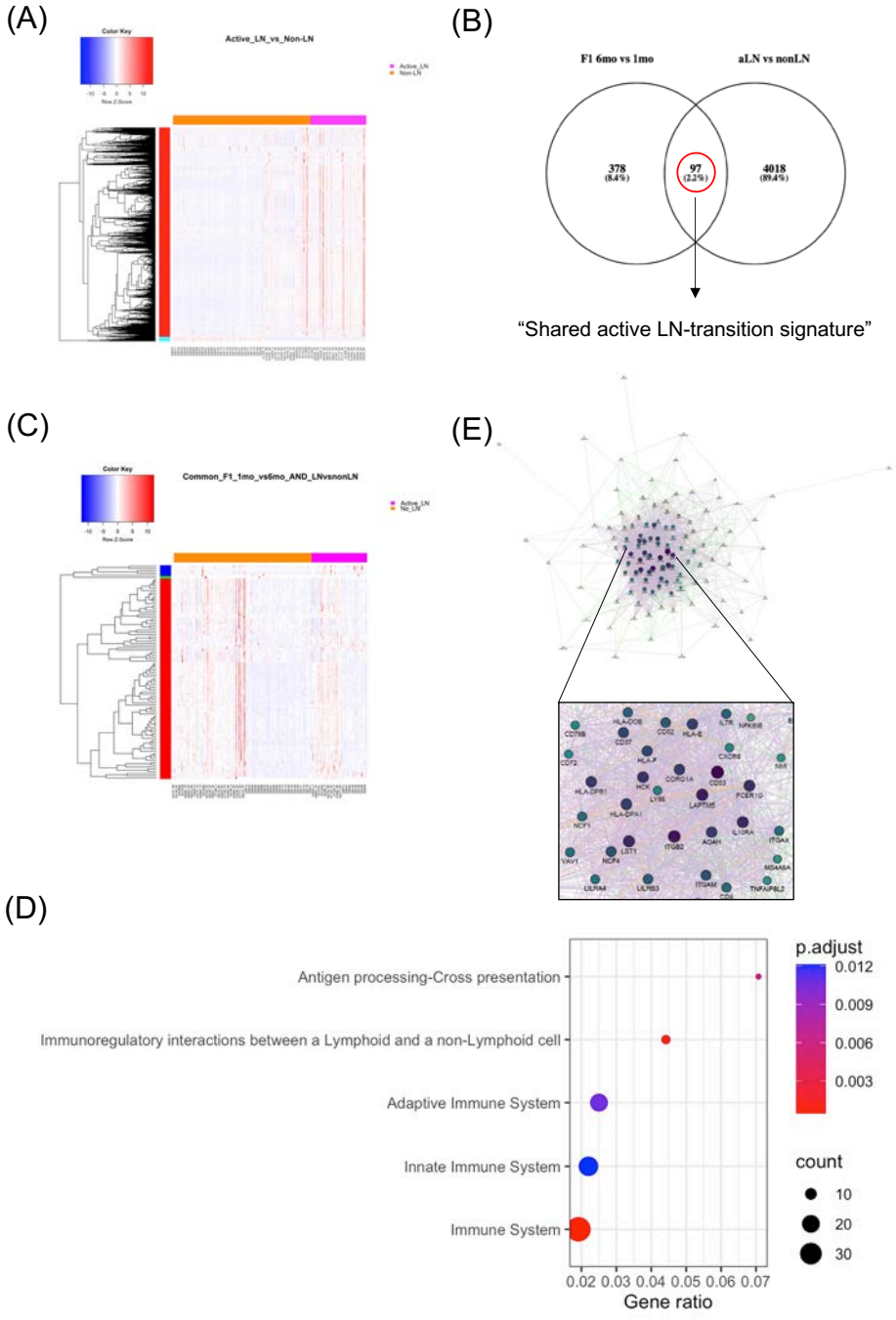


**Figure 2** Common genes between the kidney-specific gene expression profile from lupus mice at the symptomatic (nephritic) versus the asymptomatic (prepuberty) stage and the whole-blood gene expression profile from active LN (aLN) patients versus SLE patients without history of kidney involvement (non-LN) define a 'shared active LN-transition signature'. (A) Heatmap of the 4119 differentially expressed genes (DEGs) in the whole-blood from aLN patients versus non-LN patients, (B) Venn diagram demonstrating the comparison between the orthologous genes of the mouse kidney-specific DEGs from NZB/W-F1 lupus mice at the symptomatic (nephritic) versus the asymptomatic (prepuberty) stage and the whole-blood gene expression profile from aLN versus non-LN SLE patients. The 'shared active LN-transition signature' is defined by the union of the Venn diagram, corresponding to 97 common genes, (C) Heatmap of the 'shared active LN-transition signature', composed of 97 genes (67 upregulated, 30 downregulated), (D) Dot-plot diagram demonstrating functionally enriched REACTOME pathways of the 'shared active LN-transition signature', (E) gene network representation of the 'shared active LN-transition signature'. Hub genes that correspond to lupus risk loci are depicted by characters of a larger size. LN, lupus nephritis; SLE, systemic lupus erythematosus.

putative targets in LN. Complete upstream pathways of the gene signatures connecting the enriched TFs to kinases through known PPIs were also inferred (online supplemental tables S8D and S9D).

Finally, through the L1000 Characteristic Direction Signature Search Engine (L1000CDS[2]), we detected the top 50 drugs or small molecule compounds (online supplemental tables S8E and S9E) and the top 50 compound combinations that may reverse

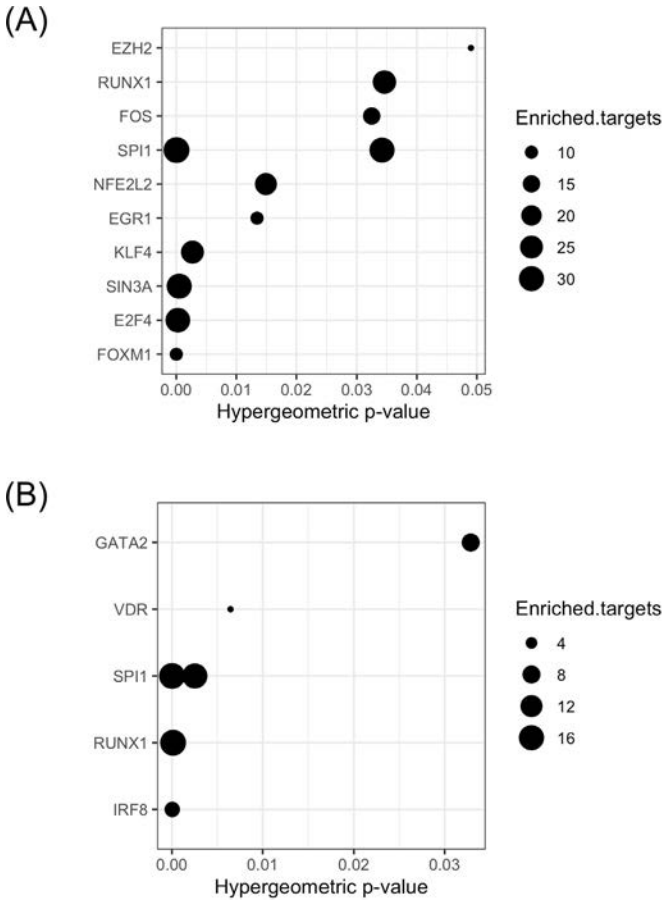


**Figure 3** Upstream regulators of the 'shared active LN signature' and the 'shared active LN-transition signature'. (A) Dot-plot diagram demonstrating the transcription factors (TF) that are predicted to reverse the common genes between the kidney-specific gene expression profile from lupus vs healthy mice at the clinical (nephritic) stage and the whole-blood gene expression profile from active LN (aLN) patients vs healthy individuals (HI). The x-axis represents the hypergeometric p value and dots correspond to the number of enriched targets of the TF, (B) Dot-plot diagram demonstrating the TF that are predicted to reverse the common genes between the kidney-specific gene expression profile from lupus mice at the clinical (nephritic) versus the preclinical (prepuberty) stage and the whole-blood gene expression profile from patients with active LN (aLN) versus SLE patients without history of kidney involvement (non-LN). The x-axis represents the hypergeometric p- value and dots correspond to the number of enriched targets of the TF. LN, lupus nephritis; SLE, systemic lupus erythematosus.

the 'shared active LN signature' and the 'shared active LN-transition signature', respectively (online supplemental tables S8F and S9F). Among these, the R(+)−6-BROMO-APB was predicted to reverse the former, and the HEMADO, norketamine hydrochloride, trichostatin A and others were predicted to reverse the latter signature, respectively, in the HA1E kidney cell line, suggesting they could be further tested in the therapy of LN.

## Eighteen genes may predict patients with active LN from healthy individuals

Demographic, clinical and serological data are imperfect in predicting the onset of kidney disease in patients with SLE. Importantly, early identification and prompt treatment have been linked to improved outcomes.[13 14] We examined whether the human orthologs of the mouse kidney-specific gene signatures and the human whole-blood gene signatures may predict those patients with SLE who will develop LN. For this, the complete mRNA-sequencing dataset was randomly split into training (70%) and validation (30%) sets, and machine-learning algorithms were applied (figure 4).

To distinguish patients with active LN from healthy individuals, we used the human orthologs of the mouse kidney-specific DEGs from lupus versus healthy mice at the nephritic stage (corresponding to the 'kidney-specific signature', composed of 726 DEGs). To remove noise and keep the smallest set of human orthologs of the mouse genes which best predicts outcome, we performed feature selection using recursive feature elimination with a random forest (machine-learning) model under a 10-fold cross-validation. Based on model accuracy, a set of 50 human orthologs were selected. Next, prediction models were fit to identify which performs best with the selected genes. The glmnet model using 18 genes—including *PLD4*, *PTPRN2*, *CASP8* and *POLE* (figure 5A, online supplemental table S10)—(32 genes had a coefficient=0 and were considered redundant in the model) best distinguished patients with active LN from healthy individuals with a 10-fold cross-validation calculated accuracy of 95.7% (95% CI (0.85% to 0.99%)], 100% sensitivity and 92.9% specificity (0.99 AUC of the ROC curve analysis) in the validation set (figure 5B,C), demonstrating an excellent model efficiency to discriminate true positive (active LN patients) from false positive (healthy individuals) cases. Inclusion of clinical factors (not included in the definition of active or responding LN), such as age, gender and the presence of anti-dsDNA, did not improve further the performance of the model. Using the validation set, principal component analysis (PCA) demonstrated that the 18 selected genes could accurately discriminate patients with active LN from healthy individuals (figure 5D). The relationship between the expression of each gene and the probability of predicting active LN is demonstrated in online supplemental figure S4. These data define a LN prognostic gene signature and demonstrate the feasibility of developing and validating an algorithm to predict patients with active LN from healthy individuals non-invasively, through machine-learning analysis of a large whole blood RNA-sequencing dataset of SLE patients using human orthologs of mouse kidney-specific genes as predictors of kidney involvement.

## Machine-learning model distinguishes LN from non-LN SLE patients

Next, we examined whether the above approach could also discriminate active LN patients from SLE patients without kidney disease (non-LN patients) in a non-invasive manner. We sought that the kidney-specific gene expression profile of lupus mice at the clinical (nephritic) versus the preclinical (prepuberty) stage of the disease (corresponding to the 'kidney-specific LN-transition signature', composed of 507 DEGs) could reflect the whole-blood gene expression profile of SLE patients with active LN versus SLE patients without history of LN (non-LN patients). Thus, we used the human orthologs of the mouse 'kidney-specific LN-transition signature' as predictors, and applied feature selection under a 10-fold cross-validation. Based on accuracy, 20 genes best predicted the outcome. Models were fit to identify which performs best with the selected genes. Model performance was further improved by the addition of age, sex and presence of anti-dsDNA, as predictors of outcome. As expected, due to the higher likelihood of patients with proliferative LN to have anti-DNA antibodies, the presence of anti-dsDNA was the most important predictor of kidney disease,

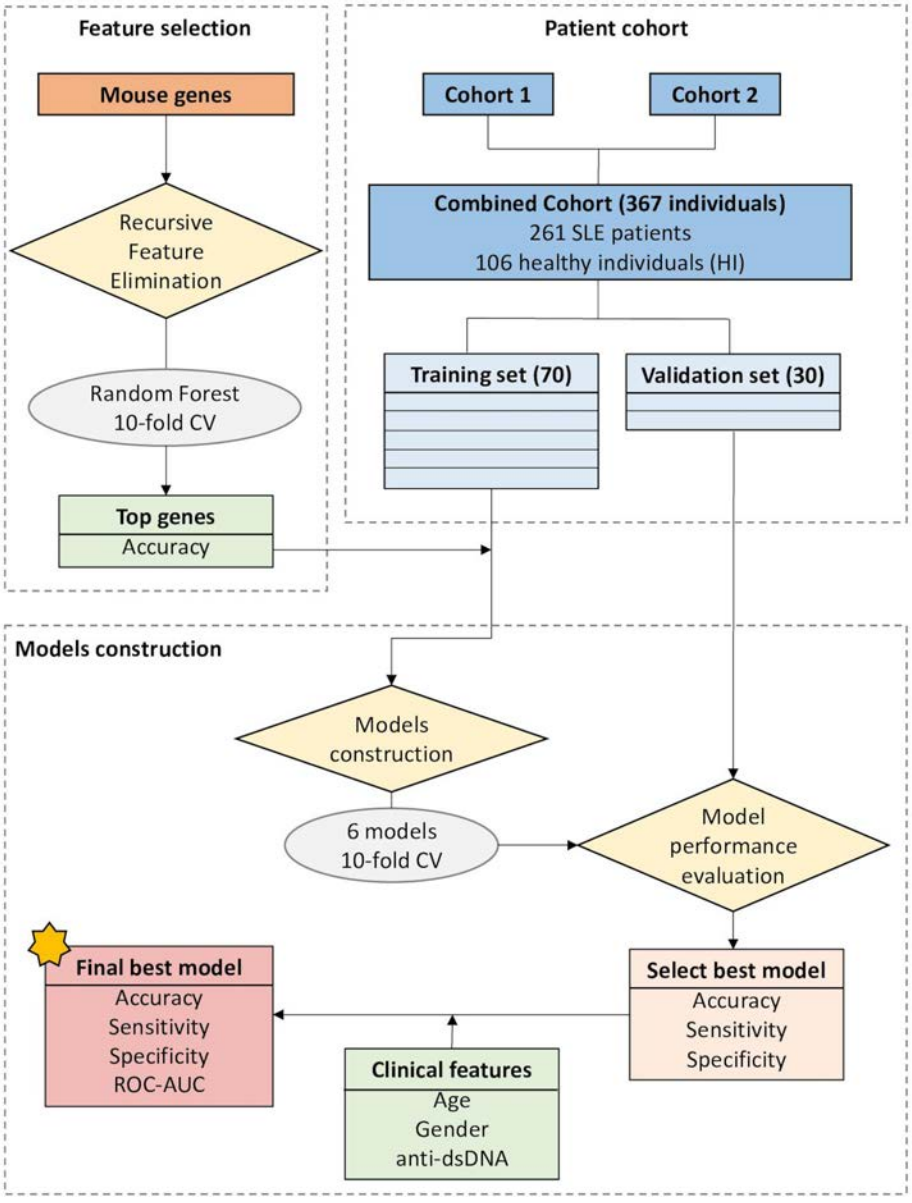


**Figure 4** Schematic overview of the machine-learning approach. RNA-sequencing data from the two human cohorts were combined and then split in training to test sets at 70:30 ratio. For each outcome measure, a corresponding gene list derived from mouse data was used. The training set was used to develop a prediction model and the test set was used to validate the results. Using the training set, feature selection was applied to remove noise and keep the smallest set of genes which best predicts each outcome based on accuracy. Then, different prediction models were fit to identify which performs best using the gene signature selected in the previous step. Once the best model was selected based on accuracy, sensitivity and specificity, the addition of age, gender and the presence of anti-dsDNA as predictors were tested if they could improve the model. The final model was validated in the test set. AUC, area under the curve; CV, cross-validation; dsDNA, double-stranded DNA; ROC, receiver operating characteristic curve.

followed by the expression of *PTPRO* gene (the lower its expression, the higher the probability of predicting active LN) and *IL10RA* gene (the higher its expression, the higher the probability of predicting active LN). Male sex and younger age of SLE patients were associated with higher probability of active LN. In the validation dataset, the glm model displayed accuracy 81.7% (95% CI (0.70% to 0.90%)), sensitivity 63.2% and specificity 90.2% (AUC 0.80) in distinguishing patients with active LN from SLE patients without history of LN (figure 6A–C, online supplemental table S11, figure S5), demonstrating that the model correctly identified SLE patients without LN (true negative cases). Using the validation set, PCA demonstrated how gene predictors could accurately discriminate patients with active LN from non-LN SLE patients (figure 6D). Together, these data demonstrate the feasibility to distinguish patients with active LN from SLE patients without kidney involvement. These gene predictors could be of prognostic value in the clinical setting following further validation studies in independent cohorts.

## DISCUSSION

Patients with LN are in need for an early diagnosis and therapeutic targeting of aberrant molecular pathways enriched within the affected kidneys. Here, we performed sequential mRNA-sequencing in three tissues of lupus and healthy mice, and in the whole-blood of SLE and healthy individuals. Through cross-tissue analysis, we defined a murine kidney-specific molecular signature and a molecular signature that underlines progression

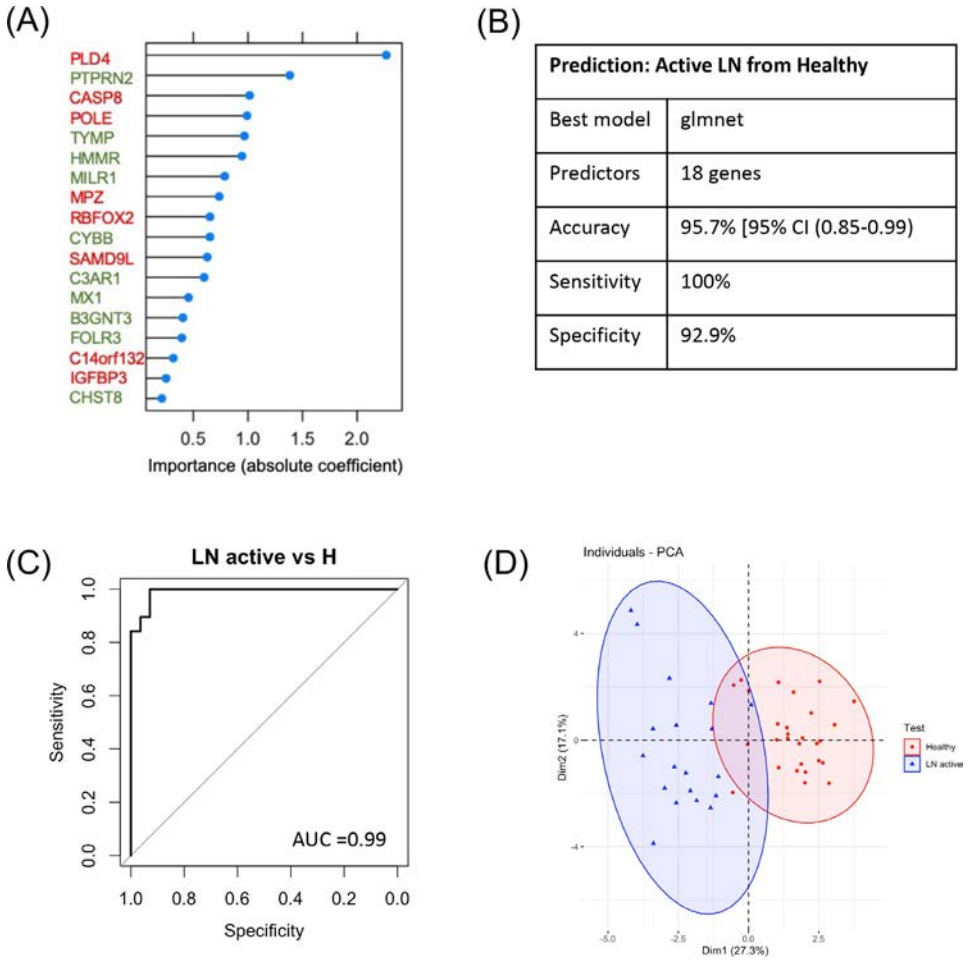


**Figure 5** Machine-learning modelling of the human whole-blood RNA-sequencing data, using mouse kidney-specific genes as predictors, distinguishes patients with active lupus nephritis (active LN) from healthy individuals (H) in a non-invasive manner and defines a LN prognostic gene signature. (A) The 18 predictors of the glmnet model distinguishing patients with active LN from healthy individuals based on their importance, as evidenced by their absolute coefficient. Gene predictors in green fonts indicate that the higher their expression the higher the probability of being a patient with active LN compared with being a healthy individual; while gene predictors in red fonts indicate that the lower their expression the higher the probability of being a patient with active LN, (B) Characteristics of the prediction model of patients with active LN from healthy individuals, (C) Receiver operating characteristic curve (ROC) analysis of the glmnet model in the validation set reveals an area under the curve (AUC) of 0.99, (D) principal component analysis (PCA) using the 18 genes.

from the predisease stage to overt clinical disease. We also demonstrated that the murine kidney transcriptome mirrors—in part—the human whole blood transcriptome of LN patients and found upstream and downstream transcriptional regulators that may be prioritised as potential therapeutic targets. Finally, we developed a blood gene-based predictive model for human LN that can be tested as an alternative, non-invasive 'liquid biopsy' marker of kidney disease in patients with SLE. Pending further confirmation, this marker could identify patients in need of monitoring for development of LN, as well as enrolment in LN prevention and early treatment studies.

To improve therapeutic interventions and optimise the use of animal models, gene expression profiling across three samples and species is important in defining how mouse biology can be extrapolated to humans.[41] To this end, the sequential cross-organ (murine spleen, kidney and brain) and cross-species (murine and human) comparative transcriptomics analysis in this paper is novel, defining unique-to-kidney molecular aberrancies in SLE that can be extrapolated to the transition from the preclinical to clinical stage of human LN. Our human transcriptomic analysis involved a large number of well-characterised patients and healthy controls which makes it the larger, single-centre, RNA-seq analysis ever performed in SLE. In addition to providing potential biomarkers for prediction and non-invasive diagnosis and monitoring, our data also reflect biological pathways involved both in the development and clinical transition of LN in a systematic and unbiased manner, without preconceived notions.

In view of the heterogeneity of lupus, we used next-generation sequencing as an unbiased and not requiring a priori hypothesis approach to uncover novel molecular pathways implicated in major end-organ injury in SLE. Initially we performed mRNA-sequencing of a peripheral lymphoid organ (the spleen, that may be used as a surrogate of peripheral blood) and two end-organ tissues (kidneys and brain) from the NZB/W-F1 lupus model at the prepuberty, preautoimmunity and nephritic stage of SLE and identified the molecular profile which is expressed uniquely within kidneys of this model—but not in other tissues studied—and the molecular profile that characterises unique-to-kidney molecular events underlying LN transition from the preclinical to clinical stage of kidney disease. In this process, we identified pathways enriched within each signature and found

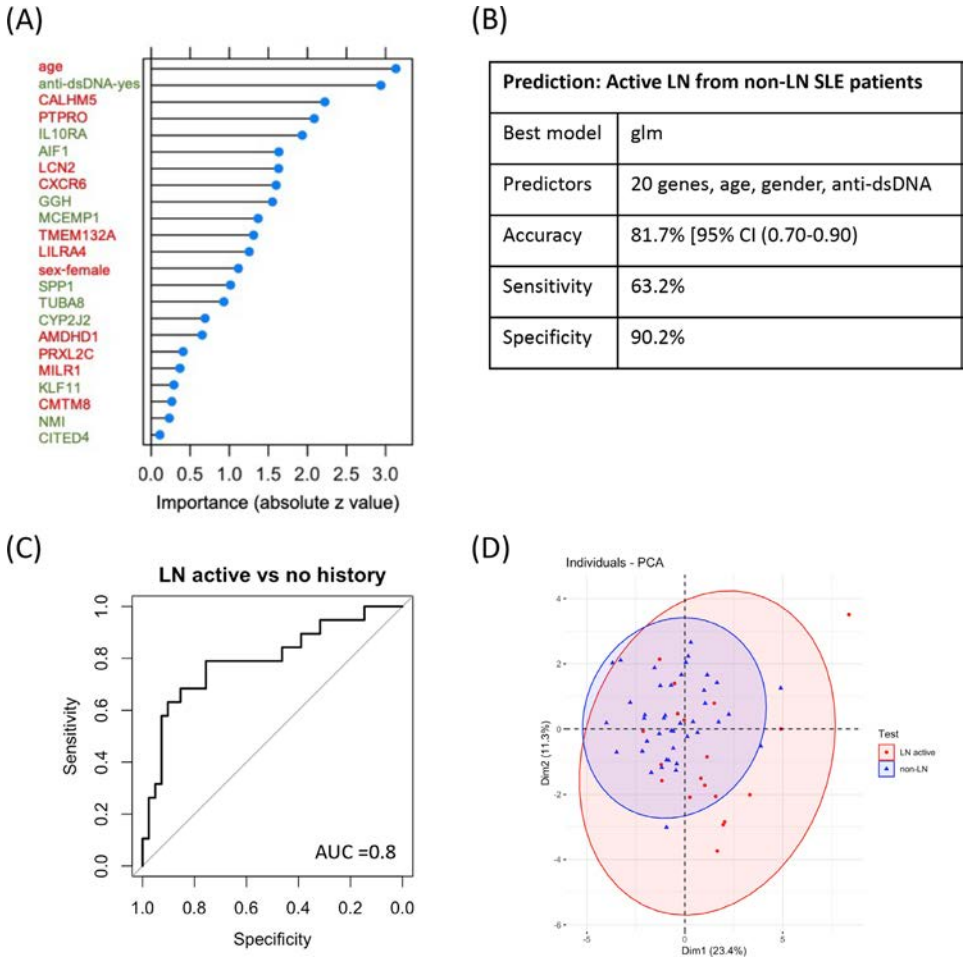


**Figure 6** Machine-learning modelling of the human whole-blood RNA-sequencing data using mouse kidney-specific LN-transition genes as predictors distinguishes patients with active lupus nephritis (active LN) from SLE patients without history of kidney disease, non-invasively. (A) The 23 predictors of the glm model distinguishing patients with active LN (active LN) from SLE patients without kidney disease (non-LN) based on their importance, as evidenced by absolute z value. Gene predictors in green fonts indicate that the higher their expression the higher the probability of being a patient with active LN compared with being non-LN patient, while gene predictors in red fonts indicate that the lower their expression the higher the probability of being a patient with active LN. The presence of anti-dsDNA (indicated in green fonts) is associated with a higher the probability of being a patient with active LN and the older age and female gender (indicated in red fonts) are associated with a lower probability of being a patient with active LN, (B) Characteristics of the prediction model of active LN patients from non-LN patients, (C) Receiver operating characteristic curve analysis of the glm model in the validation set reveals an area under the curve (AUC) of 0.8, (D) Principal component analysis (PCA) using the 20 gene-predictors. LN, lupus nephritis; SLE, systemic lupus erythematosus;

that hub genes correspond to lupus susceptibility risk loci (such as the *PTPRC*, *ITGAM*, *NCF1* and *IRF8* genes), reinforcing their pathogenic role in LN and the progression from preclinical to clinical kidney disease. Validating our results, the *VEGF*, *TLR2* and *SOCS3* genes were also differentially expressed in the kidneys from NZB/W-F1 mice 9 months old vs 6 months old as well as the kidneys from patients with LN.[36] In agreement with Arazi *et al*,[42] genes such as the *ITGAM* and *FCGR2B* were also differentially expressed in the 'kidney-specific gene signature'. The FPR2, *IL18R1*, *ITGAM* and *NCF4* genes were also differentially expressed in the myeloid lineage from paediatric patients with LN,[43] genes such as the *MDP1*, *PTGR1* and *MX2* were also differentially expressed within the kidneys from LN patients, as assessed by microarrays[44] and genes such as the *TMEM167A*, *TNFAIP8* and *VCAM1* were also differentially expressed in kidney tubular cells from LN patients.[38]

Blood transcriptome analysis identified similarities as well as differences from the molecular signatures detected within kidneys in patients with LN, underscoring that limitations exist in the use of blood for uncovering kidney disease processes.[42] However, gene expression studies have shown shared inflammatory responses within kidneys between mice and humans with LN,[36] but also shared gene signatures between kidney tubular cells and keratinocytes of LN patients.[37 38] Our data suggest that the mouse kidney transcriptome and the human whole-blood transcriptome share a common gene expression profile that corresponds to common biological processes and pathways. Lupus medications were held for 12 hours prior to sampling thus, a potential downstream effect cannot be excluded. However, validating our results, in the 'shared active LN signature', genes such as the *CEACAM1*, *TYMP*, *NCOA7* and *AIM2* were also differentially expressed in interferon stimulating genes identified through single-cell RNA-sequencing within the kidneys from LN patients[42] and *SERPINA1*, *IL1RN* and *ABCB1* genes were also differentially expressed in kidney tubular cells from LN patients.[38] We also identified hub genes of the common cross-species kidney-specific gene network corresponding to lupus-susceptibility risk loci, uncovering their cross-species pathogenic

role in LN, and identified that the pathway interactions between lymphoid and non-lymphoid cell characterises the transition from preclinical to clinical LN across species. Although we do not validate the LN blood transcriptome with the kidney transcriptome in humans, part of the mouse kidney transcriptome mirrors the human whole-blood transcriptome in patients with LN, suggesting that common genes can be prioritised as potential therapeutic targets for LN, or tested as an alternative, non-invasive 'liquid biopsy' marker of kidney disease in patients with SLE.

To decipher cross-species specific targets in LN, we used systems biology approaches and combined our experimental data with simulation-based analyses. We report upstream and downstream regulators of the cross-species kidney-specific gene signatures as specific targets in LN and describe novel cross-species drug signatures for kidney disease in lupus, suggesting non-immune-based approaches to be tested in LN therapeutics, as 'add on' therapy to conventional immune therapy. We must underscore that due to limitations in the analysis, identified TFs are not restricted to immune cells therefore therapies targeting them could have off-target effects with potential toxicity.

Although current therapeutic decisions in LN are guided by its histological classification,[20 21 45] kidney histology is an imperfect predictor of kidney outcome,[1] highlighting the need for improved biomarkers.[44] The urokinase-type plasminogen activator receptor and the decrease in urinary epidermal growth factor to creatine ratio have been identified as independent predictors of progression to chronic kidney disease in patients with glomerular diseases[46 47]; however, a biomarker for preclinical LN has not been identified. Since preclinical LN is an early stage in the natural history of the disease and improvements in the prognosis of LN have been attributed to early diagnosis and prompt therapy,[10–14] we used machine-learning approaches to identify non-invasive predictors of kidney involvement in SLE patients. Specifically, we used the 'kidney-specific gene signature' as a tool to build a machine-learning algorithm to distinguish patients with active LN from healthy individuals and demonstrated that this approach can be used successfully as a non-invasive prediction method. Then, using the murine lupus kidney-specific transcriptome, we built and validated a machine-learning algorithm that predicts patients with active LN from SLE patients without LN, to be used in the monitoring for kidney disease in such patients and enrolment in LN prevention and early treatment studies. Although validation in an independent dataset was not used, cross-validation was performed during modelling, thus reinforcing our results. These gene predictors could be of prognostic value in the clinical setting, following further validation studies in independent cohorts. Although machine-learning distinguishes patients with LN from non-LN patients accurately, yet at this point this method is not better than clinical diagnosis of LN. Moreover, sequential clinical and transcriptomic data are necessary for the prediction of patients that will flare. The prediction of patients that truly have responding LN would have also been useful; however, a kidney-specific signature corresponding to responding kidney disease (not preclinical) is not available in murine, making this algorithm not applicable for this purpose. Further validation in independent human datasets or longitudinal studies are needed to further explore these findings in human LN.

In conclusion, common cross-species, nephritis-specific genes could be used as potential therapeutic targets for LN or tested as a surrogate, non-invasive 'liquid biopsy' marker of kidney disease in patients with SLE. These kidney-specific genes can be used to design prevention and early intervention trials, following their validation in longitudinal studies.

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CLINICAL SCIENCE

# Weaning of maintenance immunosuppressive therapy in lupus nephritis (WIN-Lupus): results of a multicentre randomised controlled trial

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## ABSTRACT

**Objectives** Lupus nephritis (LN) is a frequent complication of systemic lupus erythematosus (SLE). Severe (proliferative) forms of LN are treated with induction immunosuppressive therapy (IST), followed by maintenance IST, to target remission and avoid relapses. The optimal duration of maintenance IST is unknown. The WIN-Lupus trial tested whether IST discontinuation after 2–3 years was non-inferior to IST continuation for two more years in proliferative LN.

**Methods** WIN-Lupus was an investigator-initiated multicentre randomised controlled trial. Patients receiving maintenance IST with azathioprine or mycophenolate mofetil for 2–3 years, and hydroxychloroquine, were randomised (1:1) into two groups: (1) IST continuation and (2) IST discontinuation. The primary endpoint was the relapse rate of proliferative LN at 24 months. Main secondary endpoints were the rate of severe SLE flares, survival without renal relapse or severe flare, adverse events.

**Results** Between 2011 and 2016, 96 patients (out of 200 planned) were randomised in WIN-Lupus: IST continuation group (n=48), IST discontinuation group (n=48). Relapse of proliferative LN occurred in 5/40 (12.5%) patients with IST continuation and in 12/44 (27.3%) patients with IST discontinuation (difference 14.8% (95% CI −1.9 to 31.5)). Non-inferiority was not demonstrated for relapse rate; time to relapse did not differ between the groups. Severe SLE flares (renal or extrarenal) were less frequent in patients with IST continuation (5/40 vs 14/44 patients; p=0.035). Adverse events did not differ between the groups.

**Conclusions** Non-inferiority of maintenance IST discontinuation after 2–3 years was not demonstrated for renal relapse. IST discontinuation was associated with a higher risk of severe SLE flares.

**Trial registration number** NCT01284725.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Proliferative lupus nephritis (LN) can lead to renal failure. In patients with LN, after the induction phase of immunosuppressive therapy (IST), maintenance IST aims to prevent LN relapses. The optimal duration of maintenance IST, to reduce the risk of relapse while minimising treatment-related adverse events, is unknown.

### WHAT THIS STUDY ADDS

⇒ WIN-Lupus is the first randomised controlled trial comparing maintenance IST discontinuation versus maintenance IST continuation in proliferative LN. WIN-Lupus tested the non-inferiority of maintenance IST discontinuation after 2–3 years, compared with its continuation for two more years, in proliferative LN. Non-inferiority was not demonstrated and patients who discontinued IST had a higher risk of severe flares of lupus. However, the majority of patients who discontinued IST did not experience a flare.

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

⇒ Instead of prolonging maintenance IST for all patients with proliferative LN, our results call for the development of tailored strategies, possibly involving repeat biopsy, to identify patients who can be safely weaned off maintenance IST.

## INTRODUCTION

Lupus nephritis (LN) is a frequent and severe manifestation of systemic lupus erythematosus (SLE).[1] Although the prognosis of LN has improved, substantial morbidity, partly related to treatment, is still observed.[2–5] The therapeutic strategy relies on pathological classification of renal lesions.[6 7] In patients with proliferative LN (class III or IV with active lesions, with or without associated class V,

according to the ISN/RPS 2003),[7] the treatment relies on two consecutive phases: (1) induction phase and (2) maintenance phase. The aim of induction therapy is to control renal inflammation and ideally achieve renal remission; the aim of maintenance treatment is to complete renal remission and prevent renal relapses. Gold-standard maintenance therapy is either mycophenolate mofetil or azathioprine, and progressive discontinuation of low-dose corticosteroids.[6] The addition or continuation of an antimalarial drug is also required.[6] Renal relapses nevertheless occur in 15–43% of patients after 3 years,[8–10] and 10%–20% of patients reach end-stage kidney disease (ESKD) after 10 years.[9–11] The optimal duration of immunosuppressive therapy (IST) in proliferative LN is unknown and the possibility of discontinuing IST in patients in remission is still open to debate.[3 4 12–14] While IST reduction or discontinuation before 18 months appears to be associated with a high risk of relapse,[15] with subsequent organ damage, long-term continuation of IST could be associated with higher rates of adverse events such as cardiovascular events, infections and malignancy.[2]

No randomised controlled trial (RCT) to date has prospectively assessed the possibility of maintenance IST withdrawal in proliferative LN. The hypothesis of the WIN-Lupus trial was that discontinuation of maintenance IST after 2–3 years in patients with proliferative LN who had been in remission for at least 1 year, and who were taking hydroxychloroquine, would be non-inferior to IST continuation for two more years in terms of renal relapse. The primary objective was to demonstrate non-inferiority of IST discontinuation in terms of renal relapse at 24 months. The main secondary objectives were to identify the risk factors for renal relapse and to demonstrate non-inferiority of IST discontinuation in terms of severe SLE flares (renal or extrarenal).

## METHODS

### Study design and participants

WIN-Lupus was a multicentre, two parallel-arms, randomised, non-inferiority trial conducted between February 2011 and December 2018, in 28 centres in France. The study design is described in online supplemental figure 1.

The following inclusion criteria were required: age ≥18 years, meeting at least 4/11 SLE classification criteria of the American College of Rheumatology (ACR),[16] first flare or relapse of biopsy-proven proliferative LN (ISN/RPS 2003),[7] induction treatment with high-dose corticosteroids and intravenous cyclophosphamide or mycophenolate mofetil, current maintenance IST with either azathioprine (≥50 mg/day) or mycophenolate mofetil (≥1000 mg/day or mycophenolate sodium ≥720 mg/day) for at least 2 years and a maximum of 3 years, patient in complete (proteinuria ≤0.2 g/day) or partial (proteinuria ≤0.5 g/day, or stable and considered to be related to chronic damage) renal remission, with inactive urinary sediment and normal (>90 mL/min/1.73 m²) or stable (no decrease >10%) estimated glomerular filtration rate (eGFR), as defined in the 2009 European consensus criteria,[17] for the past 12 months, current treatment with hydroxychloroquine for ≥2 months, current prednisone daily dose ≤10 mg/day, effective contraception in women of childbearing age.

Subjects were excluded from the study if they had any of the following exclusion criteria: eGFR, estimated by the Modification of Diet in Renal Disease study equation, <30 mL/min/1.73 m², pregnancy, lactation, patient wishing to become pregnant in the next 2 years, recent extrarenal flare of SLE (in the past 6 months) that required an increase in corticosteroids to >20 mg/day for at least 7 days.

The trial was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonisation Guidelines for Good Clinical Practice, addendum E6. All patients gave their written informed consent before any study-related procedure. The study, which was funded by the French Ministry of Health (PHRC 2010), was registered with the Clinical Trials identifier NCT01284725.

### Groups

The patients were divided into two groups: (1) IST continuation: maintenance IST was continued over the study period and (2) IST discontinuation: maintenance IST was tapered and discontinued over a 3-month period.

In both groups, hydroxychloroquine was continued and baseline low-dose corticosteroids (prednisone ≤10 mg/day) could be prescribed. Patients were followed up at 1 month (M1), 3 months (M3) and every 3 months thereafter until M24 postrandomisation, unless they were excluded earlier due to renal relapse, severe SLE flare or a severe adverse event.

### Randomisation

Eligible patients were randomly assigned (1:1) to the IST continuation or IST discontinuation group. The method used for randomisation was permuted block randomisation. Randomisation was stratified on a single factor: 'zone'. Three zones were defined: zone 1 including centres in the south of France, zone 2 including centres in the North of France, zone 3 including centres in the Paris region and the French West Indies. The study was not blinded to participants, investigators or data managers.

### Outcomes

The primary efficacy outcome was the percentage of patients with relapse of proliferative LN between randomisation and M24. Renal relapse was suspected in the case of confirmed proteinuria >0.5 g/24 hours, or urinary protein/creatinine ratio (UPCR) >0.5 g/g or doubling of pre-existing proteinuria, and/or recurrence of microscopic hematuria, and/or 25% increase in serum creatinine after elimination of a functional, obstructive or toxic cause. Suspected renal relapse involved a kidney biopsy to confirm (class III or class IV LN with active lesions, with or without associated class V LN) or eliminate relapse of proliferative LN.

The key secondary efficacy outcome was the percentage of patients with a severe SLE flare (renal or extrarenal), defined by the need for induction IST (high-dose steroids≥0.5 mg/kg/day and/or induction IST), between randomisation and M24.

Additional secondary outcomes included: overall patient survival, survival without renal relapse, survival without severe SLE flare, adverse events at M24 (comprising renal adverse events defined by an elevation in serum creatinine >20%, >50%, or ESKD), extrarenal SLE activity between M0 and M24 evaluated by the SLE Disease Activity Index (SELENA-SLEDAI),[18] overall exposure to corticosteroids (mean daily dose at each visit) between M0 and M24, hydroxychloroquine blood levels, health-related quality of life (QoL) (The Short Form 36 Health Survey, SF-36) and medicoeconomic impact.

### Statistical analysis

WIN-Lupus was designed as a non-inferiority trial. Non-inferiority of IST discontinuation versus continuation would be concluded if the lower limit of the 95% CI for the between-group difference was <15% for the primary outcome. At a 5% significance level, 80% statistical power and a 10% lost to follow-up

or exclusion, 100 patients per group were needed (total: 200). No interim analysis was planned.

Statistical significance was defined as $p<0.05$. The methodology was based on the extension of Consolidated Standards of Reporting Trials Statement for reporting of non-inferiority RCTs.[19]

For the primary outcome, the main analysis was performed on the per-protocol (PP) population and in the intention-to-treat (ITT) population.[20 21] Non-inferiority would be concluded if the lower limit of the 95% CI for the between-group difference (discontinuation minus continuation) was <15% renal relapses (primary analysis) in the two sets. In the case of non-significance, superiority would be tested. Renal relapse was expressed as number and percentage for each group, and as the difference D (discontinuation minus continuation) and 95% CI. For the primary outcome, survival estimates were calculated according to the Kaplan-Meier method and compared using the log-rank test.

The secondary outcomes were compared between the two groups using the $\chi^2$ test or Fisher's exact test for binary variables, and the Student's t-test for continuous variables. Evolution of the SLEDAI, exposure to corticosteroids, QoL, serum creatinine and blood hydroxychloroquine levels were compared between the two groups over the 2-year follow-up period. Risk factors for relapse were assessed by univariate analysis.

A cost analysis was also performed in which total costs were estimated and compared between the two groups. Healthcare costs related to SLE and LN management were assessed: (1) maintenance IST from inclusion to the end of follow-up; (2) hydroxychloroquine and corticosteroids; and (3) inpatient care for the management of adverse events, disease progression, disease surveillance, LN relapse or severe SLE flare. Inpatient care costs were valued based on diagnosis-related groups codes (Classification Internationale des Maladies CIM-10 coding system), and using 2018 hospital activity and associated costs from the French National Reference Costs. Indirect costs (using time lost for work activity) were also investigated.

Analysis was performed using SPSS software V.20.0.


### Role of the funding source

WIN-Lupus was an academic trial, designed by the scientific committee of the Groupe Coopératif sur le Lupus Rénal (GCLR) and funded by the French Ministry of Health (PHRC 2010). Data were collected by site investigators, compiled by the Clinical Research Department from the Assistance Publique-Hôpitaux de Marseille, and analysed by the department of Public Health from Aix-Marseille University. The scientific committee of the GCLR interpreted the data. All authors had access to the data and were responsible for the decision to submit the manuscript.


## RESULTS

A total of 125 patients were screened (figure 1) and 96 were enrolled in the trial (intention-to-treat population): 48 in the IST continuation group and 48 in the IST discontinuation group. Inclusions were interrupted after 5 years and the expected number of inclusions was not reached. As depicted in figure 1, 12 randomised patients were excluded from the study; 84 patients completed the study protocol (per-protocol population). The baseline characteristics of these 84 patients are shown in table 1. Most patients were female (84.5%), Caucasian (63.1%), and had suffered a first flare of proliferative LN (76.2%). Most patients had received low-dose intravenous cyclophosphamide as induction therapy (59.5%) and were receiving mycophenolate mofetil as maintenance IST (78.6%). The baseline characteristics of the 96 randomised patients (intent-to-treat population) are shown in online supplemental table 1.

### Primary outcome

Relapse of proliferative LN occurred in 5/40 (12.5%) patients from the IST continuation group and in 12/44 (27.3%) patients from the IST discontinuation group (p=0.710, D (95% CI): 14.8 (−1.9 to 31.5)) in the PP set and the ITT set. Non-inferiority of IST discontinuation was not demonstrated. IST continuation was not significantly superior to discontinuation in terms of relapse of proliferative LN (p=0.092). Relapses occurred after a median of 9 months (IQR: 5–17) in patients with IST continuation and 9 months (IQR: 7–14) in patients with IST discontinuation. Time to

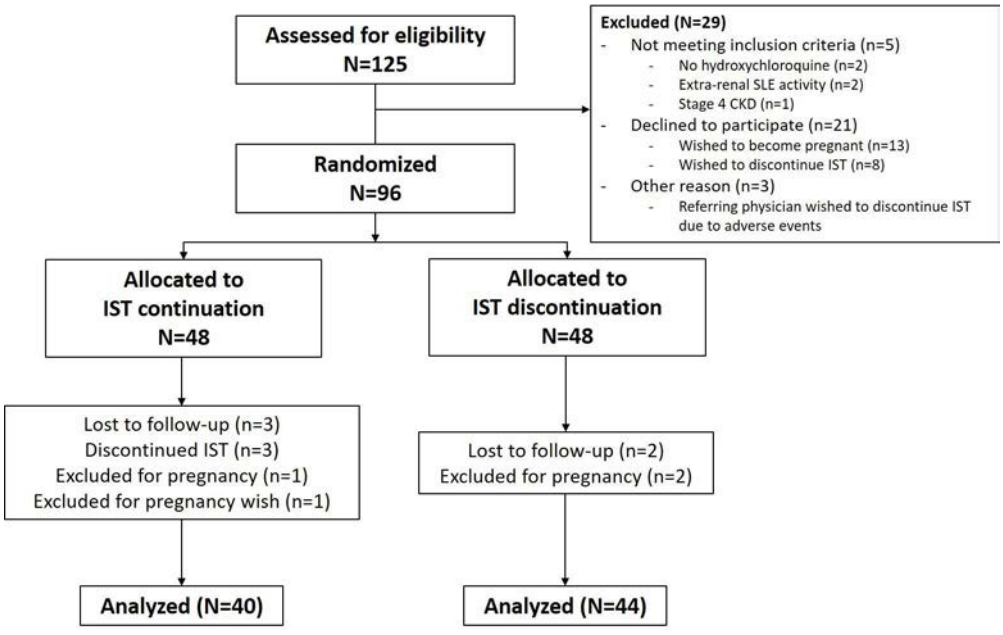


**Figure 1** Flow diagram of the study population. CKD, chronic kidney disease; IST, immunosuppressive therapy; SLE, systemic lupus erythematosus.

**Table 1** Baseline characteristics of the patients in the per-protocol population

| Characteristics | IST continuation (N=40) Mean (SD) N (%) | IST discontinuation (N=44) Mean (SD) N (%) |
|---|---|---|
| Age, years | 37.5 (14.0) | 36.7 (13.2) |
| Sex, female | 33 (82.5) | 38 (86.4) |
| Ethnicity | | |
| Caucasian | 27 (67.5) | 26 (59.1) |
| Black | 9 (22.5) | 14 (31.8) |
| Asian | 4 (10.0) | 4 (9.1) |
| SLE disease duration, years | 9.7 (10.2) | 7.6 (6.2) |
| Antiphospholipid syndrome | 5 (12.5) | 6 (13.6) |
| Menopause | 6/31 (19.4) | 10/38 (26.3) |
| Obesity (body mass index ≥30 kg/m$^2$) | 5 (12.5) | 6 (13.6) |
| Systolic blood pressure, mm Hg | 121 (13) | 116 (14) |
| Diastolic blood pressure, mm Hg | 73 (11) | 73 (10) |
| First flare of proliferative LN | 32 (80.0) | 32 (72.7) |
| Induction therapy | | |
| Low-dose intravenous cyclophosphamide | 26 (65.0) | 24 (54.5) |
| Mycophenolate mofetil | 14 (35.0) | 20 (45.5) |
| Maintenance IST | | |
| Duration, years | 2.8 (0.9) | 2.8 (0.8) |
| Mycophenolate mofetil | 30 (75.0) | 36 (81.8) |
| Azathioprine | 10 (25.0) | 8 (18.2) |
| Doses prescribed (mg/day) | | |
| Mycophenolate mofetil | 1633 (571) | 1364 (684) |
| Azathioprine | 82.5 (29) | 81.2 (39) |
| Corticosteroids | 4.3 (2.8) | 4.3 (2.8) |
| Hydroxychloroquine | 365 (89) | 334 (131) |
| Hydroxychloroquine serum level, ng/L | 861 (714) | 644 (428) |
| Serum creatinine, µmol/L | 67.7 (14.7) | 72.7 (17.2) |
| Estimated GFR, mL/min/1.73 m$^2$ | 101.6 (28.0) | 94.9 (25.8) |
| Urinary protein/creatinine ratio, g/g | 0.28 (0.38) | 0.21 (0.28) |
| Urinary protein/creatinine ratio ≤0.2 g/g | 26 (65.0) | 29 (65.9) |
| Urinary protein/creatinine ratio ≤0.5 g/g | 34 (85.0) | 42 (95.5) |
| Urinary protein/creatinine ratio ≤0.7 g/g | 35 (87.5) | 43 (97.7) |
| Serum albumin, g/dL | 4.2 (0.5) | 4.2 (0.5) |
| Haemoglobin, g/L | 1.30 (0.18) | 1.29 (0.13) |
| Leucocytes, G/L | 5.71 (1.7) | 5.58 (2.4) |
| Lymphocytes, G/L | 1.5 (0.7) | 1.39 (0.6) |
| Platelets, G/L | 255 (89) | 240 (79) |
| Low C3 | 5/38 (13.2) | 5/42 (11.9) |
| Low C4 | 4/38 (10.5) | 4/42 (9.5) |
| Positive anti-dsDNA | 24/38 (63.2) | 24/44 (54.5) |
| SLEDAI score | 2.2 (1.7) | 1.6 (1.8) |

Data are expressed as mean (SD), number (%) or number/number available (%).
GFR, glomerular filtration rate; IST, immunosuppressive therapy; LN, lupus nephritis; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index.

renal flare, compared by Kaplan-Meier survival curves (log rank test), did not differ between the groups (p=0.079) (figure 2A). The baseline individual characteristics of the 17 patients who relapsed, as well as renal presentation and pathology at the time of LN relapse, are presented in online supplemental table 2.

### Secondary outcomes

There were significantly more severe SLE flares in patients in the IST discontinuation group compared with the IST continuation group (14/44 (31.8%) vs 5/40 (12.5%) patients, p=0.035, D (95% CI) 19.3% (CI 1.3% to 35.7%)), and time to severe SLE flares was shorter in patients in the IST discontinuation group (log rank test, p=0.034) (figure 2B).

The adverse events in the 96 randomised patients are shown in table 2. There was no significant difference between the groups.

The evolution over time of several clinical (SLEDAI, dose of corticosteroids, SF-36 mental and physical component summaries) and biological (UPCR, serum creatinine, blood hydroxychloroquine levels) parameters through the study period are shown in online supplemental figure 2. Extrarenal SLE activity, evaluated by the SLEDAI and exposure to corticosteroids, did not differ between the two groups.

IST discontinuation was less costly than IST continuation in terms of maintenance therapy (−83%; p<0.001), but more costly in terms of inpatient care (+61%, p=0.027) (online supplemental table 3). No difference was found in indirect costs. Overall, patients from the IST discontinuation group had lower costs compared with the IST continuation group (−40%; p=0.001).

### Risk factors for proliferative ln relapse

The risk factors for proliferative LN relapse (univariate analysis) are shown in table 3. Antiphospholipid syndrome, higher UPCR at baseline, low C3 and higher SLEDAI at inclusion were associated with LN relapse. Higher eGFR, lower serum albumin, lower haemoglobin level and lower leucocyte, lymphocyte, and eosinophil counts were also associated with LN relapse.

The risk factors for severe SLE flares (univariate analysis) are shown in online supplemental table 4.

The risk factors for LN relapse among patients in the IST discontinuation group are shown in online supplemental table 5. The risk factors for severe SLE flares among patients in the IST discontinuation group are shown in online supplemental table 6.

## DISCUSSION

In this multicentre RCT, non-inferiority of IST discontinuation after 2–3 years was not demonstrated, although IST continuation was not significantly superior regarding LN relapse. IST discontinuation was associated with a higher risk of severe SLE flares (renal or extrarenal) requiring induction IST. No patient developed kidney failure and only two patients, with IST discontinuation, experienced an increase in serum creatinine ≥50%. Health-related costs were lower in the IST discontinuation group. Exposure to corticosteroids and adverse events did not differ between the groups.

In patients with proliferative LN, the possibility of discontinuation of maintenance IST, and the optimal timing for this discontinuation, is poorly defined. In a national survey conducted in France in 2012 among LN specialists,[22] 40% stated that they continued maintenance IST for 2 years in patients who were stable in remission, 25% continued for 3 years, 25% for 4–5 years and 9% for >5 years. Different expert recommendations on the treatment of proliferative LN were published in 2012.[23] While the European Alliance of Associations for Rheumatology (EULAR)/ERA-EDTA[24] recommended to continue maintenance IST for at least 3 years after induction therapy, the Kidney Disease Improving Global Outcome (KDIGO)[25] proposed to continue maintenance IST for at least 1 year after clinical remission before considering tapering, and the ACR[26] highlighted the need for evidence-based data to determine the optimal duration of maintenance IST. A recent update of the EULAR/ERA-EDTA recommendations proposed the gradual withdrawal of treatment

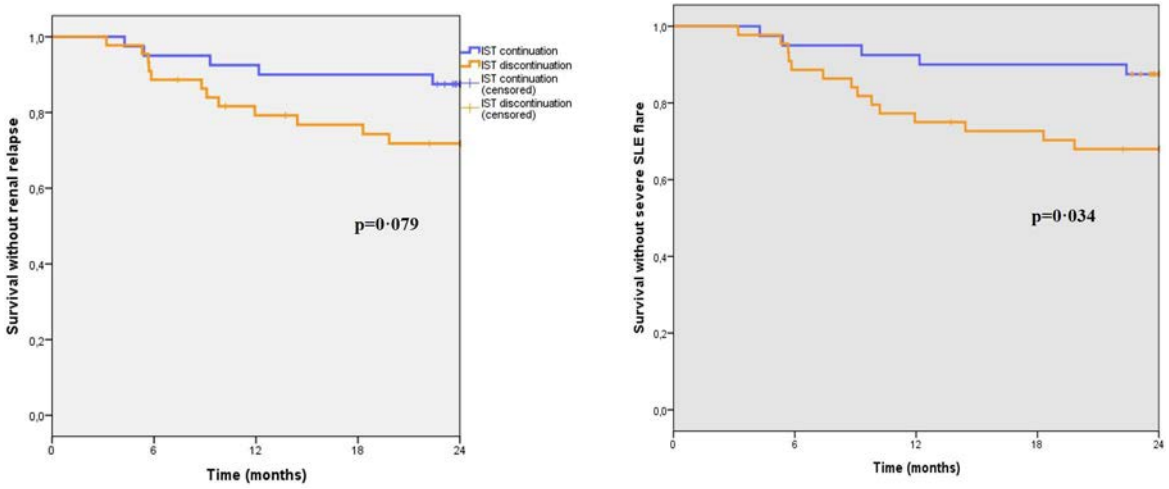


**Figure 2** Survival analyses. (A) Survival without renal relapse. (B) Survival without severe SLE flare (online supplemental material). IST, immunosuppressive therapy; SLE, systemic lupus erythematosus.

(corticosteroids first, then immunosuppressive drugs) after at least 3–5 years in patients with a complete clinical response (grade 2b/C recommendation).[6] Similarly, the KDIGO 2021 update proposed a minimum of 3 years of IST.[27]

Moroni *et al*[28] tested the discontinuation of IST and corticosteroids in 73 patients with LN who had achieved a stable clinical remission. A flare requiring treatment reinforcement was observed in 21 (29%) patients during treatment tapering, which is consistent with the 25% rate of renal relapse (and 32% of overall severe relapse) observed here in patients with IST discontinuation. Zen *et al* recently reported the outcomes of 83 patients in remission after LN, for whom IST was discontinued after a mean of 3.8 years.[29] After a mean follow-up of 9.7 years, 8 (10%) patients had an LN relapse and 19 (23%) patients experienced a flare of SLE. Younger age at IST discontinuation and the absence of HCQ prescription were associated with LN flares. This risk of relapse should be considered against the high proportion of patients who were weaned from IST without relapse.

Rather than generalising long-term continuation of maintenance IST to prevent relapses, the challenge consists of identifying patients who can safely be weaned from IST. Here, SLEDAI at inclusion, C3 consumption and lower lymphocyte count were predictive of renal relapse and of severe SLE flare. Other biological parameters, possibly associated with residual inflammation (lower serum albumin, lower haemoglobin), were also predictive of relapse and severe SLE flares. Higher eGFR was also a risk factor for relapse, which is of interest as SLE disease activity may decrease as kidney function declines.[30] Different thresholds (0.2, 0.5 or 0.7 g/g), routinely used in clinical trials to define LN complete remission or response, were predictive of risk of relapse. Proteinuria in patients with a previous flare of LN can result from persistent active lesions, or from chronic glomerular damage. No repeat kidney biopsy was warranted in WIN-Lupus to ascertain histological remission, and patients with higher initial UPCR may have presented ongoing histological activity. Of note, histological activity can be observed even in patients with complete clinical remission,[31 32] and the performance of a repeat kidney biopsy to confirm histological remission before IST weaning could become a new standard.

de Rosa *et al*[31] suggested that repeat biopsy could allow the selection of patients with pathological remission for IST discontinuation. Malvar *et al*[33] proposed a kidney biopsy-based management of maintenance IST for proliferative LN. Among 69 patients with histological remission and IST discontinuation, only 7 (10%) experienced a renal relapse.

The main motivation for IST discontinuation is to limit the burden of adverse events related to immunosuppression. Here, we evaluated the discontinuation of maintenance IST (mycophenolate mofetil or azathioprine), but all patients were on hydroxychloroquine, and low-dose corticosteroids could be prescribed. The reason we permitted the continuation of low-dose corticosteroids in this trial was to allow flexibility in controlling the extrarenal manifestations of lupus. Indeed, in a national survey,[22] 55% of physicians managing patients with LN reported continuing low-dose corticosteroids in maintenance. WIN-Lupus was not a trial of complete treatment withdrawal, as reported in recent cohort studies.[4] One of the possible reasons for clinicians to maintain IST is to prevent the need for corticosteroid use or increase,[34] which is itself associated with significant damage.[35 36] Here, the overall exposure to corticosteroids did not differ between the groups, indicating that IST discontinuation did not lead to a compensatory increase in corticosteroid dose for lupus containment. Blood levels of hydroxychloroquine were not associated with renal relapses or severe flares in this cohort, but only a minority of patients had low blood levels of hydroxychloroquine. In the present trial, the absence of a difference in adverse events between the groups could be related to the relatively short follow-up, which does not allow us to conclude on the absence of benefit of treatment withdrawal long term.

From an economic perspective, IST discontinuation was associated with cost savings due to lower maintenance therapy costs, while inpatient costs increased due to relapse care.

This study has several limitations. First, we did not reach the 200 inclusions that were expected, and the scientific committee decided to end patient recruitment after 5 years due to the slow inclusion rate (mainly related to the strict inclusion criteria, and to the exclusion of patients wishing to become pregnant within 2 years). The trial is thus underpowered, and superiority of treatment continuation could have been demonstrated with more patients. Second, block randomisation was applied to limit allocation bias, but only randomisation zone was used for stratification. Other clinically relevant factors could have been taken into account, such as induction and maintenance therapies, or initial doses of corticosteroids. Yet, these factors were well balanced between groups. Third, it was an open label and not double-blinded trial, due to budget constraints. Yet, the primary

**Table 2** Adverse events

| | IST continuation (N=48) | IST discontinuation (N=48) | All patients (N=96) |
|---|---|---|---|
| Death | 0 | 0 | 0 |
| Renal adverse events | 14 | 18 | 32 |
| Serum creatinine +20% | 14 | 16 | 30 |
| Serum creatinine +50% | 0 | 2 | 2 |
| End-stage kidney disease | 0 | 0 | 0 |
| Infections | 19 | 14 | 33 |
| Severe | 1 | 3 | 4 |
| Appendicitis | 0 | 1 | 1 |
| Malaria | 0 | 1 | 1 |
| Zoster | 1 | 1 | 2 |
| Other | 18 | 11 | 29 |
| Lower urinary tract | 6 | 4 | 10 |
| Upper respiratory tract | 4 | 4 | 8 |
| Ear, nose, and throat | 2 | 1 | 3 |
| Erysipelas | 1 | 1 | 2 |
| Dermatomycosis | 2 | 0 | 2 |
| Cervical human papillomavirus | 2 | 1 | 3 |
| Warts | 1 | 0 | 1 |
| Haematological | 41 | 48 | 89 |
| Myelodysplastic syndrome | 1 | 0 | 1 |
| Hypereosinophilia | 1 | 0 | 1 |
| Haematoma | 0 | 1 | 1 |
| Anaemia with Hb <10 g/dL | 5 | 2 | 7 |
| Anaemia with Hb <8 g/dL | 1 | 0 | 1 |
| Leucopenia <4 G/L | 16 | 17 | 33 |
| Leucopenia <3 G/L | 0 | 4 | 4 |
| Neutropenia <1.5 G/L | 3 | 7 | 10 |
| Neutropenia <1 G/L | 0 | 1 | 1 |
| Lymphopenia <1 G/L | 12 | 16 | 28 |
| Lymphopenia <0.5 G/L | 1 | 0 | 1 |
| Thrombopenia <100 G/L | 1 | 0 | 1 |
| Other | 3 | 6 | 9 |
| Cataract | 1 | 1 | 2 |
| Alopecia | 0 | 2 | 2 |
| Rash unrelated to SLE | 1 | 0 | 1 |
| New-onset hypertension | 1 | 0 | 1 |
| Obstructive sleep apnoea | 0 | 1 | 1 |
| Unexplained chest pain | 0 | 1 | 1 |
| Unexplained transient dyspnoea | 0 | 1 | 1 |

Hb, haemoglobin; IST, immunosuppressive therapy; SLE, systemic lupus erythematosus.

**Table 3** Risk factors for renal relapse at inclusion (per-protocol population)

| | Relapse (N=17) | No relapse (N=67) | |
|---|---|---|---|
| | Mean (SD) N (%) | Mean (SD) N (%) | P value |
| Age, years | 32.4 (11.7) | 38.3 (13.8) | 0.111 |
| Sex, female | 17 (100.0) | 54 (80.6) | 0.061 |
| Ethnicity | | | 0.395 |
| Caucasian | 9 (52.9) | 44 (65.7) | – |
| Black | 5 (29.4) | 18 (26.9) | – |
| Asian | 3 (17.6) | 5 (7.5) | – |
| SLE disease duration, years | 8.1 (5.9) | 8.7 (8.9) | 0.764 |
| Antiphospholipid syndrome | 5 (29.4) | 6 (9.0) | 0.041 |
| Menopause | 1 (5.9) | 15/52 (28.8) | 0.094 |
| Obesity | 2 (11.8) | 9 (13.4) | 1.00 |
| First flare of proliferative LN | 12 (70.6) | 52 (77.6) | 0.537 |
| Induction therapy with: | | | 0.947 |
| Intravenous cyclophosphamide | 10 (58.8) | 40 (59.7) | |
| Mycophenolate mofetil | 7 (41.2) | 27 (40.3) | |
| Maintenance IST duration at M0, years | 2.6 (1.0) | 2.9 (0.7) | 0.231 |
| Maintenance IST | | | 0.753 |
| Mycophenolate mofetil | 13 (76.5) | 53 (79.1) | – |
| Azathioprine | 4 (23.5) | 14 (20.9) | – |
| Doses prescribed at M0, mg/day | | | |
| Mycophenolate mofetil | 1500.0 (277.3) | 1510.2 (639.3) | 0.956 |
| Azathioprine | 75.0 (28.9) | 82.7 (35.9) | 0.676 |
| Corticosteroids | 4.8 (3.3) | 4.1 (2.6) | 0.436 |
| Hydroxychloroquine | 332 (142) | 354 (105) | 0.455 |
| Serum hydroxychloroquine level, ng/L | 787 (494) | 722 (598) | 0.464 |
| Serum hydroxychloroquine level <200 ng/L | 2/15 (13.3) | 12/53 (22.6) | 0.719 |
| Serum creatinine, µmol/L | 63.8 (10.4) | 71.9 (17.0) | 0.052 |
| Estimated GFR, mL/min/1.73 m² | 107.2 (24.0) | 95.8 (27.3) | 0.046 |
| Chronic kidney disease stage | | | 0.134 |
| Stage 1 | 14 (82.4) | 38 (56.7) | – |
| Stage 2 | 2 (11.8) | 24 (35.8) | – |
| Stage 3 | 1 (5.9) | 5 (7.5) | – |
| Urinary protein/creatinine ratio, g/g | 0.548 (0.550) | 0.169 (0.187) | 0.001 |
| Urinary protein/creatinine ratio ≤0.2 g/g | 5 (29.4%) | 50 (74.6%) | <0.001 |
| Urinary protein/creatinine ratio ≤0.5 g/g | 12 (70.6) | 64 (95.5) | 0.007 |
| Urinary protein/creatinine ratio ≤0.7 g/g | 12 (70.6) | 66 (98.5) | 0.001 |
| Serum albumin, g/dL | 3.9 (0.4) | 4.3 (0.4) | 0.004 |
| Haemoglobin level, g/L | 1.19 (0.14) | 1.32 (0.14) | 0.003 |
| Leucocyte count, g/L | 4.8 (2.4) | 5.8 (1.9) | 0.011 |
| Neutrophil count, g/L | 3.3 (2.4) | 3.8 (1.9) | 0.204 |
| Lymphocyte count, g/L | 1.0 (0.4) | 1.5 (0.6) | 0.003 |
| Basophil count, g/L | 0.01 (0.01) | 0.02 (0.02) | 0.128 |
| Eosinophil count, g/L | 0.04 (0.04) | 0.10 (0.14) | 0.049 |
| Platelet count, g/L | 289 (130) | 237 (65) | 0.109 |
| Low C3 | 6/16 (37.5) | 4/64 (6.3) | 0.003 |
| Low C4 | 3/16 (18.8) | 4/64 (7.8) | 0.194 |
| Positive anti-dsDNA | 13 (76.5) | 36 (53.8) | 0.092 |
| SLEDAI score | 3.1 (2.6) | 1.6 (1.4) | 0.025 |

Data are expressed as % or mean (SD). In the case of missing data, the number/number available (%) is indicated.
GFR, glomerular filtration rate; IST, immunosuppressive therapy; LN, lupus nephritis; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index.

endpoint was strictly defined and documented by kidney biopsy. Fourth, we did not select patients with appropriate adherence to treatment, but rather chose real-life patients, who were prescribed an antimalarial and mycophenolate mofetil or azathioprine, which they declared they were taking. In addition, low-dose corticosteroids was defined as a daily dose of ≤10 mg/day and not as a daily dose of <7.5 mg/day. Moreover, LN relapses can occur several years after IST discontinuation[28] and late relapses were not captured by this study. A 2-year follow-up may also have been too short to determine the impact of IST continuation or discontinuation on long-term kidney function. Finally, selection bias is possible, as investigators may have refrained from including patients who had previously relapsed (small proportion of patients with a previous history of relapse), or those who were in complete remission and were willing to discontinue IST.

This study also has strengths. WIN-Lupus is the first RCT to compare maintenance IST continuation with IST discontinuation in patients with proliferative LN. The patients included were

homogeneous in terms of organ involvement (biopsy-proven proliferative LN), duration of maintenance IST (2–3 years), duration of remission (≥1 year) and all patients were prescribed hydroxychloroquine. Second, although patients were included over several years, the gold-standard therapeutic strategy for proliferative LN remained the same during the study period.

To conclude, non-inferiority of maintenance IST discontinuation after 2–3 years was not demonstrated for renal relapses in patients with proliferative LN. IST discontinuation was associated with a higher risk of severe SLE flares. Nonetheless, a majority of patients did not relapse at 2 years after IST discontinuation. The most important challenge remains the identification and selection of patients who can be safely weaned from IST.

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**Contributors** NJ-C, KB, ZA and ED designed the study. NC-C, LB, SB, VC, LCh, LCo, CD, BD, SF, PG, GG, AHua, AHum, EK, AK, ML, VLG, LL, HM-L, FM, MP, VQ, PR, FS-R, DV, EH, ZA and ED included patients in the trial, revised the manuscript, and provided additional data for the revised version of the manuscript. LD supervised interpretation of kidney biopsy results. NJ-C, KB, AL and SL analysed the data, KB and AL performed the statistical analyses, SL did the medicoeconomic analyses. NJ-C wrote the draft of the manuscript. NJ-C, NC-C, KB, EH and ED revised the manuscript. All authors read the final manuscript and gave their consent for publication. Non-author contribution: Professor Jacques Pourrat and Professor Bruno Moulin participated in the initial phase of study design. Elisabeth Castanier, Sophie Tardoski and Julien Faraut collected data. Patrick Sudour supervised the conduct of the study and monitored data collection. Richard Malkoun was the data manager of the study. NJ-C is responsible for the overall content as guarantor. She accepts full responsibility for the finished 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.

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TRANSLATIONAL SCIENCE

# Hypomethylation of miR-17-92 cluster in lupus T cells and no significant role for genetic factors in the lupus-associated DNA methylation signature

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## ABSTRACT

**Objectives** Lupus T cells demonstrate aberrant DNA methylation patterns dominated by hypomethylation of interferon-regulated genes. The objective of this study was to identify additional lupus-associated DNA methylation changes and determine the genetic contribution to epigenetic changes characteristic of lupus.

**Methods** Genome-wide DNA methylation was assessed in naïve CD4$^+$ T cells from 74 patients with lupus and 74 age-matched, sex-matched and race-matched healthy controls. We applied a trend deviation analysis approach, comparing methylation data in our cohort with over 16 500 samples. Methylation quantitative trait loci (meQTL) analysis was performed by integrating methylation profiles with genome-wide genotyping data.

**Results** In addition to the previously reported epigenetic signature in interferon-regulated genes, we observed hypomethylation in the promoter region of the miR-17-92 cluster in patients with lupus. Members of this microRNA cluster play an important role in regulating T cell proliferation and differentiation. Expression of two microRNAs in this cluster, miR-19b1 and miR-18a, showed a significant positive correlation with lupus disease activity. Among miR-18a target genes, *TNFAIP3*, which encodes a negative regulator of nuclear factor kappa B, was downregulated in lupus CD4$^+$ T cells. MeQTL identified in lupus patients showed overlap with genetic risk loci for lupus, including *CFB* and *IRF7*. The lupus risk allele in *IRF7* (rs1131665) was associated with significant *IRF7* hypomethylation. However, <1% of differentially methylated CpG sites in patients with lupus were associated with an meQTL, suggesting minimal genetic contribution to lupus-associated epigenotypes.

**Conclusion** The lupus defining epigenetic signature, characterised by robust hypomethylation of interferon-regulated genes, does not appear to be determined by genetic factors. Hypomethylation of the miR-17-92 cluster that plays an important role in T cell activation is a novel epigenetic locus for lupus.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Lupus is characterised by robust DNA hypomethylation in interferon-regulated genes; however, the genetic contribution to the lupus-associated epigenotype is unknown.

### WHAT THIS STUDY ADDS

⇒ Our results suggest that genetic factors do not significantly contribute to the lupus-associated DNA methylation profiles.

⇒ We also report a novel epigenetic locus for lupus in a microRNA cluster involved in T cell function.

⇒ Furthermore, we provide a prototype example showing how a lupus risk genetic variant might mediate functional pathogenic effects through altering DNA methylation levels.

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

⇒ This study highlights the importance of non-genetic factors in determining epigenetic changes characteristic of lupus.

## INTRODUCTION

Systemic lupus erythematosus (lupus or SLE) is a heterogeneous autoimmune disease of incompletely understood aetiology. The disease is characterised by a loss of immunotolerance and the development of autoantibodies against nuclear antigens. Severe manifestations of lupus have significant impact on quality of life and can lead to organ damage and mortality in affected patients, particularly among patients of non-European genetic ancestry.[1 2] Genetic risk contributes to the development of lupus, but the estimated heritability of lupus is ~30%.[3–5] Indeed, monozygotic twin studies in lupus suggest a substantial non-genetic contribution to the aetiology of lupus.[6] Environmental exposures across the lifespan that can directly impact epigenetic regulation and cellular function are suggested to be involved in the pathogenesis of lupus.[7 8]

DNA methylation is an epigenetic mechanism that regulates gene expression through the enzyme-mediated addition of a methyl group to the cytosine bases in the genome. DNA methylation is heritable across cell generations and can promote gene silencing, making it an important component in regulating the plasticity of immune cell identity and function.[9] Early work demonstrated that adoptive transfer of CD4$^+$ T cells treated ex vivo with DNA methyltransferase (DNMT) inhibitors was sufficient to cause lupus-like disease in mice,[10] mimicking the DNA methylation inhibition in patients with drug-induced lupus.[11] Since then, other studies have

observed that CD4+ T cells of patients with lupus show a distinct shift in global DNA methylation compared with healthy individuals, potentially in part due to defective MEK/ERK signalling, suppressing DNA methyltransferase 1 (DNMT1) activity in CD4+ T cells, and leading to hypomethylation and overexpression of costimulatory genes.[12–16]

We have previously observed a robust hypomethylation signature in interferon-regulated genes defining patients with lupus.[17 18] Our initial findings in CD4+ T cells were subsequently confirmed and expanded to other cell types by our group and others.[19–21] In CD4+ T cells, we observed hypomethylation in interferon-regulated genes at the naïve CD4+ T cell stage, preceding transcriptional activity. This epigenetic 'poising' or 'priming' of interferon-regulated genes was independent of disease activity.[18] The genetic contribution to this lupus-associated epigenotype is currently unknown.

Methylation quantitative trait loci (meQTL) are genetic polymorphisms that are associated with the methylation state of CpG sites either through direct nucleotide change within the CpG dinucleotide or intermediary mechanisms. Prior studies of patients with lupus show an enrichment of meQTL associated with type I interferon genes, genetic risk loci and specific clinical manifestations in whole blood and neutrophils.[22–24] Furthermore, our previous work suggests that meQTL might at least in part explain differences in DNA methylation between African-American and European-American patients with lupus.[22]

Herein, we evaluated genome-wide DNA methylation data in naïve CD4+ T cells from a large cohort of patients with lupus compared with matched healthy controls. We integrated DNA methylation and genotyping data to better understand the influence of genetic factors on the DNA methylation changes observed in lupus.

## METHODS

### Study participants and demographics

Seventy-four female patients with lupus and 74 female healthy age-matched (±5 years) and race-matched controls were recruited as previously described[25 26] (online supplemental table 1). All patients fulfilled the American College of Rheumatology classification criteria for SLE.[27]

### Sample collection, DNA isolation and data generation

Genomic DNA samples for this study were collected from naïve CD4+ T cells as previously described.[18] Briefly, magnetic beads and negative selection was used to isolate naïve CD4+ T cells from whole blood samples collected from patients with lupus and controls. Genomic DNA was directly isolated from collected cells and bisulfite converted using the EZ DNA Methylation Kit (Zymo Research, Irvine, California, USA). The Illumina HumanMethylation450 BeadChip (Illumina, San Diego, California, USA) was used to measure DNA methylation levels at over 485 000 methylation sites across the genome.

### Epigenome-wide association study

Epigenome-wide association study (EWAS) for identifying associations between specific CpG sites and disease status was performed using GLINT.[28 29] Covariates for age, race and technical batch were included for the analysis prior to other preprocessing. No outliers beyond 4 SD were detected in the first two components of the principal component analysis (PCA) space, all 148 samples were included in the analysis. Reference-less cell type composition correction was performed using *ReFACTor*, with six components used in the downstream analysis to account for any cell-type heterogeneity in the samples. An additional covariate was included to account for effects of genetic admixture using the EPISTRUCTURE algorithm included in GLINT. Cell-type composition covariate components generated by *ReFACTor* were included at this step to reduce bias from potential cell-type heterogeneity, and polymorphic CpG sites were excluded from this step and the EWAS. Using the age, race and technical batch covariates, along with six *ReFACTor* components and one *EPISTRUCTURE* component, logistic regression for disease status was performed across all CpG sites, excluding the polymorphic and unreliable cross-reactive probes previously described in the literature, as well as CpG sites with low variance (SD <0.01).[30 31]

### Differential DNA methylation analysis of gene promoters

Raw .idat files were used to generate methylation beta value profiles across all samples using GenomeStudio (Illumina) after background subtraction and normalising to internal control probes. Missing probe values were imputed using *sklearn.impute.KNNImputer*, and *ComBat* was used to correct for batch effects associated with sample preparation.[32–34] Ensembl gene loci for hg19 were downloaded using *PyEnsembl*.[35] For each gene, loci for 1500 base pairs upstream of the transcription start site[36] to the transcription start site (TSS) were mapped to the overlapping CpG probes using *PyBedtools*, and the mean of the associated probes for each gene was used as the representative methylation value for the resulting 20 437 mapped genes.[37] Differential methylation analysis comparing patients and controls was performed on the mean TSS1500 methylation using *limma*, and false discovery rate adjustment using the Benjamini-Hochberg method was used to correct p values for multiple testing. Gene Ontology Enrichment for Biological Process terms was performed on the differentially methylated gene list using *Enrichr* with the mapped promoter gene list used as the background.[38 39]

### Trend deviation analysis

DNA methylation data derived using the Illumina 450k methylation array from 23 415 samples were downloaded from Gene Expression Omnibus (GEO).[40] To reduce batch effects, samples from experiments with fewer than 50 samples were omitted, and the remaining samples were quantile normalised.[41] A matrix of pairwise Pearson's correlation values for DNA methylation levels was computed across TSS1500 gene promoters in 16 541 samples across 37 tissues to create a multitissue correlation network (online supplemental figure 1). The differentially methylated genes in lupus-naïve CD4+ T cells were clustered by their correlation in global signature created from the GEO data. Hierarchical clustering was performed using *Scipy*'s hierarchical linkage. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed using *Enrichr*,[42] and p values were reported after false discovery rate (FDR) adjustment.

The goal of a trend deviation analysis is to detect correlation patterns among differentially methylated genes in large DNA methylation datasets. A correlation in methylation among a set of differentially methylated genes between patients and controls suggests a trend is being observed, reinforcing the significance and robustness of the differential DNA methylation detected between patients and controls.

### Genotyping

Genomic DNA isolated from naïve CD4+ T cells was used as input for the Infinium Global Screening Array-24 V.2.0 (Illumina). Single nucleotide polymorphisms (SNPs) with a genotyping call

rate <98%, minor allele frequencies (MAF) <5% and deviating from Hardy-Weinberg equilibrium (p<1E-3) were filtered out. Samples were removed if they had a genotyping call rate <95%. Sex chromosomes were not analysed. About 100 000 independent SNPs were pruned and used to perform PCA with *Eigensoft* (V.6.1.4) software.[43] Genotyping data were analysed using *PLINK* (V.1.9).[44] Genotype profiles were generated for n=63 patients and n=68 controls.

### Methylation quantitative trait loci analysis

Raw .idat files were used to generate methylation profiles using *minfi* (V.1.32.0)[45 46] and to check median intensity values and reported sex in the R statistical computing environment (V.3.6.3).[47] Probes with less than three beads and zero intensity values across all samples were removed using the *DNAmArray* package (V.0.1.1).[48] Background signal and dye bias were corrected, followed by normalisation of signal intensities using functional normalisation in the *preprocessFunnorm.DNAmArray* function[48 49] using the first three principal component values calculated from signal intensities of control probes present on all array spots to correct for technical variation. Any probe with a detection p<0.01 or returned signal intensities in fewer than 98% of samples was removed. This resulted in a total of 476 767 probes used for further analysis. Signal intensities were then converted to M values with a maximum bound of ±16. M values were used for meQTL analysis and converted to beta values (0%–100% methylation scale) using *minfi* for reporting.

We removed any probe for meeting any of the following technical criteria: a unique probe sequence of <30 bp, mapping to multiple sites in the genome, polymorphisms that cause a colour channel switching in type I probes, inconsistencies in specified reporter colour channel and extension base, mapping to the Y chromosome and/or having a polymorphism within 5 bp of the 3' end of the probe with a MAF >1% with the exception of CpG-SNPs with C>T polymorphisms which were retained.[50] Batch correction for chip ID was performed using the *ComBat* function in the *sva* (V.3.34.0) package.[51] After technical filtering, there were a total of 421 214 probes used for meQTL analysis.

We implemented a mixed correspondence analysis with the *PCAmixdata* package (V.3.1)[52] to calculate eigenvalues using patient medication data for prednisone, hydroxychloroquine, azathioprine, mycophenolate mofetil and cyclophosphamide. The top four components accounted for a cumulative 89.3% of variability in the medication data. Each component value was used as an independent variable in regression analysis to adjust for medication usage across individuals. MeQTL association analysis was performed in patients and controls separately using methylation M value profiles and corresponding sample genotypes. Age, the top 4 medication components and top 10 genotype principal components were included as covariates to build a linear model for detecting meQTL using *MatrixEQTL* (V.2.3).[53] *Cis*-meQTL were defined as CpG sites with methylation values associated with an SNP within a conservative 1000 bp of the CpG dinucleotide. We used a Benjamini-Hochberg FDR-adjusted p value cut-off of <0.05 as a threshold for significant associations. The above EWAS results were compared with the meQTL results to determine overlap of lupus-associated differentially methylated CpG sites and those CpG sites in an meQTL.

### Functional enrichment analysis

ToppGene Suite was used for functional enrichment analysis[54] of Molecular Function and Biological Process Gene Ontologies and KEGG pathways in meQTL. P values were derived using a hypergeometric probability mass function, and a Benjamini-Hochberg FDR-adjusted p value cut-off of <0.05 was used as a threshold of significance. A minimum membership of 3 genes and maximum of 2000 genes in each term was used as a threshold for inclusion. Interferon-regulated genes were identified using the set of genes associated with the CpG site in each meQTL as input using Interferome (V.2.01).[55] The type I interferon response genes were defined as genes with an expression fold change of 1.5 or greater between type I interferon-treated and untreated samples using gene expression datasets from all available $CD4^+$ T cell experiments in the Interferome database.

For the analysis of miR-18a-regulated genes, literature-based network association analysis was performed using IRIDESCENT to create a weighted network of published relationships as previously described.[56]

### MicroRNA expression microarray

MicroRNA (miRNA) expression was measured in naïve $CD4^+$ T cells from a subset of patients with lupus and healthy matched controls (n=16). Cells were immediately lysed with TRIzol Reagent (ThermoFisher Scientific, New York, USA) followed by storage at −80°C. Total RNA was isolated using the Direct-zol RNA MiniPrep Kit (Zymo Research, California, USA) following the manufacturer's directions. The Affymetrix miRNA V.4.1 Array Strip (Affymetrix, California, USA) was used to measure expression of over 2000 premature and 2500 mature human miRNA sequences. RNA sequences were polyadenylated and ligated to a biotin-labelled oligomer using the FlashTag Biotin HSR RNA Labeling Kit (Affymetrix). Biotin-labelled sequences were hybridised to array probes and washed then stained with streptavidin-phycoerythrin (PE). The Affymetrix Expression Console & Transcriptome Analysis Console V.2.0 software (Affymetrix) was used to analyse biotin/streptavidin-PE fluorescence measurements. All samples passed signal intensity, polyadenylation and ligation quality controls. Signal intensities were background adjusted and normalised. Log2-transformed expression values for each probeset was calculated using a robust multi-array average model.[23] The Pearson's r correlation coefficient for median expression values of probes for miR-17, miR-18a, miR-19a, miR-19b1 and miR-20a and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores were calculated using GraphPad Prism (V.9.3.0) (GraphPad Software, California, USA).

## RESULTS

### Differential methylation of gene promoters in naïve $CD4^+$ T cells isolated from patients with lupus

A comparison of DNA methylation profiles from circulating naïve $CD4^+$ T cells isolated from 74 patients with lupus and 74 age-matched, sex-matched and race-matched healthy controls revealed a total of 2627 CpGs, out of 334 337 total CpG sites included in the EWAS, with a significant difference in average methylation. Significant hypomethylation in interferon-regulated genes was observed, consistent with previous reports (online supplemental table 2). Average promoter methylation for each gene was calculated by including all CpG sites on the array within 1500 bp of the associated gene's TSS. A total of 51 genes showed a significant difference in average promoter methylation between patients with lupus and controls (17 hypomethylated and 34 hypermethylated in patients compared with controls) (table 1) (figure 1). Biological Process Gene Ontology enrichment analysis of differentially methylated promoter regions did not show significant enrichment compared with the background

**Table 1** Genes with differentially methylated promoter regions in naive CD4$^+$ T cells of patients with lupus compared with healthy controls

| Gene | Δβ | $-\log_{10}$ (FDR-adjusted p value) | t-statistic |
|---|---|---|---|
| *IFI44L* | −0.177 | Infinity | −10.757 |
| *DTX3L* | −0.130 | Infinity | −11.566 |
| *BST2* | −0.089 | 11.323 | −9.285 |
| *RABGAP1L* | −0.088 | 9.165 | −8.421 |
| *BCL2L14* | −0.086 | 5.520 | −6.908 |
| *MIR19B1* | −0.059 | 3.169 | −5.846 |
| *IFI44* | −0.059 | 2.057 | −5.304 |
| *MIR20A* | −0.055 | 3.088 | −5.807 |
| *MIR17* | −0.054 | 6.882 | −7.487 |
| *MIR18A* | −0.051 | 6.537 | −7.342 |
| *MIR19A* | −0.049 | 4.771 | −6.579 |
| *IKZF4* | −0.048 | 3.289 | −5.902 |
| *MX1* | −0.046 | 10.624 | −9.004 |
| *TRIM34* | −0.045 | 2.184 | −5.367 |
| *ODF3B* | −0.034 | 1.712 | −5.128 |
| *GNG2* | −0.033 | 2.138 | −5.344 |
| *FAM177B* | −0.025 | 1.897 | −5.223 |
| *MZF1* | 0.008 | 1.493 | 5.014 |
| *SSBP4* | 0.015 | 1.344 | 4.934 |
| *ATP6V0D1* | 0.018 | 2.594 | 5.569 |
| *DCUN1D1* | 0.025 | 2.068 | 5.309 |
| *C14orf93* | 0.025 | 1.922 | 5.236 |
| *TIPARP* | 0.026 | 2.069 | 5.310 |
| *LMBRD1* | 0.027 | 2.211 | 5.381 |
| *HAVCR2* | 0.027 | 2.574 | 5.560 |
| *KIAA1949* | 0.030 | 3.158 | 5.841 |
| *GPD2* | 0.032 | 1.953 | 5.251 |
| *CNTF* | 0.033 | 1.705 | 5.124 |
| *CD47* | 0.034 | 4.259 | 6.350 |
| *ARHGAP9* | 0.036 | 3.339 | 5.926 |
| *IL27RA* | 0.036 | 1.367 | 4.946 |
| *RAP1A* | 0.036 | 2.573 | 5.559 |
| *LAMA3* | 0.037 | 1.445 | 4.988 |
| *ABI3* | 0.037 | 1.436 | 4.983 |
| *FAM102A* | 0.038 | 3.161 | 5.842 |
| *CXCR5* | 0.039 | 1.439 | 4.985 |
| *DPEP2* | 0.040 | 1.889 | 5.219 |
| *DYRK2* | 0.041 | 3.924 | 6.197 |
| *TMEM71* | 0.044 | 2.757 | 5.649 |
| *ADORA2A* | 0.046 | 2.234 | 5.392 |
| *SEPT9* | 0.047 | 2.036 | 5.293 |
| *PSMB4* | 0.052 | 2.935 | 5.734 |
| *TOM1* | 0.055 | 5.415 | 6.862 |
| *PRIC285* | 0.057 | 9.934 | 8.729 |
| *LTB* | 0.062 | 2.036 | 5.293 |
| *MIR1205* | 0.067 | 1.698 | 5.121 |
| *ACER3* | 0.073 | 2.612 | 5.578 |
| *BCL9L* | 0.079 | 4.034 | 6.248 |
| *MDS2* | 0.080 | 3.149 | 5.836 |
| *SNORA5B* | 0.083 | 1.712 | 5.128 |
| *PTPRCAP* | 0.091 | 3.620 | 6.057 |

FDR correction was performed using the Benjamini-Hochberg method with an FDR-adjusted p value threshold of <0.05. Δβ: methylation difference in median methylation value of CpG sites within 1500 bp upstream of the associated gene's transcription start site (TSS1500) between patients with lupus and healthy controls. FDR, false discovery rate; TSS, transcription start site.

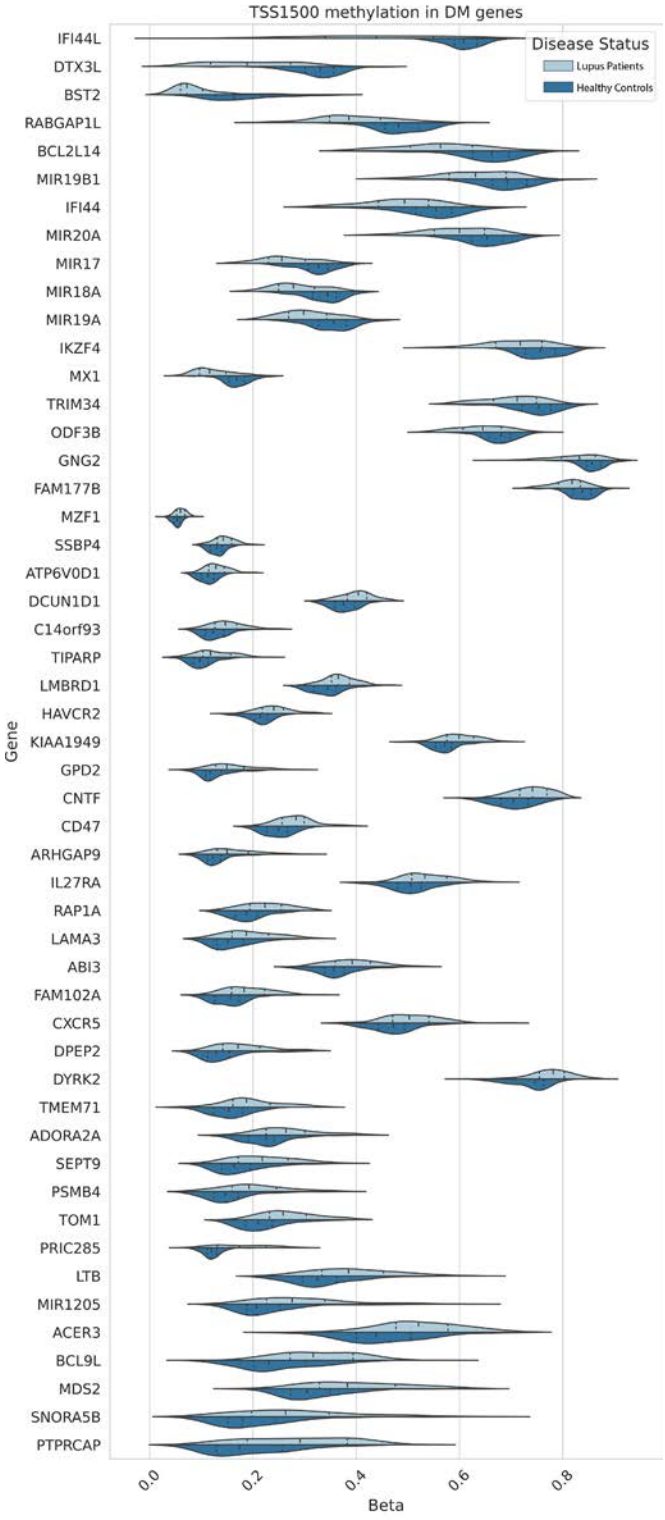


**Figure 1** Distribution of average CpG methylation levels within 1500 bp of the transcription start site (TSS1500) for the respective genes differentially methylated (DM) in naïve CD4$^+$ T cells of patients with lupus compared with healthy controls.

of all gene promoters after adjusting for multiple testing (online supplemental table 3).

The pairwise correlation of the 51 gene promoters identified above was calculated across a collection of 16 541 samples from 37 tissues available in GEO. Hierarchical clustering of correlations showed that 21 out of the 51 gene promoters were highly

**Table 2** KEGG pathway gene enrichment of 21 gene promoters highly correlated with each other in multitissue DNA methylation data constructed from 16 541 samples available through Gene Expression Omnibus

| Pathway (KEGG_2019_Human) | P value | FDR-adjusted, p value | OR | Genes |
|---|---|---|---|---|
| MicroRNAs in cancer | 1.21E-05 | 0.00039 | 20.92 | *MIR19B1;MIR20A;MIR17;MIR18A;MIR19A* |
| Cytokine-cytokine receptor interaction | 0.0034 | 0.043 | 11.28 | *CNTF;CXCR5;LTB* |
| Rheumatoid arthritis | 0.0041 | 0.043 | 23.52 | *LTB;ATP6V0D1* |

FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; OR, odds ratio.

correlated. KEGG pathway enrichment analysis showed a significant enrichment for three pathways among the 21 correlated gene promoters: 'microRNAs in cancer' (p=3.86E-04), 'cytokine-cytokine receptor interaction' (p=4.34E-02) and 'rheumatoid arthritis' (p=4.34E-02) (table 2) (figure 2). The 'microRNAs in cancer' pathway included genes encoding miR-17, miR-18a, miR-19a, miR-19b1 and miR-20a. Four of seven CpG sites used to calculate the average promoter methylation (TSS1500) in this locus showed a significant reduction in median methylation in patients with lupus compared with healthy controls (figure 3A). These sites: cg17799287 (Δβ=−5.5%; p=2.05E-03), cg07641807 (Δβ=−4.4%; p=1.71E-02), cg23665802 (Δβ=−5.8%; p=1.19E-02) and cg02297838 (Δβ=−4.9%; p=3.48E-02) were all hypomethylated in patients with lupus compared with healthy controls, and overlapped with enhancers and regions flanking TSS in peripheral naïve CD4$^+$ T cells using data collected from the Epigenome Roadmap[57] and visualised using the WashU Epigenome Browser.[58] We examined expression levels of the miRNAs included in the 'microRNAs in cancer' pathway (miR-17, miR-18a, miR-19a, miR-19b1 and miR-20a) in naïve CD4$^+$ T cells of a subset of our patients with lupus (n=16) and healthy matched controls (n=16). We did not observe a difference in expression between patients and controls. However, two miRNAs, miR-18a-5p and miR-19b1-5p, showed a significant positive correlation (hsa-miR-18a-5a p=0.038 and hsa-miR-19b1-5p p=0.042) between median expression levels and SLEDAI scores in patients with lupus (figure 3B) (online supplemental table 4).

Examining publicly available miRNA expression data from total CD4$^+$ T cells revealed overexpression of miR-18a in patients with lupus compared with healthy control individuals.[59]

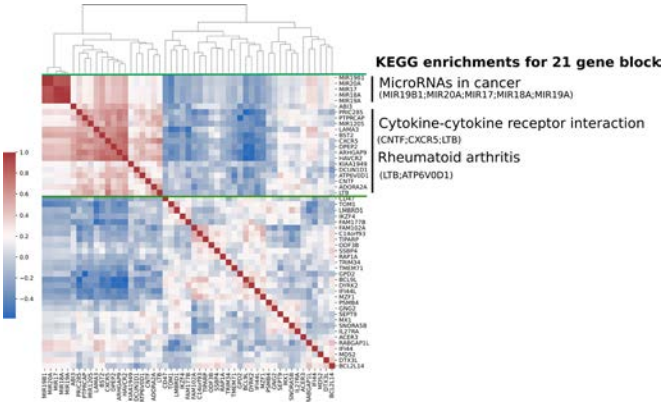


**Figure 2** Heatmap of hierarchical clustering of pairwise Pearson's correlation coefficient values of 51 differentially methylated gene promoters (transcription start site (TSS)1500) in global tissue signature derived from 16 541 samples. Range from +1 (red) to −1 (blue), represent a greater to lower correlation in global tissue, respectively. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways are significantly enriched (false discovery rate-adjusted p<0.05) in a block of 21 genes (green bars).

In these same samples, a total of 74 miR-18a-target genes were downregulated in patients with lupus compared with controls. Using a literature-based network association analysis, we identified 15 of these 74 genes with relatedness to lupus (online supplemental figure 2). *TNFAIP3*, which encodes a negative regulator of nuclear factor kappa B (NF-κB) targeted by miR-18a, was downregulated in lupus CD4$^+$ T cells compared with controls.

We examined the expression of *MIR17HG*, which is the host gene that encodes the miR-17-92 cluster, in single cell RNA-sequencing data from lupus nephritis tissue samples generated by the Accelerating Medicines Partnership (AMP) project.[60] We show evidence for MIR17HG mRNA expression in multiple immune cells infiltrating the kidneys of patients with lupus nephritis, including multiple T cell subsets, although in a small percentage of kidney infiltrating cells. While over 8% of tissue-resident macrophages in lupus nephritis tissues express

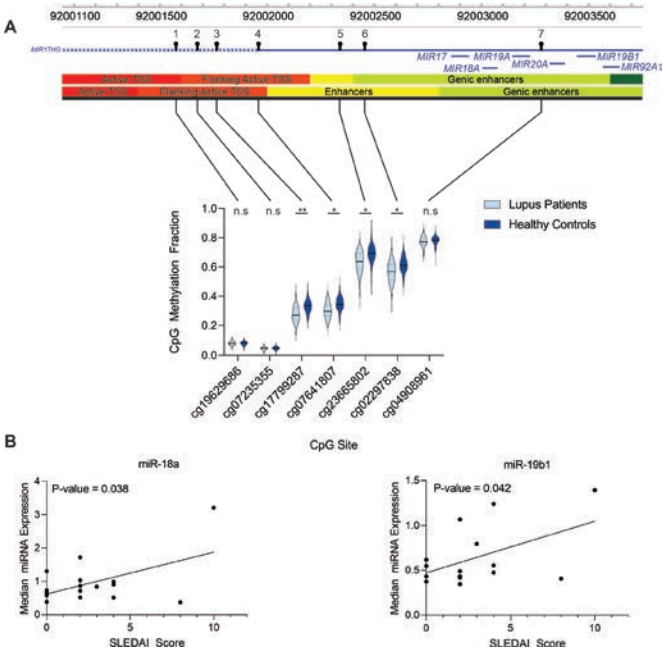


**Figure 3** (A) Violin plots of the seven CpG sites in patients with lupus and healthy controls used to calculate the average promoter methylation (transcription start site (TSS)1500) for the miR-17-92 cluster. The solid black bar represents the median value and the dashed lines the first and third quartiles. Genomic visualisation and annotation are from WashU Epigenome Browser using AuxillaryHMM tracks from peripheral naïve CD4$^+$ T cells (E038 and E039, top and bottom tracks, respectively). n.s., not significant. *P<0.05, **p<0.01. (B) Correlation of median microRNA (miRNA) expression in naïve CD4$^+$ T cells of a subset (n=16) of patients with lupus with Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score. Hsa-miR-18a-5p and hsa-miR-19b1-5p had a Pearson's correlation (r) of 0.52 (p=0.038) and 0.51 (p=0.042), respectively.

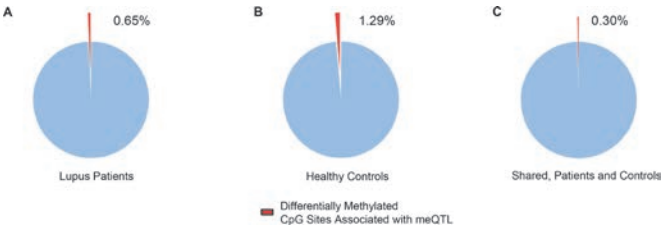


**Figure 4** Proportion of differentially methylated CpG sites in naïve CD4$^+$ T cells of patients with lupus compared with healthy controls associated with a methylation quantitative trait loci (meQTL) in (A) patients with lupus, (B) healthy controls and (C) the subset of meQTL shared between patients with lupus and healthy controls.

MIR17HG mRNA, the highest levels of expression were observed in T cell subsets (online supplemental figure 3).

### Naïve CD4$^+$ T cell methylation quantitative trait loci in patients with lupus

Global genotype profiles were generated in a subset of patients and controls and compared with global DNA methylation profiles to identify CpG sites with allele-specific methylation associations. There was no significant difference in the average age (years) between the patient (n=63) and control (n=68) subsets (patient average age=41.6; patient age SD=12.8; control average age=40.8; control age SD=12.5; t-test statistic=0.381; two-tailed p=0.704). Allele-specific DNA methylation associations were measured as meQTL, where the CpG site was within 1000 bp of the measured SNP separately in patients and controls. After adjusting for age, genetic background and medication use in patients, we identified 5785 meQTL in naïve CD4$^+$ T cells of patients with lupus with an FDR-adjusted p<0.05 (online supplemental table 5). These meQTL include 4649 unique CpG sites and 4120 unique polymorphisms.

A linear model adjusting for age and genetic background was fit to healthy controls separately. We identified a total of 7331 meQTL with an FDR-adjusted p<0.05 in controls (online supplemental table 6). These meQTL include 5885 unique CpG sites and 5138 unique polymorphisms.

Of 2627 CpG sites differentially methylated between patients and controls, we identified 17 (0.65%) and 34 (1.29%) CpG sites that overlapped with CpG sites included in meQTL in patients and controls, respectively (figure 4A,B). We examined the overlap of meQTL in patients with lupus and healthy controls and identified a total of 3957 meQTL (68.4% of lupus patient meQTL and 54.0% of healthy control meQTL) shared between both patients and controls (online supplemental table 7). This shared set of meQTL contained 8 (0.3%) CpG sites that we identified as differentially methylated between patients with lupus and controls (figure 4C).

Functional enrichment analysis was performed using genes associated with CpG sites in our meQTL shared between patients and controls. Functional enrichment analysis revealed multiple ontologies and pathways for cell adhesion ('cell-cell adhesion'; p=1.04E-12, 'biological adhesion'; p=6.80E-12, 'cell adhesion'; p=8.25E-12, 'cell adhesion molecules'; p=2.25E-06), transporter associated with antigen processing (TAP) proteins and antigen presentation ('TAP binding'; p=1.59E-7, 'peptide antigen binding'; p=4.40E-5) and immune disorder pathways ('type I diabetes mellitus'; p=1.92E-8, 'graft-versus-host disease (GVHD)'; p=4.38E-7) (online supplemental table 8).

There were 1828 meQTL detected only in patients with lupus but not in controls. These were enriched in gene ontologies and pathways related to tissue growth and development ('animal organ morphogenesis'; p=8.44E-10, 'urogenital system development'; p=1.05E-07) and gene silencing ('negative regulation of gene silencing by miRNA'; p=2.54E-6, 'negative regulation of post-transcriptional gene silencing'; p=5.41E-6) (online supplemental table 9).

We compared our list of meQTL in patients with lupus with previously identified lupus susceptibility loci from genome-wide association studies.[4 61–64] We found 41 meQTL with CpG site-associated genes that overlapped with 20 unique lupus risk loci genes (online supplemental table 10). This included interferon regulatory factor genes *IRF5* and *IRF7*. We found three meQTL in naïve CD4$^+$ T cells that included, or were in high linkage disequilibrium (LD) ($r^2 \geq 0.80$) with, a known lupus genetic risk variant (table 3).[65] We also performed a similar analysis using data previously collected from granulocytes of patients with lupus to determine if these effects were present across tissues.[22] We found meQTL associated with lupus risk variants in *CFB* (rs170942) and *IRF7* (rs1131665) in both naïve CD4$^+$ T cells and granulocytes isolated from patients with lupus. In addition, an meQTL associated with the *TMEM86B-PTPRH* locus was observed in naïve CD4$^+$ T cells. When we compared the lupus risk alleles with DNA methylation levels, we found that the presence of the risk allele at rs1270942 (*CFB*) is associated with increased DNA methylation of cg16505946. The presence of the risk allele at rs1131665 (*IRF7*) (figure 5) and rs56154925 (*TMEM86B-PTPRH*) was associated with decreased DNA methylation of cg16486109 and cg01414877, respectively. The direction of the risk allele-DNA methylation association in the *CFB* and *IRF7* meQTL was the same in both naïve CD4$^+$ T cells and granulocytes.

We examined the overlap between genes associated with CpG sites in meQTLin lupuspatients and genes that respond to type I interferon treatment in CD4$^+$ T cells, to better understand the association between genetics and type I interferon-response gene methylation differences in lupus. A total of 101 unique type I interferon-response genes were identified as meQTL in our data (online supplemental table 11).

Because IRF7 is a master regulator of type I interferon response,[66] and the lupus-associated epigenotype is dominated by hypomethylation in interferon-regulated genes, we examined if rs1131665 (*IRF7*) had an effect on the methylation levels of the 2627 CpGs differentially methylated in naïve CD4$^+$ T cells between patients with lupus and healthy controls. This *trans*-meQTL analysis revealed no significant difference in methylation levels across these CpG sites based on rs1131665 genotypes, among patients with lupus (analysis of variance, data not shown).

## DISCUSSION

We generated genome-wide DNA methylation data in naïve CD4$^+$ T cells from a large cohort of patients with lupus and matched healthy controls. Implementing an innovative trend deviation analysis, we identified a cluster of miRNAs (miR-17, miR-18a, miR-19a, miR-19b1, miR-20a) among differentially methylated loci in patients with lupus. Promoter methylation analysis revealed significant hypomethylation in this miRNA cluster in patients with lupus compared with controls. Trend deviation analysis suggested a coordinated, disease-associated change in promoter methylation for these miRNAs. Indeed, the expression of miR-18a and miR-19b1 included within this cluster positively correlated with disease activity, as measured using SLEDAI score, in our patients with lupus.

**Table 3** MeQTL in naive CD4+ T cells and granulocytes of patients with lupus that include a known lupus risk variant

| Lupus-naïve CD4+ T cell meQTL | | | | | |
|---|---|---|---|---|---|
| **CpG site** | **meQTL SNP** | **Lupus risk SNP*** | **Risk SNP-associated gene** | **Lupus risk allele** | **Direction of CpG methylation associated with risk allele** |
| cg16505946 | rs558702 | rs1270942 | *CFB* | C | ↑ |
| cg16486109 | rs1131665 | rs1131665 | *IRF7* | A | ↓ |
| cg01414877 | rs56154925 | rs56154925 | *TMEM86B-PTPRH* | C | ↓ |
| **Lupus granulocyte meQTL** | | | | | |
| **CpG site** | **meQTL SNP** | **Lupus risk SNP*** | **Risk SNP-associated gene** | **Lupus risk allele** | **Direction of CpG methylation associated with risk allele** |
| cg16505946 | rs558702 | rs1270942 | *CFB* | C | ↑ |
| cg16486109 | rs1131665 | rs1131665 | *IRF7* | A | ↓ |

*rs558702 and rs1270942 have an LD $r^2$ ≥0.80.
meQTL, methylation quantitative trait loci; SNP, single nucleotide polymorphism.

MiRNAs play an important role in post-transcriptional gene regulation by targeting specific complementary gene transcripts for degradation.[67] Peripheral blood cells in patients with lupus show altered expression of miRNAs.[68] Some dysregulated miRNAs in lupus target DNMT1, and as a result, contribute to altered DNA methylation patterns in lupus CD4+ T cells.[69–71] miR-17, miR-18a and miR-20a form the 'miR-17 family' while miR-19a and miR-19b1 form the 'miR-19 family'. These miRNAs are grouped by sequence homology and encoded in a single polycistronic miRNA gene as the 'miR-17-92 cluster'. This cluster has been well-studied as an oncogene and an immune regulator.[72] Average promoter methylation of miR-17, miR-18a, miR-19a, miR-19b1 and miR-20a was reduced by ~5% in patients with lupus compared with controls, which has not been previously described in immune cells of patients with lupus. Enterovirus 71 infection has been observed to suppress miR-17-92 cluster expression by increasing DNMT-mediated promoter methylation,[73] and chemical inhibition of DNMT1 activity in bleomycin-induced lung fibrosis mouse model increases miR-17-92 cluster expression in lung fibroblasts.[74] This suggests that miR-17-92 cluster promoter methylation plays an important role in regulating the expression of its members.

MiR-17-92 cluster genes play a vital role in regulating T cell activities including proliferation and differentiation. Overexpression of miR-17-92 cluster genes promotes lymphoproliferative disease and autoimmunity in mice by targeting critical immunotolerance regulators Bim and PTEN.[75] Conditional knockout of miR-17-92 cluster in a murine model of chronic GVHD (cGVHD) reduced disease-associated T cell infiltration and IgG deposition in the skin.[76] In cGVHD mice, miR-17-92 cluster expression in CD4+ T cells supports T helper (Th)1, Th17 and T follicular helper (Tfh) cell differentiation. Loss of miR-17-92 cluster expression leads to a corresponding reduction in Tfh-dependent germinal centre formation and plasma cell differentiation.[76] MiR-17, miR-18a, miR-19a and miR-20a are overexpressed in splenic T cells of MRL/*lpr* mice.[77] Similarly, miR-17, miR-17a, miR-18a, miR-19a, miR-19b1 and miR-20a are overexpressed in circulating CD4+ T cells of patients with lupus.[78] MiR-19b1 expression, specifically, has a significant positive correlation with disease activity as measured by SLEDAI score.[78] MiR-17 and miR-20 are downregulated in circulating peripheral blood mononuclear cells,[79] B cells[80] and as circulating free miRNAs[81] in patients with lupus compared with healthy controls, suggesting tissue-specific and miRNA-specific expression patterns. Of the miR-17-92 cluster miRNAs identified as differentially methylated in our analysis, only miR-18a and miR-19b1 showed a significant positive correlation between median expression in naïve CD4+ T cells and disease activity in patients with lupus, consistent with these prior observations. MiR-19b1 promotes proliferation of mature CD4+ T cells, Th1 differentiation and interferon-γ production, and suppresses inducible Treg differentiation.[82] MiR-18a expression increases rapidly early on in CD4+ T cell activation,[83 84] and suppresses Th17 cell differentiation through direct targeting of critical Th17 transcription factor transcripts including SMAD4, HIF1A and RORA in human CD4+ T cells in vitro and in vivo murine airway inflammation models.[83] We did not observe a difference in the expression of members in the miR-17-92 cluster between patients with lupus and controls in naïve CD4+ T cells, likely because these miRNAs are upregulated upon T cell activation. Evidence for hypomethylation in lupus in naïve CD4+ T cells suggests epigenetic priming of this locus, similar to what we previously observed in interferon-regulated gene loci in lupus.[18]

Consistent with our DNA methylation data and the epigenetic priming concept in naïve CD4+ T cells discussed above, gene expression data in total CD4+ T cells isolated from patients with lupus compared with normal healthy controls revealed upregulation of miR-18a in lupus and concomitant downregulation of several genes known to be targeted by miR-18a.[59] Of 74 miR-18a target genes downregulated in lupus CD4+ T cells, our literature-based analysis highlighted 15 genes, including *HIF1A* which is involved in T cell differentiation as discussed above. The most robustly lupus-related gene was *TNFAIP3*, which

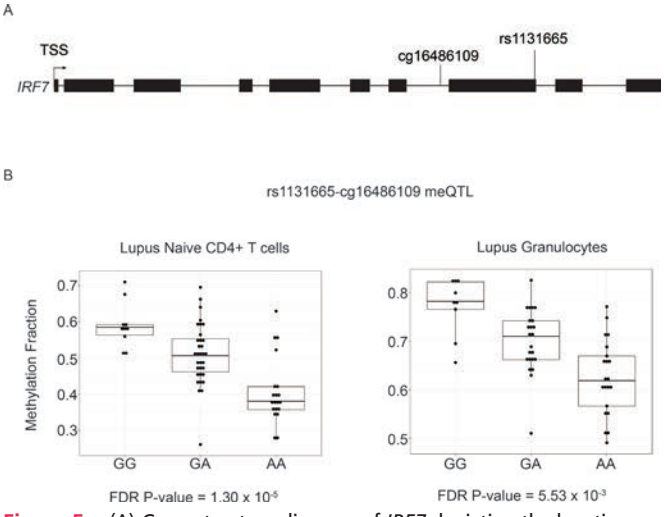


**Figure 5** (A) Gene structure diagram of *IRF7* depicting the location of rs1131665 and cg16486109. (B) The presence of the lupus risk allele at rs1131665 (allele A) is associated with significantly lower DNA methylation levels of cg16486109 located in *IRF7*. FDR, false discovery rate; TSS, transcription start site.

encodes the NF-κB negative regulator A20. Indeed, the genetic association between *TNFAIP3* loss-of-function polymorphisms and lupus has been repeatedly confirmed.[85]

Single cell RNA sequencing data from lupus nephritis kidney tissues revealed evidence for expression of *MIR17HG*, the host gene encoding the miR-17-92 cluster, in kidney-infiltrating immune cells, including multiple T cell subsets. Further studies are needed to determine if altered DNA methylation at the miR-17-92 cluster promoter is associated with expression changes with a causal role in the development of lupus, and to determine if methylation levels at this locus can be used as biomarker for monitoring disease activity.

We used analysis of meQTL to identify allele-specific DNA methylation associations across the genome of naïve $CD4^+$ T cells from patients with lupus and healthy controls. Our primary objective was to understand to what extent are DNA methylation changes associated with lupus (the lupus-defining epigenetic profile), explained by genetic factors. We found that <1% of differentially methylated sites in patients with lupus compared with healthy controls were associated with a *cis*-meQTL. This suggests that almost all of the DNA methylation alterations observed in lupus are not associated with local allelic differences in the genome, suggesting a greater contribution from non-genetic and possibly environmental factors to epigenetic dysregulation in lupus. A previous study of meQTL in whole blood of patients with lupus found that a majority of meQTLs were shared between patients and controls.[24] We observed that about 68% of meQTL in patients with lupus and 54% of meQTL in healthy controls were shared by both groups, supporting this observation.

Our prior analysis of granulocytes from a cohort of patients with lupus identified overlap in meQTL genes and lupus genetic risk loci.[22] MeQTL pairs including *ARID5B* (cg13344587-rs10821936), *HLA-DQB1* (cg13047157-rs9274477), and *IRF7* (cg16486109-rs1131665) were found in both granulocytes and naïve $CD4^+$ T cells from patients with lupus. Risk loci genes unique to naïve $CD4^+$ T cell meQTLs included *CD80* (cg06300880-rs3915166), *TYK2* (cg06622468-rs280501), *IKBKE* (cg22577136-rs17020312) and *CTLA4* (cg05092371-rs16840252, cg05092371-rs4553808). Naïve $CD4^+$ T cell-specific meQTL risk loci genes are related to signal response and activation in $CD4^+$ T cells compared with the more general DNA repair and type I interferon signalling seen in the shared meQTL risk loci genes. Disease-relevant meQTL show tissue-specific patterns which should be considered when teasing apart their potential impact.

We identified three meQTL that include SNPs previously identified as lupus genetic risk variants. One meQTL is in the complement factor B gene *CFB* (cg16505946-rs558702), where the risk allele is associated with increased DNA methylation of the nearby CpG site. Complement factor B (CFB) combines with C3 to form the C3 convertase after cleavage by complement factor D as part of the alternative complement pathway. Complement pathway defects have long been studied as a model of monogenic lupus and contribute to increased risk of polygenic lupus.[65] We identified an additional meQTL that included a known lupus risk variant in *IRF7* (cg16486109-rs1131665). Rs1131665 is a missense variant in the inhibitory domain of IRF7 (Q412R). This lupus-associated amino acid change was demonstrated to enhance IRF7-induced expression response in a luciferase reporter assay.[86] This same risk allele is also associated with decreased DNA methylation of cg16486109. Although the relative DNA methylation fractions are different between naïve $CD4^+$ T cells and granulocytes of patients with lupus, the direction of the allele-specific DNA methylation is the same. This suggests that the observed meQTL effect may be present in other lymphoid and myeloid tissues, potentially including plasmacytoid dendritic cells, which are major producers of type I interferons. We describe a direct association between a lupus risk allele and local hypomethylation of a CpG site in *IRF7* in lupus. This observation provides new insights regarding possible biological mechanisms underlying pathogenic consequences of lupus-associated genetic polymorphisms.

In summary, we investigated genome-wide DNA methylation changes in naïve $CD4^+$ T cells from an extended cohort of patients with lupus and controls, and using a methylation trend deviation analysis method, we showed promoter hypomethylation of the miR-17-92 cluster that has a significant regulatory role in T cell growth, function and differentiation. Combining genome-wide DNA methylation and genotyping data, we were able to determine genetic contribution to the lupus-defining epigenotype. Our data indicate that epigenetic changes characteristic of lupus are not under direct genetic influence. This suggests a more important role for non-genetic factors in the epigenetic dysregulation observed in patients with lupus, including the robust demethylation of interferon-regulated genes.

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**Correction notice** This article has been corrected since it published Online First. The wording in the 'Naïve CD4+ T cell methylation quantitative trait loci in patients with lupus' section has been amended.

**Contributors** All authors fulfilled the following criteria: substantial contributions to the conception or design of the work, or the acquisition, analysis or interpretation of data. Drafting the work or revising it critically for important intellectual content. Final approval of the version published. Dr. Sawalha 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** Consent obtained directly from patient(s)

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EPIDEMIOLOGICAL SCIENCE

# The effect of achieving serological remission on subsequent risk of relapse, end-stage renal disease and mortality in ANCA-associated vasculitis: a target trial emulation study

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## ABSTRACT

**Objective** To evaluate the effect of achieving a negative postinduction antineutrophil cytoplasmic antibody ANCA) assay on the risk of relapse, end-stage renal disease (ESRD) and death in ANCA-associated vasculitis (AAV).

**Methods** We emulated a target trial using observational data from the Mass General Brigham AAV cohort comparing patients who achieved versus did not achieve serological remission (negative ANCA assay) within 180 days of induction. Outcomes were relapse, ESRD or death within 5 years, obtained from medical records, the US Renal Data System and the National Death Index. We placed a 'clone' of each patient in both trial arms, censored those deviating from their assigned protocol and weighted each by the inverse probability of censoring. Outcomes were assessed by pooled logistic regression.

**Results** The study included 506 patients with AAV. The mean age was 61 years (SD 18) and the majority were women (58%), white (87%), myeloperoxidase-ANCA+ (72%) and had renal involvement (68%). Rituximab (59%) or cyclophosphamide (33%) was most often used for induction treatment. Within 5 years, 81 (16%) died, 51 (10%) had ESRD and 64 (13%) had relapse. Patients treated to a negative ANCA assay within 180 days had HR 0.55 (95% CI 0.38 to 0.81) for relapse and HR 0.87 (95% CI 0.61 to 1.25) for the composite of ESRD or death within 5 years.

**Conclusions** In this emulated target trial from a large AAV cohort, achieving serological remission within 180 days of induction was associated with lower risk of relapse, but no statistically significant difference in ESRD or mortality outcomes.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Antineutrophil cytoplasmic antibodies (ANCA) in ANCA-associated vasculitis (AAV) are useful for establishing a diagnosis of AAV and are considered pathogenic.
⇒ The utility of postinduction ANCA titres to inform management or expected outcomes in AAV remains controversial.

### WHAT THIS STUDY ADDS

⇒ Using a large cohort of patients with AAV, we performed an emulated target trial comparing patients achieving serological remission (eg, negative ANCA assay) to patients who did not achieve serological remission within 180 days.
⇒ Patients who achieved serological remission within 6 months of induction treatment had lower risk of relapse by 5 years, but no statistically significant improvement in end-stage renal disease or mortality outcomes.

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

⇒ Treatment of patients with AAV to serological remission may reduce the risk of subsequent disease relapse.
⇒ Future prospective studies should determine the utility of serial ANCA measurements to guide ANCA treatment decisions.

## INTRODUCTION

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a small-to-medium vessel vasculitis characterised by disease relapses, increased risk of end-stage renal disease (ESRD) and excess mortality.[1 2] Most patients with AAV have circulating ANCA that target proteinase 3 (PR3) or myeloperoxidase (MPO) and are considered pathogenic.[3] ANCA testing has been a central component of AAV diagnosis since the 1980s,[4 5] but the measurement of ANCA titres after treatment has been a controversial practice.

Using contemporary induction strategies, the majority of patients with AAV achieve clinical remission.[6] However, only a proportion achieve concurrent serological remission with negative serum ANCA assay.[7–10] Research on the clinical utility of post-treatment ANCA measurements has generated conflicting findings, perhaps due to heterogeneous methods that have investigated variable patient groups. Some studies focused on patients with 'persistently positive' titres, while others investigated those with rising titres or 're-emerging' ANCA after negative testing.[7–16]

Interest in using ANCA as a biomarker for disease activity stems from its potentially pathogenic role in AAV disease and early studies suggesting that rising ANCA titre may predict disease flare and relapse.[17 18] However, a subsequent meta-analysis found that repeat ANCA testing to identify patients with rising or persistent ANCA titres had limited utility for guiding patient management.[14] Despite those findings, there was a resurgence of enthusiasm for repeat ANCA testing after the adoption of rituximab for AAV induction treatment since rituximab depletes circulating precursors to ANCA-producing immune cells and significantly decreases ANCA titres.[6 19] However, recent research, including observational studies and the Maintenance of Remission using Rituximab in Systemic ANCA-associated Vasculitis (MAINRITSAN)2 randomised clinical trial have suggested that rising ANCA titres may be specific but imperfect predictors of AAV relapse.[20]

In light of these conflicting data, the impact of achieving a serological remission on later risk of relapse, ESRD and death remains unknown. To investigate the association of postinduction ANCA titres with key AAV outcomes, we emulated a target trial using observational data to examine the effect of achieving a serological remission after treatment on the subsequent risks of relapse, ESRD and death within 5 years.

## METHODS

### Study population

We used the Mass General Brigham (MGB) AAV cohort as the data source. The MGB AAV cohort is a retrospective consecutive inception cohort of patients with AAV evaluated and treated at a large multihospital, healthcare system in the Boston, Massachusetts area. The cohort contains consecutive patients with AAV who were diagnosed and received induction treatment between 1 January 2002 and 30 June 2019 identified using a previously described algorithm and confirmed to have AAV by review of electronic health records (EHRs).[21] All patients were PR3-ANCA-positive or MPO-ANCA-positive; we excluded patients with eosinophilic granulomatosis with polyangiitis. We extracted data on baseline demographics, laboratory testing and medications from the EHR. Consent was waived due to the retrospective nature of the research. Patients and the public were not involved in the design, conduct, reporting or dissemination plans of this research.

### ANCA titres

ANCA testing was performed for clinical purposes by ELISA and the assay used varied by calendar time and clinical laboratory. We extracted all available ANCA results from the EHR and classified each test as positive or negative using the associated laboratory reference values. We classified borderline results as positive. We considered a patient to be ANCA-negative if they had a negative ANCA assay (eg, titre below the assay's borderline or normal level) result within 180 days of treatment initiation, which we defined as the date of initial immunosuppression prescription for AAV.

### Outcomes: ESRD, death and relapse

The first outcome of interest was relapse (major and minor) within 5 years of induction treatment (index date). We reviewed the EHR of all patients to identify relapses. We defined relapse as an increase in Birmingham Vasculitis Score for Wegener's Granulomatosis (BVAS/WG) combined with increased immunosuppressive treatment for signs/symptoms of AAV, consistent with prior studies investigating risk factors for AAV relapse.[22] We did not consider an isolated rise in ANCA titre to represent a disease flare.

The second outcome of interest was the composite of ESRD or death within 5 years of index date. We defined ESRD as (1) Requirement of haemodialysis or peritoneal dialysis for >60 days, (2) Dialysis until death if the patient died between day 14 and day 60 of follow-up, or (3) Renal transplant. We obtained data on ESRD and renal transplant from the US Renal Data System, which is a national registry of patients with ESRD, representing an estimated 94% of patients who receive dialysis or kidney transplantation.[23] For ESRD outcome analyses, we excluded four patients who initiated renal replacement therapy >300 days prior to AAV diagnosis for other reasons. Death data were obtained from the National Death Index, a nationwide mortality index run by the Centers for Disease Control.[24] Additionally, we reviewed the EHR of all patients for vital status, ESRD or renal transplant outcomes not captured in the national databases. We also considered ESRD and death outcomes individually.

### Covariates

We extracted demographic and disease-specific features including age at diagnosis, sex, PR3-ANCA and MPO-ANCA type, induction treatment, estimated glomerular filtration rate (eGFR) and comorbidities to calculate a Charlson Comorbidity Index (CCI) from the EHR.[25] We reviewed each patient's records to determine disease manifestations and baseline BVAS/WG.[26] CCI was missing on 59 patients. There were no other missing covariate data.

### Statistical analysis

We emulated a hypothetical clinical trial comparing the 5-year risks of relapse, ESRD and death in patients who did versus did not achieve serological remission within 180 days of induction treatment. Although the goal of the treating providers may not have been to achieve a specific ANCA level, the emulated target trial assesses the impact of potential treatment strategies and minimises 'immortal time' and other biases associated with retrospective data.[27] Because the exposure of interest (ie, time to 'achieving serological remission') was the time duration to reach an exposure level, we adopted a 'cloning, censoring, and weighting' approach.[28 29] We created two trial arms, one in which patients achieved a negative ANCA assay within 180 days of induction ('Achieved serological remission') and one in which patients' treatment strategy did not result in serological remission ('Does not achieve serological remission'). We created 'clones' of each patient and assigned one duplicate to each trial arm. Censoring of a 'clone' occurred when it deviated from the assigned protocol. For example, we censored duplicates assigned to the 'serological remission' group if they did not achieve a negative ANCA assay within 180 days. Similarly, we censored duplicates assigned to the 'does not achieve serological remission' group if their ANCA assay became negative within 180 days. Because censoring may lead to selection bias, we weighted each patient by their inverse probability of censoring. Specifically, the denominator was the probability that a duplicate adhered to the assigned arm determined using a logistic regression model, which consisted of baseline age, sex, ANCA type, induction treatment regimen, BVAS/WG, eGFR and treatment with plasma exchange. This inverse probability of censoring weighting creates two pseudo-populations where group assignment is independent of prognostic factors for the outcomes of relapse, ESRD or death.

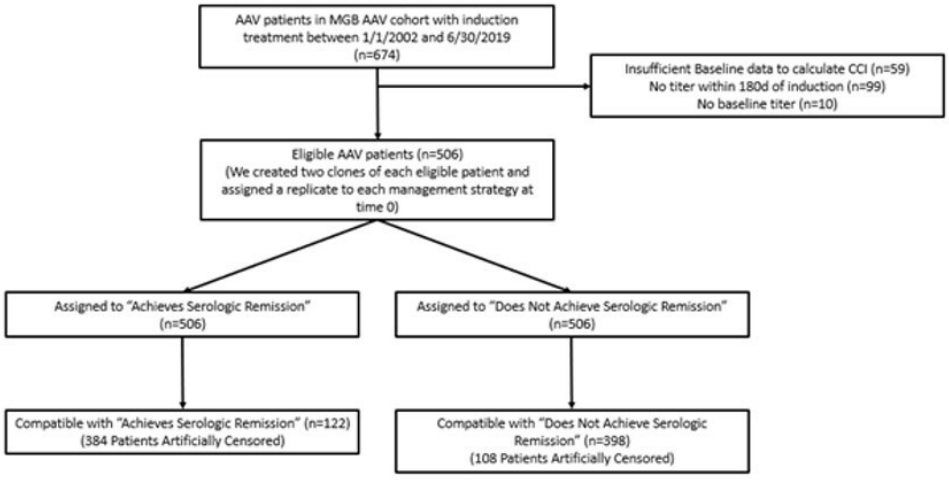


**Figure 1** Flow chart of eligible patients and target trial design (180 days). AAV, antineutrophil cytoplasmic antibody-associated vasculitis; CCI, Charlson Comorbidity Index; MGB, Mass General Brigham.

For each analysis, follow-up time among those not artificially censored ended at the earliest of: event of interest, end of follow-up at MGB (only for relapse) or 5 years after index date. For the relapse and ESRD outcomes, we accounted for the competing risk of death.[30] We fitted pooled logistic regression models for relapse and the composite outcome of ESRD or death, as well as ESRD and death individually. Because the outcomes were rare, the ORs generated from the pooled logistic regressions approximate HRs.[31] We calculated 95% CIs for the estimate of the ORs and created cumulative incidence curves for each outcome. We performed several subgroup analyses examining the effect of achieving serological remission on the risk of each outcome by PR3-ANCA+ or MPO-ANCA+ status, renal involvement, initial induction strategy (rituximab-based or cyclophosphamide-based) and use of plasma exchange.

We considered a two-sided value of $p<0.05$ as the threshold for significance, without adjustment for multiple hypothesis testing. Statistical analysis was performed using SAS V.9.4 (SAS Institute, Cary, North Carolina, USA).

### Sensitivity analysis

We performed three sensitivity analyses. First, we repeated our main analyses after using a sequential regression method to calculate baseline CCI on the 24 patients missing this baseline data.[32] Second, to test the robustness of the study finding, we repeated the main analysis for all outcomes after extending the grace period (ie, the time to achieve a serological remission) from 180 days to 365 days. Third, to protect against bias introduced by comparing results from different ANCA testing platforms, we limited the cohort to patients who had ANCA testing performed at Massachusetts General Hospital.

## RESULTS

There were 674 patients in the MGB AAV cohort screened for inclusion in the target trial. Figure 1 details patient allocation. After excluding patients lacking ANCA titre measurement within 180 days of induction and/or insufficient baseline information to calculate a CCI, we included 506 patients in this analysis (table 1). The cohort had a mean age of 61 years (SD 18) and was predominately female (293, 58%), white (442, 87%) and MPO-ANCA positive (366, 72%). Overall, 395 (78%) had major organ involvement at baseline, 342 (68%) had renal and 249 (49%) had pulmonary involvement. The mean baseline BVAS/WG Score was 5 (SD 2.2). Induction treatment included primarily rituximab in 298 (59%), cyclophosphamide in 166 (33%) or other treatments (eg, methotrexate) in 42 (8%) patients. Plasma exchange was used in 119 (24%) patients.

**Table 1** Baseline characteristics of participants (n=506)

| Characteristic | Total (n=506, %) |
|---|---|
| Age (years mean, SD) | 61 (18) |
| Male | 213 (42%) |
| Race | |
| White | 442 (87%) |
| Black | 11 (2%) |
| Asian | 6 (1%) |
| Other | 47 (9%) |
| ANCA status | |
| PR3-ANCA+ | 140 (28%) |
| MPO-ANCA+ | 366 (72%) |
| Organ Involvement | |
| Any major | 395 (78%) |
| Renal | 342 (68%) |
| eGFR (mL/min/1.72 m2) | 38.8 (14, 72) |
| Pulmonary | 249 (49%) |
| Head and neck | 213 (42%) |
| Other | 66 (13%) |
| Disease activity at diagnosis (BVAS/WG mean, SD) | 5 (2.2) |
| Charlson Comorbidity Index at diagnosis (CCI mean, SD) | 1.7 (2.3) |
| Induction treatment | |
| Included RTX | 298 (59%) |
| Included CYC | 166 (33%) |
| Included TPE | 119 (24%) |
| Other (no RTX or CYC) | 42 (8%) |
| Follow-up | |
| ANCA measurements during follow-up* (mean, SD) | 13 (9) |
| ANCA measurements within 180 of induction (mean, SD) | 3.5 (1.9) |

*Within 5 years of induction or from induction to relapse or last MGB follow-up if <5 years
ANCA, antineutrophil cytoplasmic antibody; BVAS/WG, Birmingham Vasculitis Activity Score for Wegener's Granulomatosis; CYC, cyclophosphamide; eGFR, estimated glomerular filtration rate; MGB, Mass General Brigham; MPO, myeloperoxidase; PR3, proteinase 3; RTX, rituximab; TPE, therapeutic plasma exchange.

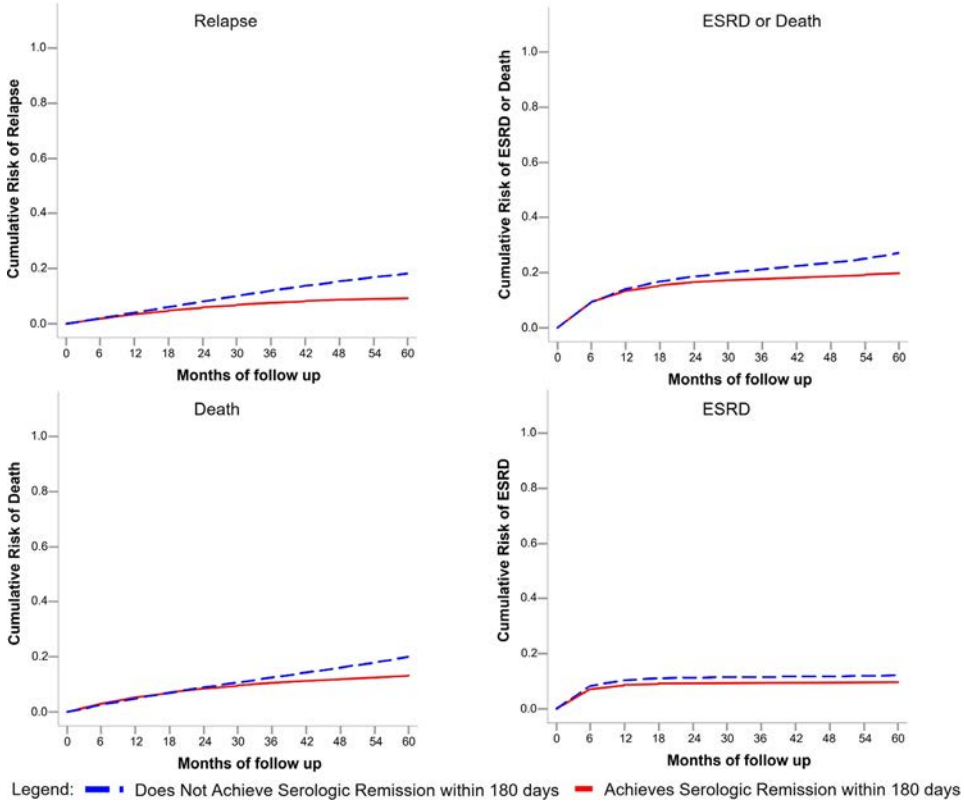


**Figure 2** Cumulative incidence of relapse, ESRD or death by postinduction ANCA status. ANCA, antineutrophil cytoplasmic antibody; ESRD, end-stage renal disease.

The median follow-up time was 49 months (IQR 20.2–60) for relapse and 60 months (IQR 21.9–60) for the assessment of ESRD and death. The mean number of ANCA measurements performed during the first 180 days after induction was 3.5 (SD 1.9). Additional details of the number of ANCA titre measurements overall and in patients with and without major organ involvement at baseline are provided in online supplemental tables 1 and 2. During the 5 years of follow-up, 81 patients (16%) died, 51 (10%) had ESRD and 64 (13%) had relapse. Among patients who had each outcome, the median times to death, ESRD and relapse were 675 days, 33 days and 539 days, respectively. Cumulative incidence curves for each outcome are detailed in figure 2.

**Table 2** The effect of achieving serological remission on risk of relapse, ESRD and death using an emulated target trial design (n=506)

| Outcome | Serological remission within 180 days | Persistently positive titre at 180 days |
|---|---|---|
| Relapse | | |
| Risk over 5 years (95% CI), *per 100* | 9.4 (3.4 to 15.4) | 18.3 (9.9 to 26.7) |
| Risk difference over 5 years (95% CI), *per 100* | **–8.9 (–17.4 to –0.4)** | Ref |
| Adjusted HR (95% CI) | **0.55 (0.38 to 0.81)** | 1.0 (Ref) |
| ESRD or death (composite)* | | |
| Risk over 5 years (95% CI), *per 100* | 19.8 (11.2 to 28.6) | 27.1 (17.0 to 37.3) |
| Risk difference over 5 years (95% CI), *per 100* | –7.3 (–15.8 to 1.2) | Ref |
| Adjusted HR† (95% CI) | 0.87 (0.61 to 1.25) | 1.0 (Ref) |
| ESRD* | | |
| Risk over 5 years (95% CI), *per 100* | 9.7 (3.6 to 15.8) | 12.3 (5.4 to 19.1) |
| Risk difference over 5 years (95% CI), *per 100* | –2.5 (–11.0 to 5.9) | Ref |
| Adjusted HR† (95% CI) | 0.93 (0.70 to 1.23) | 1.0 (Ref) |
| Death | | |
| Risk over 5 years (95% CI), *per 100* | 13.4 (6.2 to 20.5) | 20.0 (11.3 to 28.8) |
| Risk difference over 5 years (95% CI), *per 100* | –6.7 (–15.1 to 1.8) | Ref |
| Adjusted HR† (95% CI) | 0.81 (0.49 to 1.35) | 1.0 (Ref) |

Bold indicates statistical significance at a p< 0.05.
*4 patients with ESRD >300 days prior to AAV diagnosis were excluded from analyses of ESRD outcomes.
†Adjusted for baseline covariates: age, sex, ANCA type, induction treatment regimen, BVAS/WG, eGFR and treatment with plasma exchange.
AAV, ANCA-associated vasculitis; ANCA, antineutrophil cytoplasmic antibody; BVAS/WG, Birmingham Vasculitis Score for Wegener's Granulomatosis; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease.;

**Table 3** Subgroup analyses by ANCA type, renal involvement and induction treatment regimen for relapse, ESRD and death

| Outcome | Serological remission within 180 days HR (95% CI) | Persistently positive titre at 180 days |
|---|---|---|
| Adjusted HR* for relapse | | |
| PR3-ANCA+ | 0.52 (0.20 to 1.33) | 1.0 (Ref) |
| MPO-ANCA+ | **0.62 (0.40 to 0.96)** | 1.0 (Ref) |
| Renal involvement at baseline | 0.64 (0.39 to 1.03) | 1.0 (Ref) |
| RTX or RTX/CYC treated | **0.55 (0.33 to 0.92)** | 1.0 (Ref) |
| CYC only treated | 0.47 (0.21 to 1.03) | 1.0 (Ref) |
| TPE treated | 0.49 (0.10 to 2.49) | 1.0 (Ref) |
| Adjusted HR* for ESRD or death (composite)† | | |
| PR3-ANCA+ | 0.77 (0.34 to 1.74) | 1.0 (Ref) |
| MPO-ANCA+ | 0.86 (0.58 to 1.28) | 1.0 (Ref) |
| Renal involvement at baseline | 0.91 (0.63 to 1.32) | 1.0 (Ref) |
| RTX or RTX/CYC treated | 0.98 (0.61 to 1.59) | 1.0 (Ref) |
| CYC only treated | 0.95 (0.55 to 1.64) | 1.0 (Ref) |
| TPE treated | 0.95 (0.64 to 1.40) | 1.0 (Ref) |
| Adjusted HR* for ESRD† | | |
| PR3-ANCA+ | 0.55 (0.20 to 1.53) | 1.0 (Ref) |
| MPO-ANCA+ | 1.03 (0.75 to 1.41) | 1.0 (Ref) |
| Renal involvement at baseline | 1.00 (0.74 to 1.34) | 1.0 (Ref) |
| RTX or RTX/CYC treated | 0.80 (0.53 to 1.21) | 1.0 (Ref) |
| CYC only treated | 1.28 (0.87 to 1.90) | 1.0 (Ref) |
| TPE treated | 0.91 (0.58 to 1.44) | 1.0 (Ref) |
| Adjusted HR* for death | | |
| PR3-ANCA+ | 1.04 (0.35 to 3.14) | 1.0 (Ref) |
| MPO-ANCA+ | 0.73 (0.41 to 1.31) | 1.0 (Ref) |
| Renal involvement at baseline | 0.88 (0.49 to 1.57) | 1.0 (Ref) |
| RTX or RTX/CYC treated | 1.04 (0.55 to 1.96) | 1.0 (Ref) |
| CYC only treated | 0.73 (0.27 to 1.96) | 1.0 (Ref) |
| TPE treated | 1.18 (0.69 to 2.02) | 1.0 (Ref) |

*Adjusted for baseline covariates: age, sex, ANCA type, induction treatment regimen, BVAS/WG, eGFR and treatment with plasma exchange.
†4 patients with ESRD >300 days prior to AAV diagnosis were excluded from analyses of ESRD outcomes.
AAV, ANCA-associated vasculitis; ANCA, antineutrophil cytoplasmic antibody; BVAS/WG, Birmingham Vasculitis Score for Wegener's Granulomatosis; CYC, cyclophosphamide; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; MPO, myeloperoxidase; PR3, proteinase-3; RTX, rituximab; TPE, plasma exchange.

In the target trial analysis for relapse, 122 patients achieved a negative ANCA assay within 180 days of induction and were compatible with the 'serological remission' group, while 398 patients were compatible with the 'does not achieve serological remission' group. Censoring of clones in both trial arms is detailed in figure 1.

The 5-year cumulative incidence of relapse was 9.4 per 100 patients in the group achieving serological remission and 18.3 in the group that did not achieve serological remission within 180 days of induction. The corresponding risk difference was −8.9 (95% CI −17.4 to −0.4) per 100 and the HR was 0.55 (95% CI 0.38 to 0.81) (table 2, figure 2). Achieving serological remission was not significantly associated with decreased risk of death or ESRD. The HR for the composite outcome of death or ESRD within 5 years was 0.87 (95% CI 0.61 to 1.25) for the group that achieved a serological remission.

We observed similar results in subgroup analyses stratifying by ANCA type, baseline renal involvement and induction treatment (table 3). Achieving serological remission within 180 days was associated with a statistically significant reduction in the risk of relapse in the MPO-ANCA+ (HR 0.62 95% CI 0.40 to 0.96) and rituximab-treated (HR 0.55 95% CI 0.33 to 0.92) groups. Our sensitivity analyses confirmed the robustness of the findings after imputing data for those with missing baseline CCI, extending the time to achieve a serological remission from 180 days to 365 days, and limiting ANCA testing to a single laboratory (table 4).

## DISCUSSION

In this target trial emulation study using observational data from a large cohort of patients with AAV, achieving serological remission (negative ANCA assay) within 180 days of induction was associated with decreased risk of relapse, but was not associated with statistically significant reduction in the risk of ESRD or death within 5 years. We observed similar results when stratifying by ANCA type and induction treatment strategy. These findings suggest that achieving a negative ANCA assay during and after induction may result in fewer subsequent disease relapses.

Our study investigates an ongoing controversy in AAV care that has led to varying ANCA testing practices following diagnosis. Previous studies have yielded conflicting results, in part due to significant heterogeneity of study designs investigating the association of relapses with rise in ANCA titre,[11–14] re-emergence

**Table 4** Sensitivity analyses examining target trial outcomes of ESRD, relapse and death within 5 years

| | Serological remission within 180 days HR (95% CI) | Persistently positive titre at 180 days |
|---|---|---|
| **Sensitivity analysis 1: Imputation of missing baseline data (n=530)*** | | |
| All patients with imputed baseline data (n=530)* | | |
| Relapse | **0.62 (0.43 to 0.89)** | 1.0 (Ref) |
| ESRD or death* | 0.85 (0.61 to 1.19) | 1.0 (Ref) |
| ESRD* | 0.85 (0.67 to 1.06) | 1.0 (Ref) |
| Death | 0.79 (0.48 to 1.29) | 1.0 (Ref) |
| **Sensitivity analysis 2: extending grace period to 365 days (n=506)*** | | |
| | **Serological remission within 365 days HR (95% CI)** | **Persistently positive titre at 180 days** |
| All patients with complete data (n=506)* | | |
| Relapse | **0.73 (0.54 to 0.99)** | 1.0 (Ref) |
| ESRD or death* | 0.94 (0.68 to 1.28) | 1.0 (Ref) |
| ESRD* | 0.96 (0.72 to 1.28) | 1.0 (Ref) |
| Death | 0.73 (0.46 to 1.17) | 1.0 (Ref) |
| **Sensitivity analysis 3: ANCA testing performed at Massachusetts General Hospital** | | |
| | **Serological remission within 180 days HR (95% CI)** | **Persistently positive titre at 180 days** |
| All patients with ANCA performed at MGH (n=453)* | | |
| Relapse | **0.61 (0.40 to 0.93)** | 1.0 (Ref) |
| ESRD or death* | 0.95 (0.65 to 1.39) | 1.0 (Ref) |
| ESRD* | 1.00 (0.73 to 1.38) | 1.0 (Ref) |
| Death | 0.90 (0.53 to 1.51) | 1.0 (Ref) |

*4 patients with ESRD >300 days prior to AAV diagnosis were excluded from analyses of ESRD outcomes.
AAV, ANCA-associated vasculitis; ANCA, antineutrophil cytoplasmic antibody; ESRD, end-stage renal disease; MGH, Massachusetts General Hospital.

of ANCA titre,[10] ANCA persistence[7–9 14 16] or a combination.[20] Many studies were also conducted prior to the introduction of rituximab, which renewed enthusiasm for ANCA as a clinical biomarker, since rituximab targets the B cell lineage that ultimately produces ANCA.[19] Recent investigations have suggested that the utility of ANCA titres as a marker of relapse risk may vary by ANCA subtype and specific disease manifestations.[13 16 33]

We expand on these studies using a contemporary cohort of newly diagnosed patients undergoing remission induction, many with rituximab, and applying methods to address immortal time bias and confounding. We focused on the impact of achieving serological remission (negative ANCA assay) within 6 months of remission induction. This is an important time point in AAV care that typically marks the end of 'remission induction' and a transition from induction immunosuppression to maintenance therapy. Achieving a negative ANCA assay at this time may have prognostic significance and inform the choice and intensity of maintenance therapy or subsequent monitoring by identifying patients with favourable AAV treatment response and low risk of subsequent relapse. Our findings remained rather consistent across subgroups stratified by ANCA titre and induction treatment, in contrast to previous studies. Additional studies are needed to evaluate the association of rising ANCA titres with relevant outcomes using similar methodologies to address potential confounding and immortal time bias.

Significant basic and translational research has demonstrated the importance of ANCA for AAV disease pathogenesis. ANCA have been shown to bind to autoantigens and activate neutrophils, leading to microvascular injury.[2] In light of the recognition of the effect that ANCA have on immune cells in animal models and in vitro studies, the inconsistent association between ANCA levels and disease activity remains incompletely understood. Two recent studies suggest that post-translational modification of ANCA immunoglobulins may correlate with differences in disease activity. Espy and colleagues demonstrated that sialylation of PR3-ANCA increased in patients with inactive disease[34] whereas Lardinois and colleagues demonstrated that glycosylation of the Fc segment of IgG was reduced in PR3-ANCA+patients with active disease.[35] Our findings indicate that, at least in some patients, persistent ANCA beyond remission induction are pathogenic given their effects on relapse risk. However, it is also known that not all patients with a persistent ANCA titre will experience a relapse. More detailed examination of ANCA expression, including post-translational modification, may offer further insights into disease risk in AAV.

Strengths of our study include the use of a large AAV cohort and the assessment of the clinically meaningful outcomes of relapse, ESRD and death. There has been minimal prior research on the association between ANCA titres and renal and mortality outcomes.[36] We obtained outcome data from comprehensive sources including EHR review, the US Renal Data System and the National Death Index. Another strength was the inclusion of a majority of MPO-ANCA +patients. Prior literature on the utility of ANCA titres to predict disease flares and outcomes have focused primarily on patients with granulomatosis with polyangiitis who are often PR3-ANCA+.[14] The use of an emulated target trial design with cloning, censoring and weighting was also a strength of our study. This approach allowed for assessment of the impact of treatment to serological remission using observational data without the cost of a prospective clinical trial. This technique also leveraged a rich observational data set while minimising the effects of immortal time bias, baseline confounding and selection bias in the weighting step.[37]

Our study has certain potential limitations. First, we relied on observational data from a single healthcare system, which may limit the generalisability of the results. However, the MGB system includes community and tertiary care hospitals, primary care and other specialty clinics throughout many sites in the New England area. Second, we adjusted our analysis for patient baseline factors, but the possibility of residual confounding remains. Third, because we used ANCA test results from multiple reference laboratories and information about the specific assay used was not always available, we were unable to examine if ELISA type impacted the observed associations and directly compare baseline ANCA values between assays.[13] Fourth, we specified a 180-day 'grace period' for patients to achieve serological remission in our target trial, but this window may miss differences between patients who have serological response early or late within that time period. Fifth, we assessed for relapse outcomes using clinical notes and defined a relapse as intensification of therapy with rise in BVAS/WG Score. Although these criteria are agnostic to ANCA titre, the treating providers were not blinded to ANCA results and we cannot account for differences in subsequent treatment and monitoring. Additionally, the relapse rate that we observed was lower than reported in some AAV clinical trials.[6 38 39] This is likely multifactorial, including the MPO-ANCA predominance of our cohort, which has a lower risk of flare,[40] as well as the enrolment of patients with relapse into some clinical trials and therefore selection for patients at higher risk of relapse, or other factors. Further prospective studies investigating the effect of achieving serological remission using structured assessment of disease activity are needed. Finally, we observed an association between achieving serological remission with decreased risk of relapse and a trend towards decreased ESRD or mortality that did not reach statistical significance. It is possible that our study was underpowered to detect differences in ESRD and death outcomes, which may be long-term consequences of recurrent disease activity. However, our study represents one of the largest published AAV cohorts and was relatively enriched for these outcomes with 23% of subjects experiencing ESRD or death during follow-up. Alternatively, significant morbidity and mortality in patients with AAV may be less related to disease activity in the modern treatment era. Although we adjusted for induction immunosuppression in our analyses, the induction regimen was not randomly selected and was instead chosen at the discretion of the treating physician based on clinical and other patient factors. Therefore, our findings regarding the prognostic significance of postinduction ANCA titres should not be used to guide clinical management decisions regarding the choice or intensity of induction immunosuppression or subsequent treatment. This represents an important avenue for future prospective research.

In conclusion, we found that achieving serological remission (negative ANCA assay) during the first 180 days after induction was associated with a decreased risk of relapse within 5 years. We did not observe a statistically significant difference in the risk of ESRD or death within 5 years comparing patients who achieved serological remission to those who did not achieve serological remission. We observed similar results after stratifying by ANCA type and induction treatment strategy. These findings suggest that achieving a serological remission within 180 days of induction is associated with a decreased risk of AAV relapse but may have lower impact on ESRD and mortality outcomes. Further studies are needed to investigate how postinduction ANCA titres and other disease biomarkers may guide AAV management strategies.

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**Contributors** GM, YZ and ZSW had access to the study data, developed the figures and tables and vouch for the data and analyses. XF and YZ performed the statistical analyses and contributed to data quality control, data analysis and interpretation of the data. GM, ZSW, CC, CA, BD, JH, JHS, HKC and YZ contributed to data collection, data analysis and interpretation of the data. ZSW directed the work, designed the data collection methods, contributed to data collection, data analysis

and interpretation of the data and had final responsibility for the decision to submit for publication. All authors contributed intellectual content during the draft and revision of the work and approved the final version to be published. ZSW accepts full responsibility for the finished work and/or the conduct of the study, had access to the data and controlled the decision to publish.

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CLINICAL SCIENCE

# Risk of irAEs in patients with autoimmune diseases treated by immune checkpoint inhibitors for stage III or IV melanoma: results from a matched case–control study

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## ABSTRACT

**Objective** To quantify the risk of immune-related adverse events (irAEs) in patients with pre-existing autoimmune disease (pAID) treated by immune checkpoint inhibitors (ICIs) for stage III or IV melanoma.

**Methods** Case–control study performed on a French multicentric prospective cohort of patients with melanoma, matched for irAE risk factors and oncological staging. Risk of irAE was assessed by logistic regression.

**Results** 110 patients with pAID were included and matched with 330 controls, from March 2013 to October 2020. Over a median follow-up period of 7.2 months for cases and 6.9 months for controls, the ORs of developing all-grade and grade ≥3 irAEs among cases compared with controls were 1.91 (95% CI (1.56 to 2.27)) and 1.44 (95% CI (1.08 to 1.82)), respectively. Patients with pAID had an increased risk of multiple irAEs (OR 1.46, 95% CI (1.15 to 2.67)) and a shorter time to irAE onset. In contrast, there were no difference in irAE-related mortality nor in the rate of treatment discontinuation, and a landmark analysis revealed a better survival at 24 months among cases (p=0.02). Thirty per cent of cases experienced a pAID flare during follow-up, and baseline immunosuppression did not prevent irAE occurrence. Last, we report associations between the pAID clinical subsets and organ-specific irAEs.

**Conclusion** In our study, patients with pAID were at greater risk of all-grade, severe and multiple irAEs, yet had a better 24-month survival than controls. Thus, patients with pAID should be eligible for ICI therapy but benefit from a close monitoring for irAE occurrence, especially during the first months of therapy.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Patients with pre-existing autoimmune diseases (pAID) are thought to be at greater risk of immune-related adverse events (irAEs), but previously published data are discordant.

⇒ Precise quantification of this risk, independently of the other known risk-factors of irAEs is lacking.

### WHAT THIS STUDY ADDS

⇒ - Patients with pAID had a higher risk of developing both all-grade and grade ≥3 irAEs, but also of multiple irAEs, occurring in a shorter time than controls, but contrasting with a better overall survival at 24 months. Subsetting pAID into clinical subgroups highlighted distinct associations with organ-specific irAEs.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Patients with pAID benefit from ICI but should be closely monitored for irAEs, especially during the first months of therapy. The knowledge of distinct associations between pAID subsets and organ-specific irAEs can improve the early detection of irAEs.

## INTRODUCTION

Immune checkpoint inhibitors (ICIs) are one of the major therapeutic advances in oncology in the past 10 years. Since their first approval in metastatic melanoma[1] and non-small cell lung cancer (NSCLC),[2] ICI indications have broadened and now extend to more than 50 different cancer types.[3] The most prescribed ICIs are anti-PD-1/PD-L1 (programmed cell death protein 1/programmed cell death ligand 1) agents, and their prescription is likely to increase with up to 2975 active clinical trials of September 2019.[4] ICI restore anticancer immunity by targeting tumour-driven expression of immune checkpoints to mount an effective antitumoral immune response. However, as these pathways are physiologically involved in the downregulation of T cell responses and act as gatekeepers to prevent excessive T-cell activation, ICIs subsequently expose to the risk of T-cell-driven autoimmunity.[5] ICIs' side effects include a large range of autoimmune manifestations (immune-related adverse events, referred to as irAEs),[6] estimated to occur in 54%–76% of the patients.[7] Management depends on the severity

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grade: while most CTCAE (Common Terminology Criteria for Adverse Events) grade 1 irAEs do not require therapeutic intervention; grade 2 irAEs may require ICI temporary interruption and patients with grade ≥3 should receive corticosteroids.[5] Lethal irAEs occur in 0.3%–1.3% of cases,[8] and symptoms may persist after ICI cessation and cause long-term sequelae.[9 10] Thus, identifying patients at higher risk of developing irAEs is crucial to early diagnosis and improved care

Autoimmune diseases (AIDs) are frequent, with an estimated prevalence of 4.5%,[11] and co-occurrence with cancer is not uncommon, as recent studies reported a 14%–25% frequency of pre-existing AID (pAID) in 210 509 patients with lung cancer,[12] and of 1.6% in 311 patients treated by anti-PD-1 agents.[13] As irAEs often mimic AID manifestations,[14–17] patients with pAID were thought to be at higher risk of irAEs and excluded from the first clinical trials of ICI.[18–20] Several studies described the safety and oncological outcomes of ICI therapy in patients with pAID. Overall, they reported a frequency of pAID flare of 23%–47%, a frequency of irAEs of 29%–44% and a frequency of grades 3–4 irAEs of 10%–44%. Most pAID flares and irAEs were managed by corticosteroid therapy, leading the authors to conclude that ICI therapy in pAID patients was safe but required close monitoring.[21–25] In a recent study published by Tison *et al*,[25] of 112 patients with pAID treated by ICI, 71% presented with an immune toxicity (pAID flare, irAE or both), and ICI was permanently withdrawn for 21%. Moreover, treatment of immune toxicities with immunosuppressive drugs was associated with a lower progression-free survival. In contrast, in a recent study from a prospective Dutch nationwide melanoma registry, the incidence of grade ≥3 irAEs did not differ between 415 patients with pAID and controls.[26] These results pinpoint the need for a precise evaluation of the risk of irAEs in patients with pAID.

We present the results of a large case–control matched study of patients included in a prospective cohort, evaluating the risk of irAEs among patients with pAID compared with patients without pAID matched for irAEs' risk factors and disease stage.

## Study design

This study was conducted using the French multicentric prospective and longitudinal cohort MelBase (registered NCT02828202), which is dedicated to the prospective follow-up in 26 participating centres of adults with unresectable stage III or IV melanoma at the time of declaration of metastasis. MelBase prospectively records data regarding oncological progression and survival, treatment introduction and discontinuation, adverse events, and their management, as well as demographic data including age, sex and medical history. Inclusion criteria require an age of over 18 years old, the availability of a tumour sample for histological confirmation of advanced primary melanoma (unresectable stage III or stage IV) and the absence of prior systemic treatment other than adjuvant treatment. Additional data regarding baseline immunosuppressive therapy and pAID flares in cases were collected retrospectively through a questionnaire sent to all recruiting centres.

## Cases and controls

Cases were defined as patients treated by anti-PD-1 and/or anti-CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) antibodies for metastatic melanoma with a history of AID prior to immunotherapy, prospectively recorded at inclusion and were referred to as pAID. Specifically, during the inclusion process of the MelBase cohort, the dermato-oncologist in charge of the patient declared if his/her patient had an history of an AID (detailed in the online supplemental annex). For most of these patients, the decision of initiating an ICI therapy was taken collegially by multidisciplinary boards specialised in the management of immunotoxicities due to their history of AIDs and weighted while considering the diagnostic criteria and AID activity. All cases of pAID were centrally reviewed by the authors at the conception of the study.

**Table 1** Cases and controls characteristics

| | Cases (N=110) | Controls (N=330) | P value |
|---|---|---|---|
| Age (years old, mean (SD)) | 65 (14) | 65 (18) | 1 |
| >65 years old (no (%)) | n=65 (59) | n=195 (59) | 1 |
| Gender (no (%)) | | | 1 |
| Female | n=58 (53) | n=174 (53) | |
| Male | n=52 (47) | n=156 (47) | |
| Body mass index (kg/m$^2$, mean (SD)) | 26 (21) | 25 (20) | 0.8 |
| Melanoma stage | | | 0.3 |
| M1a | n=9 (8%) | n=51 (15%) | |
| M1b | n=22 (20%) | n=55 (17%) | |
| M1c | n=59 (54%) | n=172 (52%) | |
| IIIB | n=5 (5%) | n=8 (2%) | |
| IIIC | n=15 (14%) | n=44 (13%) | |
| BRAF status (no (%)) | | | 0.8 |
| Wild type | n=67 (61) | n=195 (59) | |
| Mutated | n=43 (39) | n=135 (41) | |
| LDH at baseline | | | |
| >1x ULN | n=29 (26%) | n=87 (26%) | 1 |
| >2x ULN | n=4 (4%) | n=17 (5%) | 0.7 |
| ECOG status at baseline | | | **0.02*** |
| 0–1 | n=84 (76%) | n=285 (86%) | |
| >1 | n=26 (24%) | n=45 (14%) | |
| Number of metastases (no (%)) | | | 1 |
| <3 | n=16 (15) | n=48 (15) | |
| ≥3 | n=94 (85) | n=282 (85) | |
| Hepatic metastases | | | 1 |
| Yes | n=30 (27%) | n=90 (27%) | |
| Cerebral metastases | | | 1 |
| Yes | n=28 (25%) | n=84 (25%) | |
| First-line immunotherapy | | | 1 |
| Yes | n=81 (74%) | n=243 (74%) | |
| No | n=29 (26%) | n=87 (26%) | |
| Number of previous therapeutic lines | | | 1 |
| 0 | n=81 (74%) | n=243 (74%) | |
| 1 | n=19 (17%) | n=57 (17%) | |
| ≥2 | n=10 (9%) | n=30 (9%) | |
| Immunotherapy regimen | | | 1 |
| Anti-PD-1 monotherapy | n=86 (78%) | n=258 (78%) | |
| Pembrolizumab | n=40 (36%) | n=120 (36%) | |
| Nivolumab | n=46 (42%) | n=138 (42%) | |
| Anti-CTLA-4 monotherapy | n=15 (14%) | n=45 (14%) | |
| Anti-PD-1+anti-CTLA-4 combination | n=9 (8%) | n=27 (8%) | |

* p<0.05

ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; PD-1, programmed cell death protein 1; ULN, upper limit of normal.

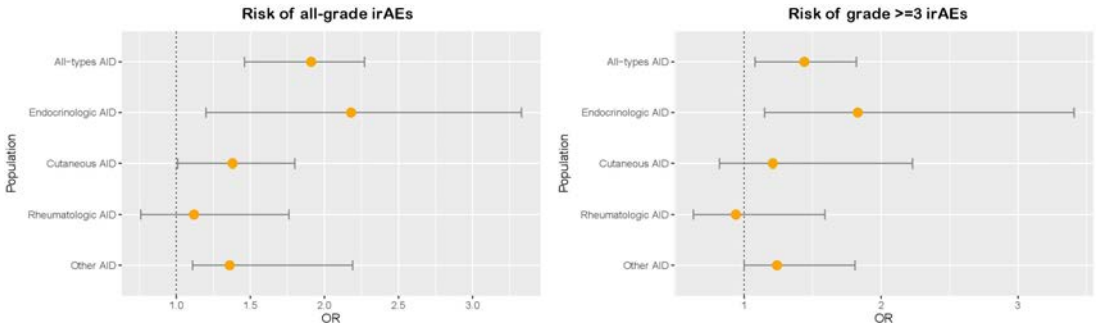


**Figure 1** Risk of all-grade irAEs and risk of grade ≥3 irAEs in patients with pre-existing autoimmune diseases matched to controls. AID, autoimmune disease; irAEs, immune-related adverse events.

### Definitions and classification of irAEs

IrAEs were defined as an event or laboratory test abnormality, considered to be possibly, probably or certainly linked to the immunotherapy, following the WHO-UMC (WHO-Uppsala Monitoring Centre) causality assessment guidelines, and recorded prospectively during the follow-up. Severity was determined using the CTCAE v4.0. The clinical subtypes of irAEs were regrouped as follows: cardiovascular, endrocrinological, rheumatological, cutaneous, pulmonary, haematological, neurological, psychiatric, renal, ophthalmological, musculoskeletal and general symptoms.

### Statistical analysis and case–control matching

Baseline demographic and disease characteristics were summarised as numbers and percentages for categorical variables, and as mean, SD, median, IQR and range for continuous variables, as appropriate. Case–control matching was performed on R statistical software V.4.0.4, on a 3-controls-for-1-case ratio, using the following criteria: age (by 2-year range classes), sex, immunotherapy regimen (anti-PD-1 monotherapy, anti-CTLA-4 monotherapy, or anti-PD-1 and anti-CTLA-4 associated therapy), number of previous therapeutic lines, baseline lactate dehydrogenase (LDH) values (by quartiles), existence of liver metastasis and of cerebral metastasis and number of metastasis (< or ≥3 sites). Comparison of variable distribution between cases and controls was performed using either $\chi^2$, Fisher's exact test and Kruskal-Wallis's test when appropriate. Logistic regression was used to calculate OR of developing irAEs. Patients censored before 12 months were patients lost to follow-up (most commonly patients who had completed their follow-up). Missing data were handled by multiple imputation using chained equations. Twenty datasets were imputed and analysed separately, and results were then pooled into a final estimate. Survival analysis was performed using log-rank test and Kaplan-Meier method with associated 95% CI was used to generate survival curves and estimate overall survival and time-to-first-irAE. Data analyses were conducted using R statistical software V.4.0.4 and the R MICE (Multivariate Imputation by Chained Equations) package to address missing data (The R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set to $p<0.05$.

### Patient's consent

The French Ethics Committee approved MelBase protocol (Comité de protection des personnes Ile-de-france XI, no 12027, 2012). MelBase is registered in the National Institute of Health clinical trials database (NCT02828202). Written informed consent was obtained from all patients.

### Patient's and public involvement

None.

## RESULTS

### Patient's characteristics

One hundred and ten patients with pAID were identified among the 2227 patients included in the prospective cohort MelBase from March 2013 to October 2020, and matched with 330 controls, on a 1-case-for-3-controls ratio. The median duration of follow-up was 7.24 months (IQR (3.67–23.41)) for cases and 6.86 months (IQR (3.62–20.83)) ($p=0.30$) (online supplemental figure).

The mean age was 65 years old for cases and controls. There was no difference for the gender repartition, nor for the mean body mass index (table 1). Thirty-nine per cent of the cases harboured a BRAF mutation, and 41% of the controls ($p=0.8$). The proportion of patients with an ECOG (Eastern Cooperative Oncology Group) performance status score >1 was higher in cases than in controls (24% vs 14%, $p=0.02$) (table 1).

Seventy-eight per cent of the patients were treated by anti-PD-1 monotherapy (42% by nivolumab, 36% by pembrolizumab), 14% by anti-CTLA-4 monotherapy and 8% by the association of anti-PD-1 and anti-CTLA-4 therapy (combination therapy). Seventy-four per cent received ICI as first-line immunotherapy.

### Pre-existing autoimmune diseases

Of the 110 cases, 47 (43%) had a history of autoimmune thyroiditis, 18 (16%) of psoriasis, 11 (10%) of rheumatoid arthritis, 8 (7%) of vitiligo, 3 (3%) of sarcoidosis, 3 of Raynaud disease, 3 of spondyloarthritis, 3 of multiple sclerosis, 2 (2%) of Crohn's disease, 2 of thrombopenic idiopathic purpura, 2 of giant cell arteritis, 2 of myasthenia, 1 (1%) of Guillain-Barré syndrome, 1 of systemic sclerosis, 1 of polymyositis, 1 of dermatomyositis, 1 of autoimmune hepatitis and 1 of IgA nephropathy. The median time from AID diagnosis to first ICI infusion was 103 months (IQR (37–241)). Seventy cases (63%) were treated for their pAID in the 3 months before ICI initiation, and 19 (17%) by immunosuppressive or immunomodulatory agents (13 by systemic corticosteroid therapy and 8 by methotrexate). We separated pAID into four subgroups: endocrinological pAID (autoimmune thyroiditis), cutaneous pAID (psoriasis and vitiligo), rheumatological pAID (rheumatoid arthritis and spondyloarthritis) and others. Endocrinological and cutaneous pAID were more often treated by anti-CTLA-4 monotherapy or combination therapy, and rheumatological and other pAID were more often treated by immunosuppressive agents at baseline (online supplemental table).

**Table 2** Characteristics of the irAEs presented by cases and controls

| | Cases (N=110) | Controls (N=330) | P value |
|---|---|---|---|
| Duration of follow-up from first infusion (months, median (IQR)) | 7.24 (3.67–23.41) | 6.86 (3.62–20.83) | 0.3 |
| Number of cures before first irAEs (mean, SD) | 1.4 (0.76) | 1.3 (0.49) | 0.1 |
| Time between first infusion and first irAEs (months, mean (SD)) | | | |
| All-grade | 4.8 (12.1) | 4.3 (11.2) | 0.12 |
| Grade ≥3 irAEs | 8.3 (16.4) | 8.9 (17.4) | 0.16 |
| Immunotherapy interruption due to irAEs | | | |
| Temporarily | 15 (14%) | 36 (11%) | 0.6 |
| Definitively | 8 (7%) | 14 (4%) | 0.3 |
| Number of irAEs per patient (mean, SD) | 7.2 (1.2) | 5.1 (1.1) | **0.04*** |
| Number of grade ≥3 irAEs per patient (mean, SD) | 1.04 (0.3) | 0.40 (0.10) | 0.05 |
| Death due to irAEs (no. (%)) | n=2 (1.8) | n=6 (1.8) | 1 |
| Total number of irAEs | n=794 | n=1683 | 0.56 |
| Type of irAEs (no (%)): | | | |
| Cardiological | 16 (2.0) | 30 (1.8) | |
| Cutaneous | 94 (11.8) | 184 (10.9) | |
| Digestive tract | 166 (20.9) | 402 (23.9) | |
| Endocrinological | 48 (5.6) | 56 (3.3) | |
| Haematological | 33 (4.2) | 69 (4.1) | |
| Neurological | 47 (5.9) | 106 (6.3) | |
| Pulmonary | 33 (4.2) | 76 (4.5) | |
| Rheumatological | 21 (2.6) | 23 (1.4) | |
| Renal | 17 (2.1) | 29 (1.7) | |
| Psychiatric | 15 (1.9) | 31 (1.8) | |
| Musculoskeletal | 22 (2.8) | 53 (3.1) | |
| Ophthalmological | 17 (2.1) | 32 (1.9) | |
| General symptoms | 265 (33.4) | 592 (35.2) | |
| Total number of grade ≥3 irAEs | n=114 | n=132 | 0.74 |
| Type of grade ≥3 irAEs (no (%)) | | | |
| Cardiological | 4 (3.5) | 6 (4.5) | |
| Cutaneous | 6 (5.3) | 9 (6.8) | |
| Digestive tract | 32 (28.0) | 42 (31.8) | |
| Endocrinological | 12 (10.5) | 10 (7.6) | |
| Haematological | 8 (7.0) | 10 (7.6) | |
| Neurological | 3 (2.6) | 4 (3.0) | |
| Pulmonary | 8 (7.0) | 10 (7.6) | |
| Rheumatological* | 2 (1.8) | 3 (2.3) | |
| Renal | 5 (4.4) | 2 (1.5) | |
| Psychiatric | 5 (4.4) | 2 (1.5) | |
| Musculoskeletal* | 1 (0.9) | 4 (3.0) | |
| Ophthalmological | 3 (2.6) | 3 (2.3) | |
| General symptoms | 25 (21.9) | 27 (20.4) | |

*Rheumatological irAEs included arthralgia and arthritis. Musculoskeletal irAEs included myalgia, myositis, muscle weakness, CPK (Creatinine phosphokinase) increase and bone pain.
irAEs, immune-related adverse events.

## Risk of irAEs and severe irAEs

Overall, 72% of the 118 cases and 77% of the 354 controls presented with at least one irAE of all-grades, and 57% and 37% with at least one irAE of grade ≥3. There was no difference for the number of immunotherapy cures before irAEs between cases and controls (1.4 vs 1.3, p=0.1). The mean time from first infusion of immunotherapy to first irAEs was 4.5 months (SD 3.0) in the whole population, 4.8 months for cases and 4.3 months for controls (SD 2.8 and SD 2.6, respectively, p=0.12).

Cases had a higher risk of all-grade irAE occurrence (OR 1.91, 95% CI (1.56 to 2.27), p=0.03), along with a higher risk of grade ≥3 irAEs (OR 1.44, 95% CI (1.08 to 1.82), p=0.04) when compared with matched controls (figure 1). When considering only organ-specific irAEs (ie, after exclusion of general symptoms of irAEs), cases still had a higher risk of all-grade irAE occurrence (OR 2.26, 95% CI (1.22 to 4.39), p=0.03) and of grade ≥3 irAEs (OR 1.71, 95% CI (1.09 to 2.34), p=0.05).

The risk of multiple irAEs was also higher among cases (OR 1.46, 95% CI (1.15 to 2.67)) with a mean number of irAEs per patient of 7.2 (SD 1.2) versus 5.1 for controls (SD 1.1) (p=0.04, table 2). Both all-grade and grade ≥3 irAEs occurred earlier in cases (log-rank test, p=0.002 and p=0.01, respectively;

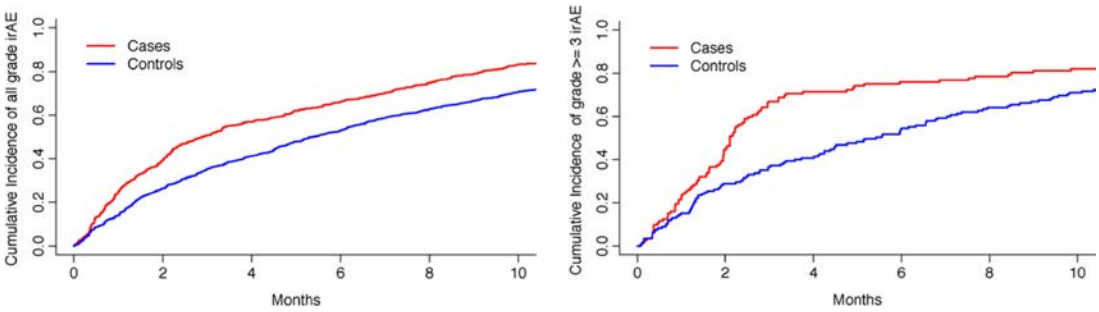


**Figure 2** Cumulative incidence of all-grade irAE and grade ≥3 irAE among patients with pre-existing autoimmune diseases and matched controls. Cases: patients with pre-existing autoimmune disease, controls: patients without pre-existing autoimmune disease. The x axis represents the percentage of patients who developed 100% of their irAEs at a given follow-up time, represented on the y axis. Both cases and controls curves will reach a plateau at 1.0 at the end of follow-up. irAE, immune-related adverse event.

figure 2). No difference was seen for irAEs-related mortality, and the proportion of patients requiring definitive immunotherapy cessation after irAEs was not higher in cases than in controls (7% vs 4%, p=0.30, table 2). The existence of a baseline immunosuppressive therapy in cases was not associated with a significant protective effect on all-grade or grade ≥3 irAEs (OR 1.18, 95% CI (0.66 to 2.13), p=0.57 and OR 0.91, 95% CI (0.38 to 2.21), p=0.85, respectively).

### Risk of irAEs depending on the ICI regimen

The higher risk of all-grade irAEs in patients with pAID was consistent in patients treated by anti-PD-1 monotherapy (OR 2.47, 95% CI (1.40 to 4.92), p=0.03), anti-CTLA-4 monotherapy (OR 1.82, 95% CI (1.02 to 4.51), p=0.05), combination therapy (OR 2.31, 95% CI (1.18 to 5.10, p=0.04), as well as the higher risk of grade ≥3 irAEs in patients treated by anti-PD-1 monotherapy (OR 1.80, 95% CI (1.19 to 2.47), p=0.04), anti-CTLA-4 monotherapy (OR 1.50, 95% CI (1.03 to 2.42) p=0.05) and combination therapy (OR 1.49, 95% CI (1.07 to 2.46), p=0.05).

### Risk of irAEs and pAID flares among pAID subgroups

When considering pAID subgroups (online supplemental table), endocrinological pAID were associated with a higher risk of all-grade irAEs (OR 2.18, 95% CI (1.20 to 3.33) p=0.03) and of grade ≥3 irAEs (OR 1.83, 95% CI (1.15 to 3.41), p=0.04) (figure 1). Neither cutaneous nor rheumatological pAID were associated with a higher risk of all-grade irAEs (OR 1.38, 95% CI (1.01 to 1.80), p=0.05, and OR 1.12, 95% CI (0.76 to 1.76), p=0.16, respectively) nor with a higher risk of grade ≥3 irAEs (OR 1.21, 95% CI (0.82 to 2.23), p=0.10, and OR 0.94, 95% CI (0.63 to 1.59), p=0.13). The subgroup of 'others' pAID was associated with a higher risk of all-grade irAEs and of grade ≥3 irAEs (OR 1.36, 95% CI (1.11 to 2.04), p=0.04, and OR 1.24, 95% CI (1.09 to 1.81), p=0.04, respectively). Overall, 33 (30%) cases experienced a flare of their pAID: 12 (25%) with endocrinological pAID, 8 (31%) in patients with cutaneous pAID, 7 (50%) in patients with rheumatological pAID and 6 (26%) in patients with others pAID.

### Clinical subtypes of irAEs

The distribution of the clinical irAE subtypes did not differ between cases and controls, neither for all-grade irAEs nor for grade ≥3 irAEs (table 2). The most frequent grade ≥3 irAEs were digestive tract, endocrinological, haematological and pulmonary (table 2).

### Distribution of irAE subtypes depending on pAID subgroup

We compared the distribution of the irAE clinical subtypes between patients with endocrinological, cutaneous, rheumatological and others pAID. The overall distribution of all-grade (but not grade ≥3) irAE clinical subtypes was significantly different between pAID subgroups (p=0.04) (table 3). Patients with rheumatological pAID had a higher frequency of gastrointestinal tract and rheumatological all-grade irAEs (p=0.04 and p<0.0001, respectively) as well as of severe irAEs (p=0.002). Patients with endocrinological pAID had the higher frequency of all-grade neurological irAEs (p<0.0001) but not of severe neurological irAEs. Musculoskeletal irAEs were more frequent among patients with others pAID (p=0.02) (table 3).

### Survival analysis

Cases had an increased overall survival when compared with controls (log-rank test, p=0.02; figure 3). However, many of the survival data were censored due to restricted follow-up duration. The estimated landmark overall survival at 24 months was 64.8% (IQR (56.2–74.7) for cases and 45.9% (IQR (40.4–52.1)) for controls. We compared cases treated with immunosuppressive agents or not and did not find a difference in survival (log-rank test, p=0.68).

## DISCUSSION

While previous studies reported a high risk of irAEs and/or AID flare in patients with pAID treated by ICI, this study is to our knowledge the first to compare these patients with matched controls for irAE risk factors and oncological status, in a prospective cohort of patients treated for metastatic melanoma. The strengths of our study are the prospective collection of data and the case–control design allowing for precise quantification of the risk of irAEs depending on the existence of a history of AID.

The main limitations are the absence of data on the irAEs' therapeutical management apart from checkpoint inhibitor therapy discontinuation, and the limited follow-up duration which might have underestimated the risk of delayed irAEs. Another potential limitation is the absence of a systematic set of diagnostic criteria fulfilment at the inclusion of a patient with pAID in the MelBase cohort. This could restrict the interpretation of our observations in small groups of complex AIDs. The theoretical risk of misclassification of a control as a case, despite central reviewing, would result in negative bias and thus does not prevent the extrapolation of our results.

In our study, patients with pAID had two times higher risk of developing both all-grade irAEs than controls, independently of

**Table 3** Distribution of the clinical subtypes of irAEs depending on the subgroup of pre-existing autoimmune disease

| irAE subtype | Endocrinological AID | Cutaneous AID | Rheumatological AID | Others AID | P value for the distribution |
|---|---|---|---|---|---|
| Median number of all-grade irAEs (IQR) | 4.1 (0–13) | 1.5 (0–8) | 3 (1–6) | 4(1–13) | **0.04*** |
| Median number of grade 3+ irAEs (IQR) | 1 (0–1) | 1 (0–1) | 1 (0–1) | 2 (0–4) | 0.16 |
| Endocrinological irAEs (no (%)) | | | | | |
| All-grade | 19 (5.2) | 8 (5.4) | 6 (7.3) | 15 (7.5) | 0.65 |
| Grade 3+ | 2 (5.6) | 1 (3.8) | 1 (7.1) | 8 (20.5) | 0.12 |
| Digestive tract irAEs (no(%)) | | | | | |
| All-grade | 65 (17.9) | 32 (21.8) | 22 (26.9) | 47 (23.4) | **0.04*** |
| Grade 3+ | 12 (33.3) | 6 (23.1) | 4 (28.6) | 10 (25.6) | 0.83 |
| Cutaneous irAEs (no (%)) | | | | | |
| All-grade | 53 (14.6) | 16 (10.9) | 7 (8.5) | 18 (9.0) | 0.16 |
| Grade 3+ | 2 (5.6) | 2 (7.7) | 0 (0) | 3 (7.7) | 0.86 |
| Haematological irAEs (no (%) | | | | | |
| All-grade | 12 (3.3) | 6 (4.1) | 8 (9.8) | 7 (3.5) | 0.09 |
| Grade 3+ | 5 (13.9) | 2 (7.7) | 0 (0) | 1 (2.6) | 0.21 |
| Neurological irAEs (no (%)) | | | | | |
| All-grade | 33 (9.1) | 3 (2.0) | 0 (0) | 11 (5.5) | **<0.001*** |
| Grade 3+ | 1 (2.8) | 1 (3.8) | 0 (0) | 1 (2.6) | 1.0 |
| Pulmonary irAEs (no (%)) | | | | | |
| All-grade | 18 (4.9) | 7 (4.8) | 1 (1.2) | 7 (3.5) | 0.47 |
| Grade 3+ | 2 (5.6) | 2 (7.7) | 1 (7.1) | 3 (7.7) | 1.0 |
| Cardiovascular irAEs (no (%)) | | | | | |
| All-grade | 7 (1.9) | 3 (2.0) | 0 (0) | 6 (3.0) | 0.50 |
| Grade 3+ | 1 (2.8) | 2 (7.7) | 0 (0) | 1 (2.6) | 0.67 |
| Rheumatological irAEs* (no (%)) | | | | | |
| All-grade | 7 (1.9) | 6 (4.1) | 8 (9.8) | 0 (0) | **<0.001*** |
| Grade 3+ | 0 (0) | 0 (0) | 2 (14.2) | 0 (0) | **0.002*** |
| Renal irAEs (no (%)) | | | | | |
| All-grade | 9 (2.) | 5 (3.4) | 1 (1.2) | 2 (1.0) | 0.43 |
| Grade 3+ | 2 (5.6) | 2 (7.7) | 0 (0) | 1 (2.6) | 0.69 |
| Psychiatric irAEs (no (%)) | | | | | |
| All-grade | 3 (0.8) | 4 (2.7) | 1 (1.2) | 7 (3.5) | 0.09 |
| Grade 3+ | 1 (2.8) | 1 (3.8) | 1 (7.1) | 2 (5.1) | 0.92 |
| Musculoskeletal irAEs* (no (%)) | | | | | |
| All-grade | 5 (1.4) | 4 (2.7) | 1 (1.2) | 12 (6.0) | **0.02*** |
| Grade 3+ | 0 (0) | 1 (3.8) | 0 (0) | 0 (0) | 0.35 |
| Ophthalmological irAEs (no (%)) | | | | | |
| All-grade | 8 (2.2) | 1 (0.7) | 3 (3.7) | 5 (2.5) | 0.42 |
| Grade 3+ | 2 (5.6) | 0 (0) | 1 (7.1) | 0 (0) | 0.13 |
| General symptom irAEs (no (%)) | | | | | |
| All-grade | 125 (34.3) | 52 (35.4) | 24 (29.3) | 64 (31.8) | 0.74 |
| Grade 3+ | 6 (16.7) | 6 (23.1) | 4 (28.6) | 9 (23.1) | 0.77 |

*: p<0.05

*Rheumatological irAEs included arthralgia and arthritis. Musculoskeletal irAEs included myalgia, myositis, muscle weakness, CPK increase and bone pain.
AID, autoimmune disease; irAEs, immune-related adverse events.

known risk factors of irAEs, such as age, sex,[27 28] the immunotherapy regimen[29] and/or of disease status, such as the melanoma stage, the existence of hepatic or cerebral metastasis, the number of metastasis and the LDH values at baseline.[30 31] Additionally, there were no difference between cases and controls for the body mass index values.[32] We were not able to perform a matching of the ECOG status because of a higher proportion of ECOG 0 patients among control candidates. Thus, cases were more often classified as ECOG >1 than controls, which could, however, have undermined our results, as low ECOG has been associated with an increased risk of irAEs and of multisystem irAEs.[33 34] We also reported a higher risk of multiple irAEs, and a shorter time to both all-grade and grade ≥3 irAE onset in patients with pAID, as previously reported.[22]

The most frequently represented AID among cases were autoimmune thyroiditis, psoriasis, rheumatoid arthritis and vitiligo, in a relative proportion close to the one described in the general population.[35] We regrouped the cases into four pAID subgroups based on their clinical expression, a choice that might not reflect common disease pathogenesis but rather help categorise patients in the real-world practice. Patients with endocrinological and others pAID had an increased risk of all-grade and grade ≥3

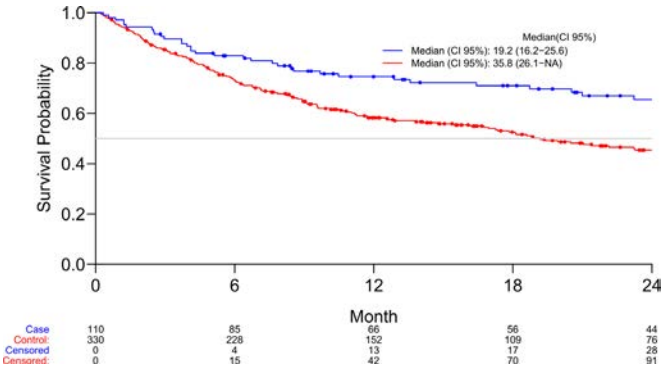


**Figure 3** Overall survival among patients with pre-existing autoimmune diseases and matched controls. Cases: patients with pre-existing autoimmune disease, controls: patients without pre-existing autoimmune disease. NA, not applicable.

irAEs, but not patients with cutaneous or rheumatological pAIDs. This difference could be explained by the high proportion of immunosuppressive treatment at baseline in patients with rheumatological pAID or be the testament of physiopathological differences. Thirty per cent of the cases experienced a pAID flare during follow-up. As the pAID flares were all declared as irAEs, this might have overestimated the number of irAEs in cases—however, they only represent a small fraction of the irAEs developed over follow-up in this group (n=794) and were the most frequent in patients with rheumatological irAEs, whose risk of irAEs was not increased when compared with controls in the subgroup analysis. Importantly, we noticed a difference in the clinical subtypes of all-grade irAEs depending on the subgroup of pAID, suggesting a predisposition for certain irAEs. These patterns of irAE clinical subtypes' susceptibility could help monitoring patients with pAID treated by ICI and improve the screening for irAEs in this population.

Both cases and controls presented with a rather high number of irAEs compared with previously published data. This might be explained by the systematic and prospective recording of all-grade irAEs and by the inclusion of general symptom irAEs, which were declared as linked to the immunotherapy but might also reflect cancer evolution. Importantly, after exclusion of general symptom irAEs, the ORs of developing all-grade and grade ≥3 irAEs in cases compared with controls were even greater. Despite of a higher risk of grade ≥3 irAEs, the proportion of lethal irAEs did not differ between cases and controls, and cases had an increased overall survival rate. This result could possibly be linked to the increased occurrence of multiple irAEs, which have reportedly been associated with increased survival in patients with NSCLC.[34] Moreover, in patients treated for metastatic melanoma or NSCLC, the occurrence of irAEs seems to be associated with an increased oncological survival.[36 37]

Our findings are in line with previously published data from retrospective case-series and prospective cohorts. Menzies *et al* were the first to address the risk of irAEs in patients with pAID and found an incidence of 29% of all-grade irAEs among 51 patients with pAID followed for a median of 4.7 months and a 8% ICI discontinuation rate.[21] Danlos *et al* reported a grade ≥2 irAE incidence of 44% over a 5.1-month period of follow-up, along with an ICI discontinuation rate of 11.1%, in 45 patients with pAID compared with 352 patients without pAID from the REISAMIC prospective registry.[22] The higher incidence of all-grade irAEs in our study can be explained by the prospective design and systematic recording of all-grade irAEs, the differences in ICI regimen[6 38] and longer follow-up. In a large multicentric retrospective study, Cortellini *et al* found a 65.9% incidence of all-grade irAEs in 85 patients with pAID followed for a median of 14.7 months, along with an ICI discontinuation rate of 7%.[33] Interestingly, both inactive and active pAID were associated with a higher risk of all-grade irAEs. Moreover, in accordance with our results, the risk of all-grade irAEs was greater in patients with endocrinological pAID and lower in patients with rheumatological pAID. In a large multicentric retrospective study, Tison *et al* reported a 71% incidence of all-grade irAEs and/or autoimmune flares in 112 patients with pAID followed for a median of 8 months. The ICI discontinuation rate (21%) was greater than in our study, possibly reflecting a higher proportion of potentially severe AIDs, such as inflammatory bowel disease.[25] Our results however contrast with those of a recent prospective cohort study published by Van der Koij *et al*, which did not find an increase in the risk of grade ≥3 irAEs among patients with pAID treated by checkpoint inhibitors for advanced melanoma, a difference possibly explained by the absence of a matched case–control design for irAEs risk factors and by the high proportion of patients with AID treated by corticosteroids at inclusion.[26]

Overall, while our results pinpoint an increased risk of irAEs and severe irAEs in patients with pAID, we did not find an increased risk of lethal irAEs and reported an increased overall survival when compared with controls, further confirming they should be considered for ICI therapy. Patients with pAID should, however, be closely monitored for irAEs, which are more frequent and occur earlier. As the risk of irAEs differs depending on the nature of the pAID, specific guidelines for autoimmune flares and irAEs should be considered.[39] Ongoing clinical trials will provide valuable data to confirm the safety and efficacy of ICI in patients with pAID.[40]

## CONCLUSION

In this case–control study, the existence of a history of AID was associated with an increased risk of all-grade irAEs, severe irAEs and multiple irAEs, yet was associated with an increase in overall survival. Patients with a history of AID should be closely monitored during checkpoint inhibitor therapy.

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CLINICAL SCIENCE

# Gain-of-function mutations in *ALPK1* cause an NF-κB-mediated autoinflammatory disease: functional assessment, clinical phenotyping and disease course of patients with ROSAH syndrome

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## ABSTRACT

**Objectives** To test the hypothesis that ROSAH (retinal dystrophy, optic nerve oedema, splenomegaly, anhidrosis and headache) syndrome, caused by dominant mutation in *ALPK1*, is an autoinflammatory disease.

**Methods** This cohort study systematically evaluated 27 patients with ROSAH syndrome for inflammatory features and investigated the effect of *ALPK1* mutations on immune signalling. Clinical, immunologic and radiographical examinations were performed, and 10 patients were empirically initiated on anticytokine therapy and monitored. Exome sequencing was used to identify a new pathogenic variant. Cytokine profiling, transcriptomics, immunoblotting and knock-in mice were used to assess the impact of *ALPK1* mutations on protein function and immune signalling.

**Results** The majority of the cohort carried the p.Thr237Met mutation but we also identified a new ROSAH-associated mutation, p.Tyr254Cys.
Nearly all patients exhibited at least one feature consistent with inflammation including recurrent fever, headaches with meningeal enhancement and premature basal ganglia/brainstem mineralisation on MRI, deforming arthritis and AA amyloidosis. However, there was significant phenotypic variation, even within families and some adults lacked functional visual deficits. While anti-TNF and anti-IL-1 therapies suppressed systemic inflammation and improved quality of life, anti-IL-6 (tocilizumab) was the only anticytokine therapy that improved intraocular inflammation (two of two patients). Patients' primary samples and in vitro assays with mutated ALPK1 constructs showed immune activation with increased NF-κB signalling, STAT1 phosphorylation and interferon gene expression signature. Knock-in mice with the *Alpk1* T237M mutation exhibited subclinical inflammation.
Clinical features not conventionally attributed to inflammation were also common in the cohort and included short dental roots, enamel defects and decreased salivary flow.

**Conclusion** ROSAH syndrome is an autoinflammatory disease caused by gain-of-function mutations in *ALPK1* and some features of disease are amenable to immunomodulatory therapy.

**WHAT IS ALREADY KNOWN ON THIS TOPIC**

⇒ The p.Thr237Met variant in *ALPK1* has been associated with a dominantly inherited form of progressive blindness. ALPK1's role in human physiology and immune regulation is still under investigation but the protein is known to act as a sensor for bacterial sugars.

### WHAT THIS STUDY ADDS

⇒ This is the first study to demonstrate that retinal dystrophy, optic nerve oedema, splenomegaly, anhidrosis and headache (ROSAH) syndrome is an autoinflammatory disease caused by gain-of-function mutations in *ALPK1* and it identifies a second *ALPK1* mutation associated with human disease. The study also establishes that ROSAH syndrome can present with a range of systemic features including recurrent fever, uveitis, deforming arthritis, AA amyloidosis, meningeal enhancement and premature mineralisation of the basal ganglia, substantia nigra and red nuclei on MRI and many manifestations of disease are amenable to modulation with anticytokine therapy.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

⇒ This study introduces ROSAH syndrome as a new autoinflammatory disease and emphasises the need for broader awareness of the disease to facilitate early diagnosis so that patients can be evaluated for immunomodulatory treatment before they suffer irreversible damage from chronic inflammation. It also lays the foundation for future studies to investigate the specific impact of IL-6 inhibition on disease course given its success in reducing intraocular inflammation for two patients.

## INTRODUCTION

A heterozygous missense variant p.Thr237Met (T237M) in the *alpha kinase 1* gene (*ALPK1*) has been shown to cause a syndrome termed retinal dystrophy, optic nerve oedema, splenomegaly, anhidrosis and headache (ROSAH), denoting the features of ROSAH.[1] Nevertheless, ALPK1's role in human biology is still under investigation, and little has been reported about the mechanism through which the *ALPK1* mutation causes ROSAH syndrome.

The initial paper describing families with ROSAH focused on the ophthalmologic manifestations of the disease and proposed that, like many other forms of heritable retinal degeneration, ROSAH syndrome may be a ciliopathy.[2] In support of this hypothesis, the authors showed that ALPK1 localises to the ciliary basal body in retinal pigment epithelial cells, and primary cilia formation is dysfunctional in primary patient cells.[1]

However, there is increasing evidence that ALPK1 plays a role in innate immune activation.[3–8] ALPK1 has been shown to act as an intracellular sensor for metabolites produced by a variety of bacteria including *Helicobacter pylori*, *Shigella flexneri* and *Burkholderia cenocepacia*. Specifically, the N-terminal domain of ALPK1 binds bacterial sugars, including ADP-beta-D-manno-heptose (ADP-heptose). On activation, the kinase domain of ALPK1 phosphorylates TRAF-interacting protein with fork head-associated domain, leading to enhanced NF-κB signalling.[3] Wild-type mice injected with subcutaneous ADP-heptose demonstrated massive neutrophil recruitment and increased production of NF-κB-induced cytokines and chemokines, whereas these responses were compromised in ALPK1 knock-out mice.[3] ALPK1 has also been linked to inflammatory conditions in humans. Single-nucleotide polymorphisms in *ALPK1* have been associated with an increased risk of gout, while rare variants have been identified in patients with recurrent periodic fevers.[9–11] These data suggested that patients with ROSAH may have an inflammatory signature. However, systematic analysis of inflammatory features in humans or mice harbouring activating mutations in *ALPK1* has not been performed.

Given ALPK1's role as an innate immune sensor, we hypothesised that ROSAH syndrome is an autoinflammatory disease. To test this hypothesis, we characterised a large cohort of molecularly diagnosed patients and analysed the effect of *ALPK1* pathogenic variants on protein function and immune signalling.

## METHODS

This cohort study included 27 patients with ROSAH syndrome from 8 countries. Twenty patients from 12 unrelated families were confirmed to carry the T237M variant. Six individuals who were a first-degree relative of a proband and had at least two of three features of optic nerve oedema or advanced retinal degeneration, anhidrosis and splenomegaly secondary to red pulp congestion were also included in the cohort. One patient with the ROSAH syndrome phenotype lacked the T237M variant but was found to carry a previously unreported heterozygous missense variant, *ALPK1* p.Tyr254Cys (figure 1A,B).

Between September 2019 and April 2022, information on demographics, clinical manifestations, laboratory parameters and disease course were compiled through interviews of patients or first-degree relatives and review of medical records. During this same period, 11 of these patients were also evaluated at the National Institutes of Health (NIH) Clinical Centre using clinical, radiographic, ultrasonographic and functional examinations. Biological specimens were collected for functional analyses and patients were empirically given immunomodulatory therapies including adalimumab, anakinra, canakinumab and tocilizumab when clinically appropriate and acceptable to the patient.

Additional details are provided in the supplement and include methods for identifying the previously unreported Y254C variant and methods for measurement of soluble biomarkers, gene-expression studies, luciferase assay and development of a knock-in mouse model.

## RESULTS

### Patient population

Twenty-seven patients with ROSAH syndrome were included in this cohort (online supplemental table 1 and figure 1).

### Identification of Y254C variant in an individual with ROSAH syndrome

Exome sequencing in patient F13.1 led to identification of a novel heterozygous missense substitution NM_025144.4: c.761A>G; p.Tyr254Cys (Y254C). The patient is of European ancestry, and she is the only clinically affected member in her family. The variant was absent in her mother, and no samples were available from her deceased father or first-degree relatives.

The variant occurs in the ligand binding domain, is predicted to be damaging to protein function by multiple in silico algorithms, including Varsome, PolyPhen-2, SIFT and CADD (24.3) and is absent in the population database gnomAD.[12] The Tyr254 residue is evolutionarily conserved in vertebrates (figure 1C).

### Clinical features of ROSAH syndrome

ROSAH patients presented with variable clinical features; however, penetrance was complete in all identified family members. Prominent clinical characteristics are summarised in figure 1D, and representative images are provided in figure 2A–G.

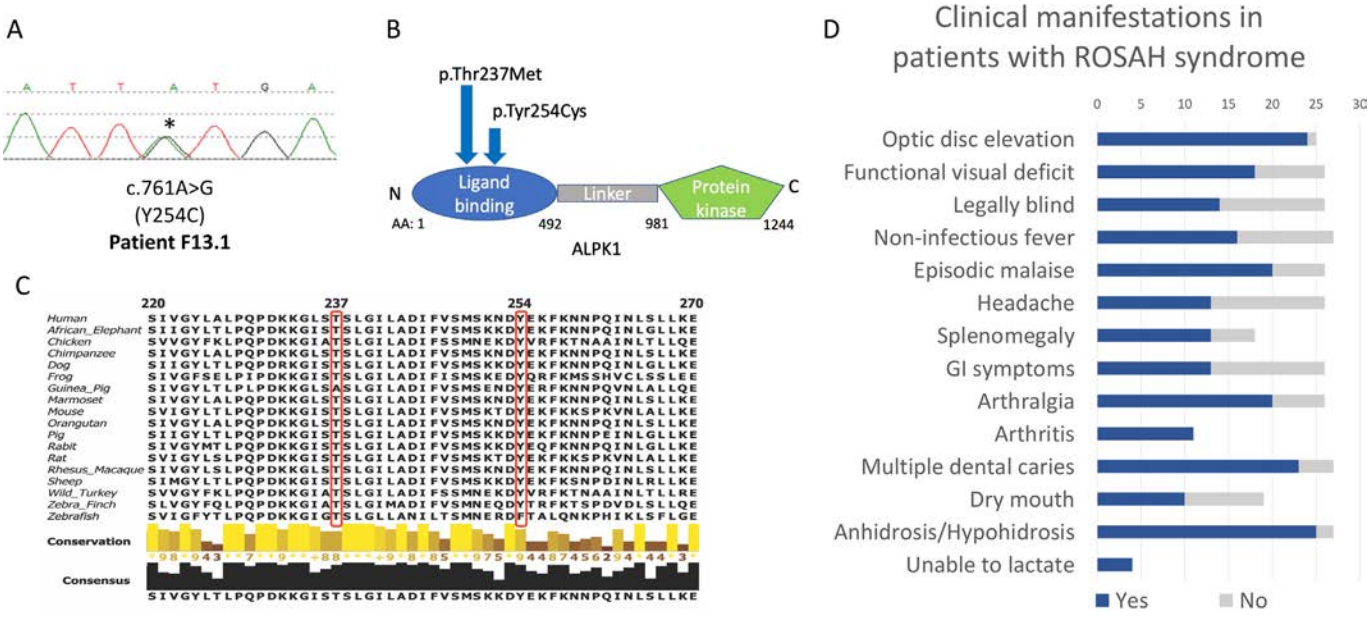


**Figure 1** Heterozygous missense mutations of *ALPK1* in the cohort and overview of clinical manifestations observed in patients with ROSAH syndrome. (A) Electropherogram of the previously unreported Y254C mutation for patients F13.1. (B) Domain structure of ALPK1 protein, indicating the location of the observed ROSAH-associated mutations (T237M and Y254C). (C) Schematic showing cross-species conservation of ALPK1 in the regions flanking the T237M and Y254C mutations. Sequences were obtained from Uniprot and multiple sequence alignments were created on Clustal Omega. (D) Bar chart indicating the prevalence of clinical manifestations reported in our ROSAH syndrome cohort. Patient F2.4 has cerebral palsy and was unable to provide any information about subjective clinical features. Blue shading indicates yes, and grey shading indicates no. Splenomegaly as determined by ultrasounds and arthritis as demonstrated by X-ray. Arthritis was present in all individuals evaluated by X-ray. ROSAH, retinal dystrophy, optic nerve oedema, splenomegaly, anhidrosis and headache.

For all families in this cohort, the genetic testing of the proband was prompted by findings on ophthalmologic examination. Ocular manifestations included optic nerve elevation, uveitis, retinal vasculitis and retinal degeneration (online supplemental table 1). For most patients, the initial ophthalmological examination was prompted by subjective visual changes and, in addition to bilateral optic disc elevation, patients were noted to have intraocular inflammation. However, patient F9.1 had no subjective visual symptoms, but bilateral optic disc elevation was observed on a routine health screening examination at age 32. It is also notable that the onset and course of ophthalmological disease varied considerably even within families, and several adults (patients F3.2, F5.2, F9.1, F11.2) lacked subjective visual deficits. The mean age at which patients initially recognised subjective visual deficits was 14.9 years of age (online supplemental figure 2). As an example of the variability in ocular disease, patient F11.3 began experiencing problems with her vision at age 15 and was legally blind by age 21, while her 27-year-old brother (patient F11.2) remains without any significant visual impairment and her 51-year-old mother (patient F11.1) only has decreased night vision. Additionally, patient F5.3 developed retinal detachment leading to left eye blindness by the age of 7, while his 42-year-old father (patient F5.2) remains without any visual deficit. Optic disc elevation was nearly universal in this cohort and was often dramatic but was remarkably subtle in some patients (figure 2A).

Nearly all patients exhibited at least one inflammatory feature which included recurrent fever, malaise, episodic abdominal pain, headaches, transient cytopenias and uveitis with retinal vasculitis. Most patients experienced episodic malaise and many patients experienced non-infectious low-grade fevers. The fever episodes lasted less than 24 hours before resolving spontaneously.

Arthralgia was common (77% (20/26)), with patients reporting involvement of the hands, wrists, elbows, spine, knees, ankles and feet. Nine adults had deforming joint disease that was grossly appreciable on clinical examination or as erosive changes visible on X-ray (figure 2B). In some patients, joint disease was the first clinical manifestation. Patient F12.1 had prominent knee and ankle arthritis by age 4 years, and, after developing debilitating arthritis at age 7, patient F13.1 was evaluated for systemic juvenile idiopathic arthritis (sJIA).

Gastrointestinal symptoms were reported in 14 patients and ranged from episodic abdominal pain, gastro-oesophageal reflux disease (GERD) and dysphagia to constipation and ileus. On endoscopy performed for dysphagia, patient F2.2 was found to have linear oesophageal furrows, gastric erythema and duodenal erosions consistent with ongoing inflammation (figure 2C). Similar erythema of the gastric mucosa was noted by endoscopy in patients F5.2 and F12.1. Most patients evaluated by abdominal ultrasound (72% (13/18)) had splenomegaly, and seven patients underwent splenectomy for abdominal discomfort or cytopenias. Splenic tissue was notable for red pulp expansion (figure 2D, online supplemental table 2). Five patients had hepatomegaly. Transabdominal ultrasound with Doppler imaging, transient liver elastography and abdominal MRI were not consistent with a diagnosis of portal hypertension. Patient F4.1 was found to have microalbuminuria and AA amyloid present on a fat pad biopsy.

Cognitive deficits were rare (4% (1/27)) and only present in patient F2.4 who has cerebral palsy after preterm delivery that was complicated by severe intraventricular haemorrhage. However, recurrent headaches were experienced by many patients (50%, (13/26)) and abnormalities on brain MRIs were common. We reviewed brain MRIs for eight adults who had

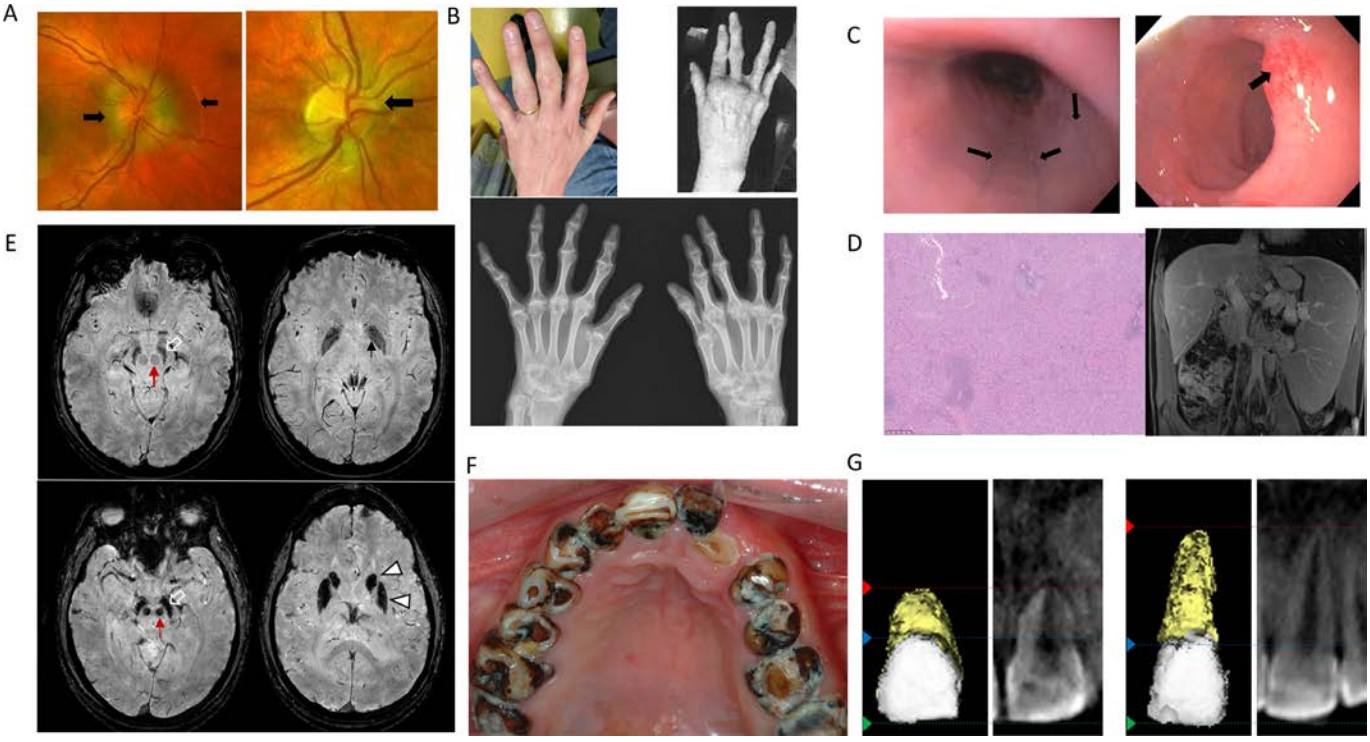


**Figure 2** Clinical manifestations associated with ROSAH syndrome. (A) Optic disc elevation. Fundus photographs demonstrating flagrant optic disc oedema (black arrow) in patient F9.2 (left) and more subtle changes in patient F9.3 (right). (B) Inflammatory arthritis with erosive changes in patient F3.3 (top left) and patient F13.1 (top right). X-ray demonstrating advanced diffuse changes of inflammatory arthritis involving wrist, metacarpophalangeal and interphalangeal joints with evolving joint deformities for patient F13.1 (bottom). (C) Gastrointestinal inflammation. Patient F2.2's endoscopy for dysphagia revealed oesophageal linear furrows (left, arrows) and erythematous duodenal mucosa (right, arrow). (D) Massive splenomegaly. Splenic histology showing red pulp expansion with mild histiocytic hyperplasia from patient F2.2 (resected at age 13, 26×15×6 cm, weighing 1320 grams) (left). Abdominal MRI from patient F1.1 at age 13 demonstrating hepatosplenomegaly with spleen craniocaudal diameter of 22.5 cm and liver craniocaudal diameter of 18.6 cm (right) in the coronal plane (normal range for age: spleen 8–12 cm, liver 8.5–14 cm).[27] (E) Premature basal ganglia and brainstem mineralisation. Susceptibility weighted imaging from brain MRIs showing decreased signal intensity consistent with premature mineralisation of the globus pallidi (small black arrow), substantia nigra (open white arrows) and red nuclei (red arrows). The mineralisation worsens with age eventually involving the caudate nuclei and putamina (white arrow heads) (top row: patient F1.1, bottom row: patient F7.1). (F) Dental caries. Sjögren's disease-like pattern of dental caries in patient F4.1. (G) Short dental roots. Three-dimensional rendering and two-dimensional slice of the maxillary central incisor from F5.3 (left) and an age matched healthy control (right). The crown length is similar between the teeth (green line to blue line). However, the root length is one third in F5.3 (blue line to red line). ROSAH, retinal dystrophy, optic nerve oedema, splenomegaly, anhidrosis and headache.

imagining performed as part of evaluation for headaches or for deep-phenotyping of the cohort. None of the of the patients endorsed extrapyramidal symptoms such as parkinsonism or involuntary movements, although, on clinical examination, patient F7.1 had subtle cog wheel rigidity elicited only with reinforcement manoeuvres. However, seven of the patients had premature mineralisation/calcification of the globus pallidi, red nuclei and substantia nigra, worsening with age, eventually involving the rest of the basal ganglia (figure 2E). White matter abnormalities have also been reported and patient F10.1 initially received a diagnosis of multiple sclerosis after she presented with loss of colour vision and was reported to have multiple lesions on MRI. Focal areas of hyperintensity were noted on fluid-attenuated inversion recovery (FLAIR) in the subcortical white matter of three patients (online supplemental figure 3a). Among the subjects who received post contrast FLAIR imaging (n=8), four showed foci of meningeal enhancement (online supplemental figure 3b), suggesting the possibility of ongoing central nervous system (CNS) inflammation. Additionally, MRI of the orbits showed disc oedema and/or thickening/enhancement of the posterior aspects of the globes in 7 out of 10 subjects, suggesting retinal/choroidal inflammation (online supplemental figure 3c). Two patients had small optic nerves, likely secondary to atrophy, and one subject had chronic retinal detachment.

Dental abnormalities were prevalent. Most patients (85% (23/27)) had multiple dental caries (figure 2F), 5 adults were edentulous, and all seven patients who underwent dental examination at the NIH had some degree of enamel defects with the severity increasing with age. Younger individuals had mild defects in the form of enamel pitting and grooves. Older individuals had severe enamel defects including loss of enamel from the tooth surface. Radiographic examination revealed presence of short and stunted roots in five of the seven patients (online supplemental figure 4a-d). Notably, two of the youngest individuals (both 14 years old) had the shortest roots with root length being less than half of the crown length (figure 2G). Enlargement of the pulp cavity leading to short dental roots in a pattern known as taurodontism was noted in four of seven individuals, with mandibular second molars being the most affected.[13]

Because patients with ROSAH syndrome had a pattern of dental caries that was reminiscent of Sjögren's Disease (SjD), four patients underwent formal evaluation for SjD (online supplemental table 3).[14 15] Patients had evidence of hyposalivation with decreased mean unstimulated and stimulated salivary

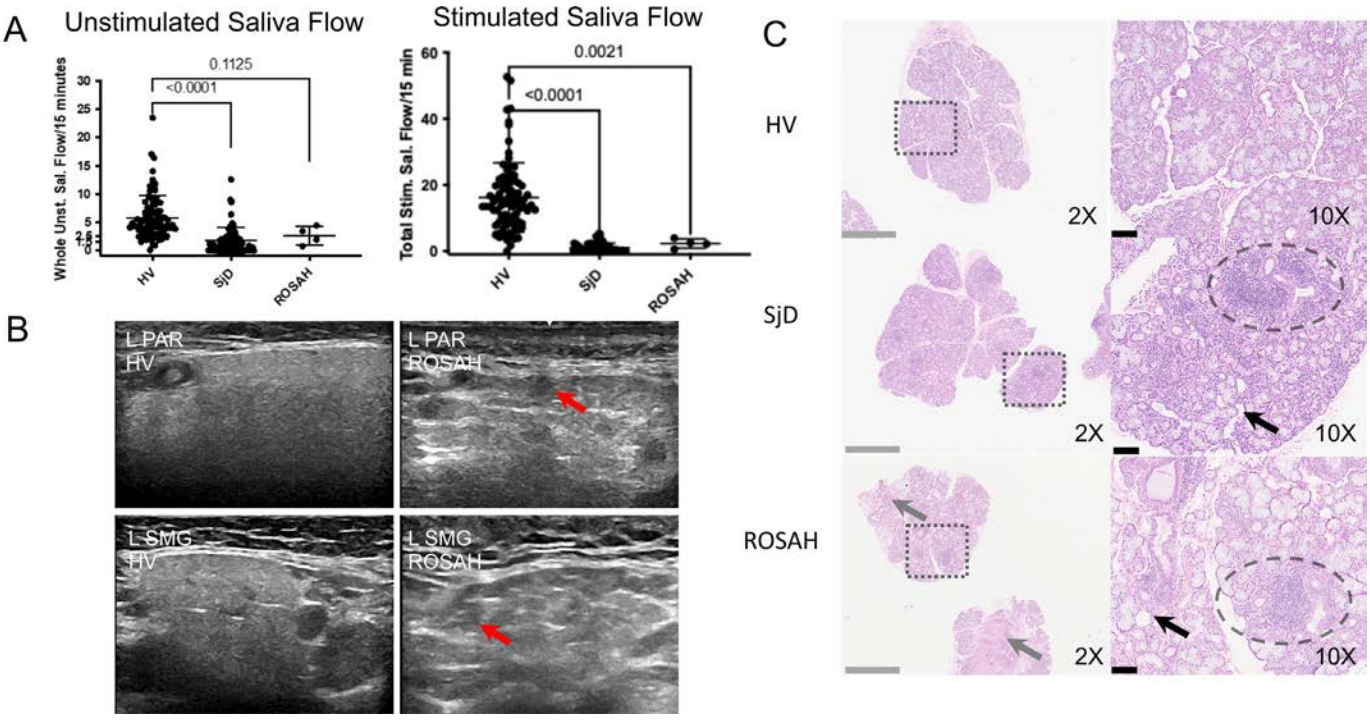


**Figure 3** ROSAH patient salivary glands demonstrate salivary hypofunction, altered echoarchitecture and histopathological evidence of inflammation, atrophy and fibrosis. (A) Whole unstimulated saliva flow and total stimulated saliva flow (TSSF, collected while stimulating with 2% citric acid every 30 s) were measured in 4 patients with ROSAH and compared with patients with Sjögren's disease (SjD) and healthy volunteers (HV). Like SjD, unstimulated and stimulated salivary flow rates were reduced in patients with ROSAH as compared with HV. Statistical significance was only reached for TSSF. (B) The parotid (PAR) and submandibular (SMG) salivary gland ultrasound (SGUS) of ROSAH patients exhibited abnormal echogenicity and homogeneity compared with HV. The most striking finding were isolated (<25% total surface area) to scattered (>50% total surface area) round, hypoechoic lesions which ranged in size from 1.5 mm to 6.5 mm (average of 3–3.5 mm; red arrows). These differ from hypoechoic lesions seen in SjD in shape, size, and distribution and are most likely attributable to pockets of trapped saliva (ie, sialectasias). (C) Labial minor salivary glands (LSG) were inspected using light microscopy. HV LSG are typified by mixed seromucous and mucous acinar cells, and associated ducts, with minimal atrophy or fibrosis and only minimal scattered, typically plasmacytic, inflammation. Alternately, SjD LSG exhibit overall architectural distortion with decreased proportions of seromucous >mucous acinar cells and increased proportion of immune infiltrates (eg, periductal focal lymphocytic sialadenitis (dashed ellipsis) with enhanced diffuse non-sialadenitis). Additional features included: atrophy (eg, decreased lobular size, decreased acinar size), fibrosis (eg, increased interlobular and intralobular collage deposition), adipocyte infiltration ('fatty infiltration'; black arrow). SMG from patients with ROSAH syndrome exhibit features similar to SjD including periductal focal lymphocytic sialadentis (two of three cases; dashed elipsis), decreased seromucous acinar cells (three of three), prominent increased periductal fibrosis (three of three) and atrophy and increased fatty infiltration (three of three). Additional features included perivascular inflammation, and damage to ducts with mucous extravasation reaction was observed in two cases. ROSAH, retinal dystrophy, optic nerve oedema, splenomegaly, anhidrosis and headache.

flow (figure 3A). The cause of the decreased stimulated salivary flow in ROSAH syndrome is unclear but ultrasonic imaging of the parotid and submandibular salivary glands was notable for round, hypoechoic lesions that may represent pockets of trapped saliva (figure 3B). Histopathologic analysis of ROSAH labial salivary glands revealed focal inflammation (foci of lymphocytic infiltrate), glandular atrophy with adipocytic replacement and focal mild fibrosis (figure 3C, online supplemental figure 4e). However, subjective eye dryness was not prominent, and features of dry eye disease were not appreciated on slit lamp exam. However, of the 3 patients that had fluorescein ocular staining and Schirmer testing, one individual had an ocular staining score consistent with SjD and one individual had Schirmer testing consistent with SjD.

Dysfunctional production of sweat and breast milk were also common features among patients with ROSAH syndrome. Hypohidrosis or anhidrosis was a nearly universal, present from birth. In this cohort, four parous women were unable to lactate after a total of eight live births.

### Immune system dysregulation

C reactive protein (CRP) levels were highly variable in untreated patients and significant elevations occurred without change in systemic symptoms and resolved without intervention (figure 4A). For patients with a spleen, episodes of CRP elevation were typically associated with transient cytopenias (online supplemental figure 5a). Seven patients experienced transient neutropenia. Eight individuals underwent bone marrow biopsy for evaluation of cytopenias and there was no evidence of hypocellularity. While cytopenias appeared to improve after splenectomy (online supplemental figure 5b), elevated CRPs were observed after splenectomy in patients F2.2, F11.3 and F12.1 at 101 mg/L, 65 mg/L and 217 mg/L, respectively. Lymphocyte phenotyping by flow cytometry was performed for 12 patients (online supplemental figure 6). Lymphopenia was present in 41% of the patients (5 of 12) without clear predilection for a specific lineage. There was no evidence of increased susceptibility to documented bacterial infections.

Among immune cells, *ALPK1* transcription is highest in neutrophils (online supplemental figure 7a).[16 17] Therefore, neutrophil function was assessed using assays to evaluate phagosome formation and

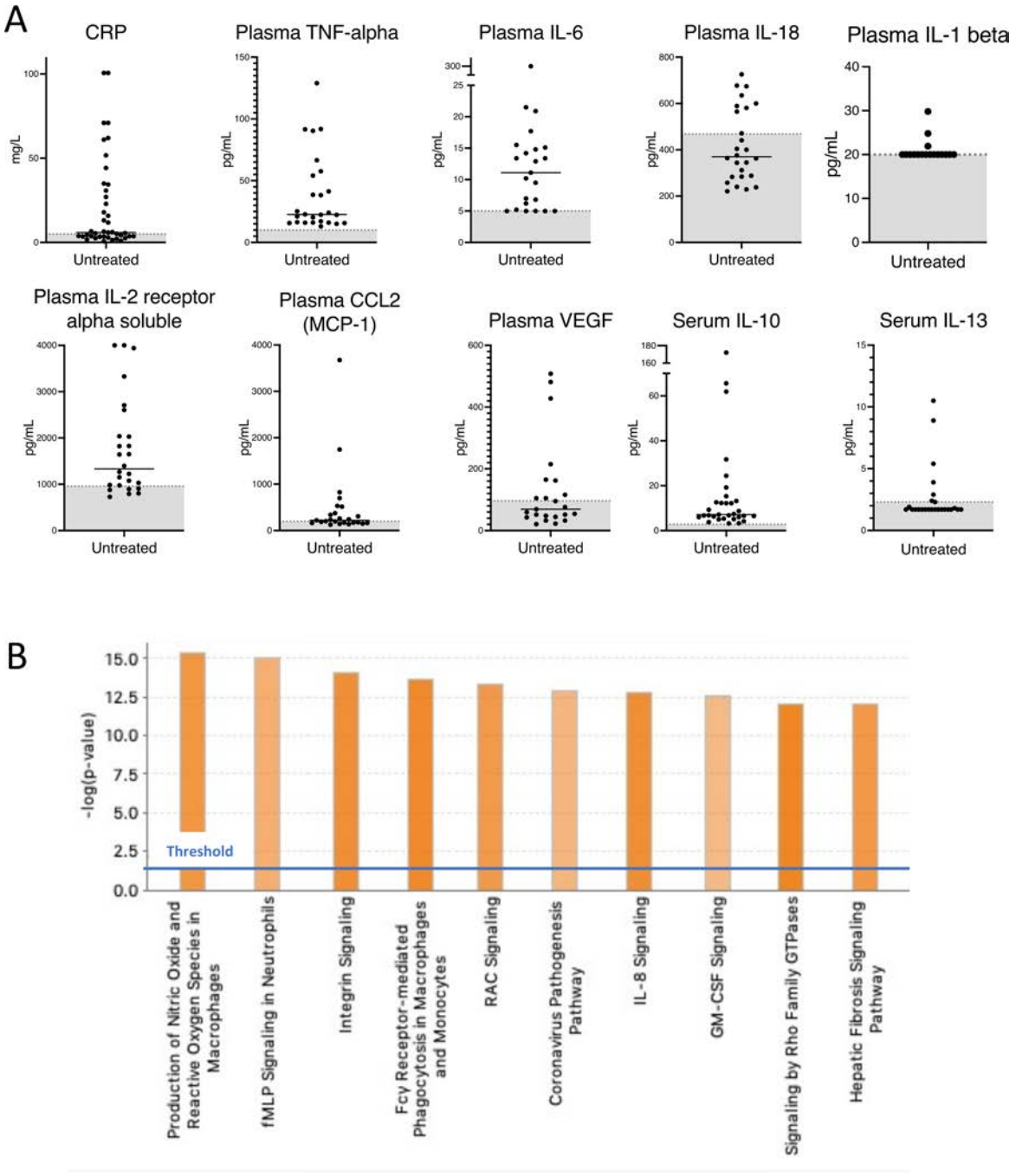


**Figure 4** Inflammatory signature in untreated patients with ROSAH syndrome. (A) CRP, cytokine and chemokine levels in serum (n=7) and plasma (n=5) of untreated patients with ROSAH syndrome. Grey zone indicates normal range. (B) Top 10 activated canonical pathways predicted based on differentially expressed genes from whole blood RNA of untreated adults with ROSAH syndrome (n=4) based on Ingenuity Pathway Analysis. Bars denote the different pathways based on Z-scores. CRP, C reactive protein; GM-CSF: granulocyte-macrophage colony-stimulating factor; ROSAH, retinal dystrophy, optic nerve oedema, splenomegaly, anhidrosis and headache.

oxidative burst (online supplemental figure 7b). Based on ingestion of *Staphylococcus aureus* labelled with pH-sensitive dye, neutrophils from one untreated patient (F2.4) demonstrated an early increase in phagocytosis as compared with neutrophils from her affected relatives on cytokine inhibitors and two healthy controls. No difference in phagocytic activity was appreciated in monocytes (online supplemental figure 7c). Dihydrorhodamine flow cytometric assay did not detect any abnormalities in NADPH oxidase activity before or after stimulation with phorbol myristate acetate (PMA).

Pretreatment immunoglobulin levels were normal in most patients (online supplemental figure 8), and consistent with other diseases of autoinflammation, most ROSAH syndrome patients lacked high-titre autoantibodies (online supplemental table 4). Specifically, relevant to the poor dentition observed in patients with ROSAH syndrome, patients had normal IgA levels and lacked anti-SSA and anti-SSB antibodies.

### Inflammatory signature

Untreated patients had recurrent elevations of CRP as well as proinflammatory cytokines and chemokines (figure 4A). Plasma TNF levels were persistently elevated in untreated patients and

IL-6, CCL2 (MCP-1), soluble IL-2 receptor alpha and IL-10 were also frequently elevated (online supplemental figure 9a). Patients with ROSAH syndrome also demonstrated elevations of additional cytokines and chemokines including plasma CXCL10 (interferon gamma-induced protein 10 (IP-10)) and serum CXCL1 (GRO-alpha (previously known as neutrophil-activating protein 3)) (online supplemental figure 9b-c). However, no elevations in intracellular IFN-gamma, TNF-alpha or IL-4 were observed after stimulation of peripheral blood cells from two patients (online supplemental table 5).

Analysis of cerebral spinal fluid (CSF) was suggestive of CNS inflammation (online supplemental table 6). CSF neopterin, produced by immune cells after interferon stimulation and shown to correlate with CSF interferon-alpha titres in Aicardi-Goutières syndrome (AGS), was measured in patients F2.2 and F3.4 and found to be elevated.[18–20] CSF cytology was performed on patient F10.1's sample and was notable for numerous cells consistent with activated monocytes and lymphocytes. Patient F2.2 had a banked sample available for CSF cytokine analysis and the results were notable for elevation of IL-13 and soluble IL-2 receptor alpha (online supplemental table 7).

RNA extracted from whole blood for four untreated adults with ROSAH had a distinct transcriptomic signature as compared with healthy controls (figure 4B). Many of the differentially expressed genes are involved in innate immune signalling pathways. Production of nitric oxide and reactive oxygen species in macrophages, fMLP signalling in neutrophils, integrin signalling and Fcγ receptor-mediated phagocytosis were among the top upregulated canonical pathways.

### ROSAH syndrome mutations are gain of function and result in enhanced NF-κB signalling

We assessed the activity of mutant ALPK1 using an NF-κB luciferase assay. Transiently transfected mutant proteins had increased constitutive NF-κB activity relative to the wild-type protein. Additionally, the previously unreported Y254C variant showed a significantly higher NF-κB activity than the mutant T237M plasmid (figure 5A).

To explore the effect of the ALPK1 mutation ex vivo, we used T237M patient-derived fibroblasts from two unrelated patients to study the activity of the canonical NF-κB pathway in response to stimulation with the ALPK1 agonist ADP-heptose (the patient with the Y254C mutation declined skin biopsy). As compared with healthy controls, stimulated patients' cells showed increased phosphorylation of IκBα, IKKα/β, and MAP kinases p38 and JNK, which are hallmarks of the activated canonical NF-κB pathway (figure 5B). Additionally, we observed higher mRNA expression of *ALPK1* and increased expression of NF-κB regulated genes in RNAseq data from patients with ROSAH syndrome (figure 5C and online supplemental figure 10).

### ROSAH syndrome mutations are associated with increased signal transducer and activator of transcription phosphorylation and expression of interferon-regulated genes

Based on the presence of premature CNS basal ganglia mineralisation reminiscent of that seen in classic type-I interferonopathies including AGS, we were interested in assessing signal transducer and activator of transcription (STAT1) phosphorylation and expression of interferon-regulated genes.[21] Immunoblotting for phospho-STAT1 in patient fibroblasts and in 293T cells transfected with WT and mutant constructs revealed that ROSAH-associated mutations result in constitutive STAT1 activation (figure 6A,B). Unstimulated monocytes isolated from a pre-treatment ROSAH patient demonstrated increased STAT1 phosphorylation as compared with cells isolated from a healthy control and monocytes from a ROSAH patient treated with a TNF inhibitor (figure 6C).

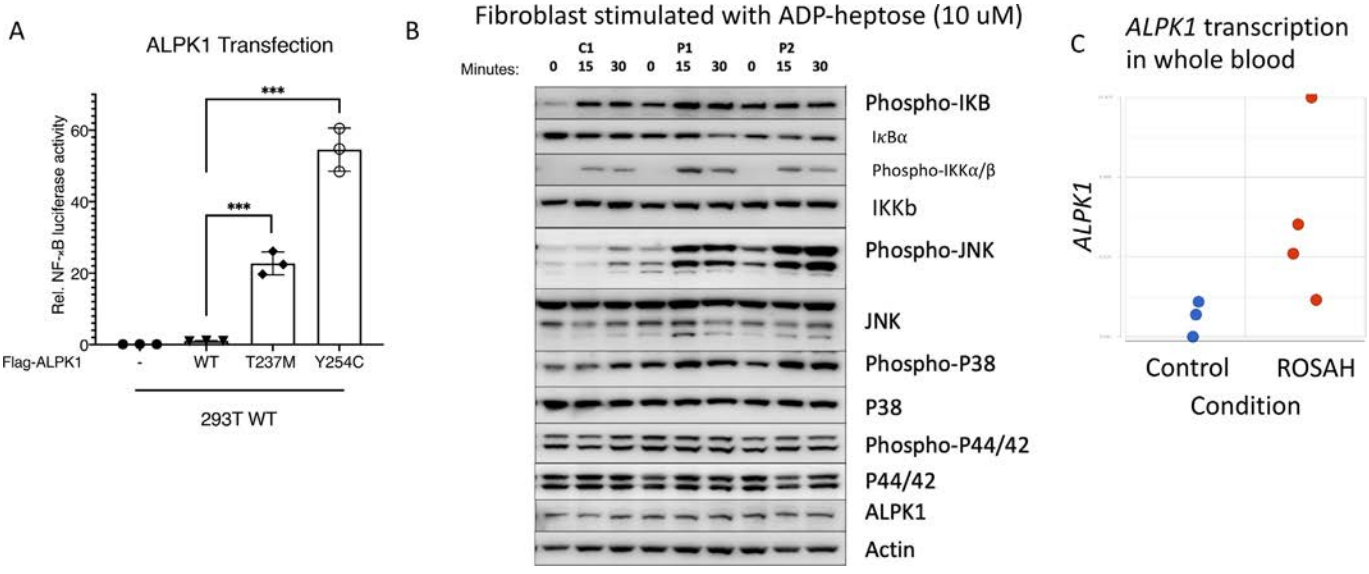


**Figure 5** Gain-of-function mutations in *ALPK1* are associated with enhanced NF-κB activation in transfected cells and fibroblasts from patients with ROSAH syndrome. (A) 293T cells were transiently cotransfected with an NF-B–responsive luciferase reporter gene and Flag-ALPK1 (wild-type or disease-associated mutant [T237M or Y254C]). Luciferase assay of NF-κB activation is shown as mean±SD. From three technical replicates (two-tailed unpaired Student's t-test, ***p<0.001). (-) reflects transfection with empty vector. (B) Fibroblasts derived from patients with ROSAH syndrome were stimulated with ADP-heptose and whole cell lysates were immunoblotted against respective target proteins. Patient derived fibroblast showed increased levels of phospho-IκBα, increased degradation of IκBα, increased phospho-IKKα/β and increased MAPK activity (p38 and JNK). (C). Whole blood RNASeq data demonstrating ALPK1 mRNA expression was higher in untreated patients with ROSAH syndrome (red dots, n=4) as compared with controls (blue dots, n=3). ROSAH, retinal dystrophy, optic nerve oedema, splenomegaly, anhidrosis and headache.

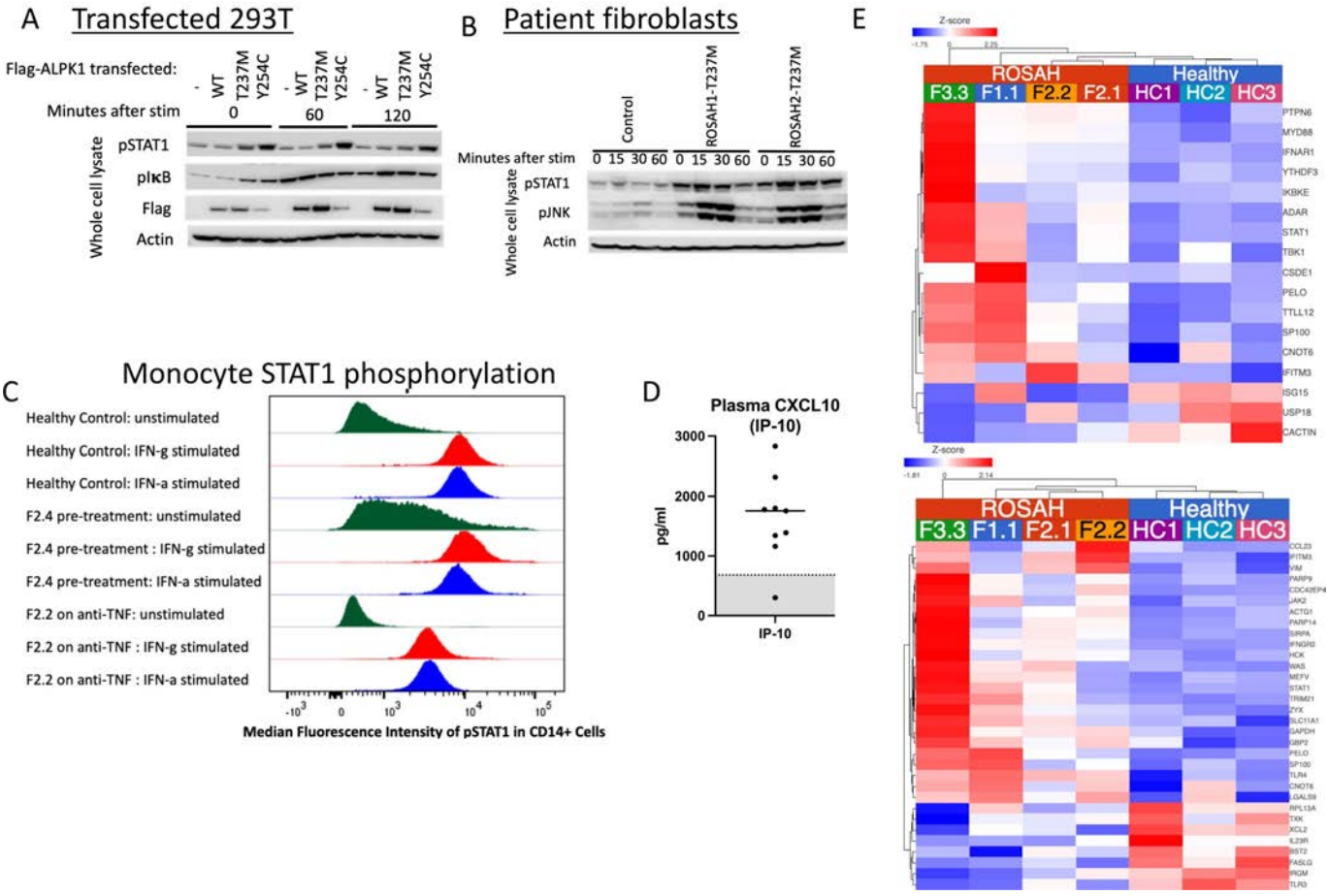


**Figure 6** *ALPK1* mutations affect STAT1 phosphorylation, plasma levels of interferon-induced cytokines and transcription of interferon-regulated genes. (A, B) 293T cells transiently transfected with ALPK1 variants (A) and ROSAH patient derived fibroblasts (B) were stimulated with ADP-heptose (5 uM) and whole cell lysates from both experiments were subjected to Western blotting for indicated proteins. Constitutive STAT1 phosphorylation (pSTAT) was observed in both transfected cells and patient fibroblasts. (-) reflects transfection with empty vector. (C) CD14-labelled monocytes from an untreated ROSAH patient (F2.4, middle of panel) showed constitutively phosphorylated STAT1 (pSTAT1) as compared with healthy control (top) and ROSAH patient treated with TNF-inhibitor (F2.2, bottom of panel). (D) Plasma CXCL10 (interferon-inducible protein 10 (IP-10)) as measured in patients F1.1, F2.2, F2.3, F2.4 and F7.1. Grey shaded area represents the mean plus or minus 2 SD from 114 healthy controls. (E) Heat map showing increased expression of interferon-regulated genes (type I: top (GO:0060337) and type II: bottom (GO:0034341)) in four untreated patients with ROSAH syndrome as compared with three healthy controls. Upregulated genes are shown in red and down-regulated genes in blue. ROSAH, retinal dystrophy, optic nerve oedema, splenomegaly, anhidrosis and headache; STAT1, signal transducer and activator of transcription.

We also observed elevated levels of CXCL10 in the peripheral whole blood samples (n=5 patients) and increased expression of interferon-regulated genes (n=4 patients) (figure 6D,E).

### Mouse model

Consistent with the observations in patients with ROSAH syndrome, knock-in mice with the *Alpk1* T237M mutation had elevated serum levels of CXCL1, CXCL10 and CCL2 (online supplemental figure 11a). At 16 weeks, mice did not have an increase in spleen size or weight (online supplemental figure 11b) and mice did not exhibit visual decline (online supplemental figure 11c, d) or evidence of retinal degeneration due to *Alpk1* mutation at up to 12 months of age (online supplemental figure 11e). However, we cannot exclude the possibility that retinal abnormalities could manifest in mice at a later age.

### Response to therapy

Ten patients have been treated with anti-cytokine therapy (online supplemental table 1), and seven patients with systemic symptoms reported subjective improvement in at least one clinical feature of ROSAH syndrome. Patients F3.4 and F5.3 lacked subjective symptoms, and patient F13.1 denied subjective benefit but discontinued anti-IL-1 therapy (anakinra) after less than 1 week secondary to intolerable injection site reactions. Anti-TNF therapy (adalimumab) led to improvement in fatigue, headache, or arthralgia for four of four patients in whom these features were present. Additionally, three of these patients were noted to have normalised CRPs (figure 7A) and a decline in inflammatory cytokines (figure 7B) while on therapy. Six patients were treated with anti-IL-1 therapy (anakinra or canakinumab) and reported some improvement in subjective symptoms; however, serum CRP levels were not consistently suppressed.

Whole blood RNA sequencing was performed on paired pre- and post-treatment samples from patients F1.1 and F2.2. Prior to the initiation of treatment, patients with ROSAH syndrome demonstrated increased expression of many genes linked to inflammation (figure 7C, online supplemental table 8). After initiation of anti-TNF therapy, both patients had transcriptome changes consistent with decreased inflammation. Patients F2.1

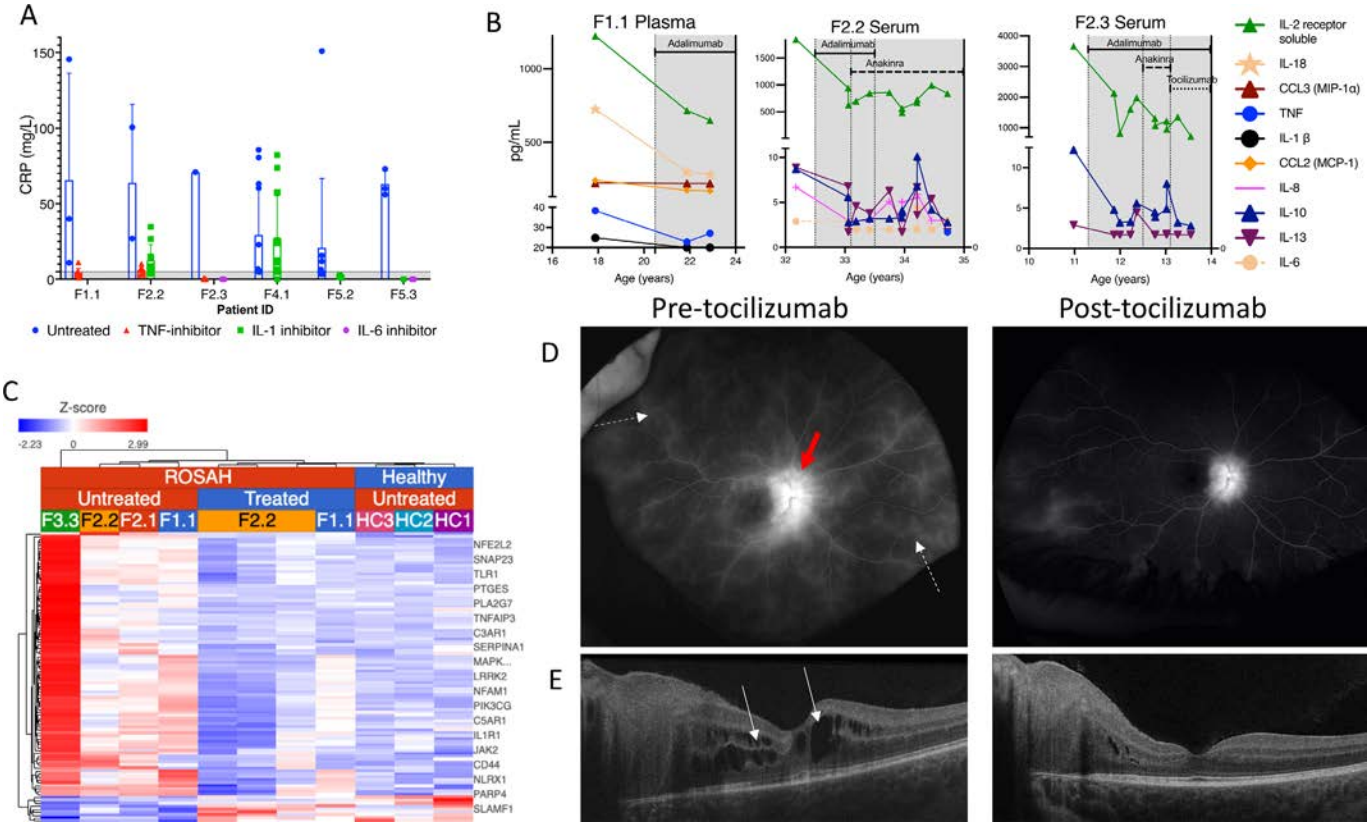


**Figure 7** Response to anticytokine therapy. (A) Pretreatment and post-treatment CRPs for patients initiated on anticytokine therapy (n=6). Shaded zone represents normal range. (B) Pretreatment and post-treatment cytokines from patients F1.1 (plasma), F2.2 (serum), F2.3 (serum). Shaded area indicates time on anti-cytokine therapy. Specific therapies are as indicated in the figures. (C) Heatmap showing differentially expressed inflammatory response genes (GO: 0006954) in whole blood of pre-treatment (n=4) and post-adalimumab (n=2) patients with ROSAH syndrome. Patient F2.2 had post-treatment samples collected on three separate visits. Upregulated genes are shown in red, and downregulated genes in blue. Complete list of genes in online supplemental table 9. (D) Fluorescein angiography from patient F2.3 demonstrating retinal vasculitis (dotted white arrows) and disc leakage (solid red arrow) that improved after initiation of tocilizumab. (E) Optical coherence tomography from patient F2.3 demonstrating cystoid macular oedema (white arrows) that improved after initiation of tocilizumab. CRP, C reactive protein; ROSAH, retinal dystrophy, optic nerve oedema, splenomegaly, anhidrosis and headache.

and F3.3 are already blind and have declined treatment with anticytokine therapy.

Our ability to determine the impact of therapy on ocular disease was limited because most patients in this cohort already exhibited advanced retinal disease at the time of evaluation. However, two patients (F2.3 and F5.3) had substantially decreased intraocular inflammation after starting the IL-6 receptor antagonist, tocilizumab. Patient F2.3 had almost complete resolution of her cystoid macular oedema after 3 months of treatment with tocilizumab and this was maintained at 9 months on tocilizumab and fluorescein angiography showed significant improvement in retinal vascular leakage (figure 7D,E). After 5 months of tocilizumab treatment, patient F5.3 had decreased retinal vascular leakage and this improvement was maintained during 14 months of treatment. Patients F1.1 and F4.1 had continued visual decline with progressive constriction of visual fields despite adalimumab and canakinumab monotherapies, respectively. Subsequently, patient F4.1 was switched from canakinumab to sarilumab, the only subcutaneously administered IL-6 receptor antagonist locally available to the patient but within 1 week of the first 150 mg subcutaneous dose, developed grade 4 neutropenia (absolute neutrophil count $<500/\mu L$). Neutropenia persisted the following week and sarilumab was suspended (online supplemental figure 5a).

## DISCUSSION

Although the initial report of ROSAH syndrome emphasised the visual manifestations associated with the disease, our work additionally establishes ROSAH syndrome as a disease of systemic inflammation caused by gain-of-function mutations in the innate immune receptor ALPK1. This conclusion is supported by both our in vitro work as well as our systematic analysis of inflammatory features in the largest cohort of patients reported to date. These findings have important implications for both basic science and clinical practice.

Our discovery of a second ROSAH-associated mutation occurring in the ligand-binding domain of ALPK1 emphasises the importance of this domain in protein activation and provides a solid foundation for establishing the pathogenicity of missense mutations affecting the region. While the exact impact of these mutations on protein structure and function remains to be elucidated, there is clearly a strong phenotypic overlap between patients with the recurrent p.T237M variant and the patient with the p.Y254C mutation. Additionally, our in vitro work demonstrates that both mutations are associated with increased innate immune activation as shown with enhanced NF-κB signalling and STAT1 phosphorylation in transfected cells and ADP-heptose stimulated patient fibroblasts.

The findings from this large, international cohort study provide valuable insights on the clinical spectrum of disease associated with mutations in *ALPK1* and highlight the possibility that patients with ROSAH syndrome may be currently unrecognised in cohorts of more common inflammatory disorders. We have found that ROSAH syndrome can present with periodic fevers, malaise, headaches, uveitis, deforming joint disease, abdominal pain, premature CNS mineralisation and focal meningeal enhancement on brain MRI and it has mimicked diseases including sJIA, sarcoidosis, neuro-Behçet's disease, SjD and multiple sclerosis. We also found that untreated patients with ROSAH syndrome had frequent elevations of CRP and proinflammatory plasma cytokines including TNF and IL-6. Additionally, we have seen that diagnosis may be aided by the presence of ocular involvement, splenomegaly, decreased or inability to sweat or multiple dental caries, but none of these features is universal. While advanced retinal degeneration was common among adults in our cohort, three adults lacked significant visual impairment but suffered from other systemic inflammatory manifestations of the disease.

Patients' clinical improvement on anticytokine therapies also highlights the role of immune activation in disease pathogenesis and emphasises the importance of referring patients with ROSAH syndrome for multidisciplinary evaluation and care. While all index patients in this cohort had routine ophthalmology care at the time of initial contact with the NIH, most patients did not have a regular provider experienced in management of systemic inflammation. Yet thorough examination revealed that many patients had elevations of serum CRP and non-ophthalmological indications to consider systemic immunomodulatory treatment including recurrent headaches, disabling episodes of fatigue, arthritis, abdominal pain, and AA amyloidosis. Most patients who received anti-TNF or anti-IL1 therapy reported subjective improvement in systemic symptoms. While these therapies may be appropriate for treating non-ocular inflammatory manifestation, there is no evidence that they are efficacious for treating intraocular inflammation or that they can influence progressive vision loss. Thus, additional prospective studies are needed to determine optimal treatment for this disease, but providers should consider alternative therapies in patients with active, vision-threating intraocular inflammation.

The IL-6 inhibitor tocilizumab has shown very promising results in patients F2.3 and F5.3. Both patients had intraocular inflammation that was unresponsive to TNF and IL-1 inhibition but showed dramatic improvement on tocilizumab, and we are actively seeking to determine if these results can be replicated in additional patients. It should also be noted that patients with ROSAH syndrome have an interferon gene expression signature, premature basal ganglia mineralisation and elevated CNS neopterin that suggest the disease may be an interferonopathy, which might indicate that patients would benefit from treatment with a JAK-inhibitor.[21 22]

Deep-phenotyping of this cohort illustrates the potential for monogenic diseases to advance our understanding on ALPK1's role in human biology. Several clinical features of ROSAH syndrome, including short dental roots as well as the aberrant production of sweat, breast milk and saliva are not classically associated with inflammation but may reflect ALPK1's role in ciliary functioning. Indeed, primary cilia are present in the dental epithelium and mesenchyme at various stages of tooth development, and the clinical and radiographic features of teeth in this cohort were very similar to past reports of dental anomalies in ciliopathy disorders.[23 24] While, to date, no monogenic diseases have been categorised as both an autoinflammatory disease and a ciliopathy, this nosology may change as there are an increasing number of proteins that were initially labelled as 'ciliary' but have now also been observed at the innate immune synapse.[25 26]

The prevalence of clinical manifestations is this cohort was likely biased by the fact that all diagnostic genetic testing in the cohort was prompted by ophthalmological examination findings, and the prevalence of specific clinical features is likely to change as more patients without prominent ocular manifestations are screened for mutations in *ALPK1*. Additionally, the prevalence of disease features for deceased patients was limited to what could be recalled by their surviving children.

In conclusion, we have demonstrated that ROSAH syndrome is an autoinflammatory disease that can manifest with a spectrum of inflammatory features including recurrent fever, uveitis, deforming arthritis and cyclical cytopenias. For patients with advanced retinal degeneration, TNF inhibitors and IL-1 inhibitors can be considered for treatment of non-ocular disease manifestations including fevers, headaches, and arthritis. However, for patients with active intraocular inflammation, our findings indicate that tocilizumab may be the preferred treatment and future studies should be pursued to determine if this result is reproducible in additional patients. Continued study of ALPK1 function and ROSAH syndrome may also provide valuable insights for more common disorders of inflammation, such as gout or periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis (PFAPA), where endogenous ligands may play a role as damage-associated molecular patterns.

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TRANSLATIONAL SCIENCE

# NOD/RIPK2 signalling pathway contributes to osteoarthritis susceptibility

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## ABSTRACT

**Objectives** How inflammatory signalling contributes to osteoarthritis (OA) susceptibility is undetermined. An allele encoding a hyperactive form of the Receptor Interacting Protein Kinase 2 (RIPK2) proinflammatory signalling intermediate has been associated with familial OA. To test whether altered nucleotide-binding oligomerisation domain (NOD)/RIPK2 pathway activity causes heightened OA susceptibility, we investigated whether variants affecting additional pathway components are associated with familial OA. To determine whether the *Ripk2*$^{104Asp}$ disease allele is sufficient to account for the familial phenotype, we determined the effect of the allele on mice.

**Methods** Genomic analysis of 150 independent families with dominant inheritance of OA affecting diverse joints was used to identify coding variants that segregated strictly with occurrence of OA. Genome editing was used to introduce the OA-associated *RIPK2* (p.Asn104Asp) allele into the genome of inbred mice. The consequences of the *Ripk2*$^{104Asp}$ disease allele on physiology and OA susceptibility in mice were measured by histology, immunohistochemistry, serum cytokine levels and gene expression.

**Results** We identified six novel variants affecting components of the NOD/RIPK2 inflammatory signalling pathway that are associated with familial OA affecting the hand, shoulder or foot. The *Ripk2*$^{104Asp}$ allele acts dominantly to alter basal physiology and response to trauma in the mouse knee. Whereas the knees of uninjured *Ripk2*$^{Asp104}$ mice appear normal histologically, the joints exhibit a set of marked gene expression changes reminiscent of overt OA. Although the *Ripk2*$^{104Asp}$ mice lack evidence of chronically elevated systemic inflammation, they do exhibit significantly increased susceptibility to post-traumatic OA (PTOA).

**Conclusions** Two types of data support the hypothesis that altered NOD/RIPK2 signalling confers susceptibility to OA.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ A coding variant that elevates the proinflammatory activity of the Receptor Interacting Protein Kinase 2 (RIPK2) signal transducer has been associated with familial early-onset osteoarthritis (OA), raising the possibility that perturbations of nucleotide-binding oligomerisation domain (NOD)/RIPK2 signalling may confer susceptibility to OA.

### WHAT THIS STUDY ADDS

⇒ We discover alleles affecting several components of the NOD/RIPK2 signalling pathway are associated with multiple forms of familial OA, supporting the novel hypothesis that the pathway is a common vulnerability factor for OA.

⇒ Introduction of the OA-associated hyperactive *Ripk2*$^{104Asp}$ allele into the mouse genome causes changes in the basal physiological status of the joint in ways that presage a definitive OA state.

⇒ Although *Ripk2*$^{104Asp}$ mice display no evidence of systemic inflammation or histological evidence of joint degeneration, they displayed significantly increased sensitivity to experimentally induced OA.

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

⇒ By showing that altered NOD/RIPK2 signalling is predictive of susceptibility to multiple forms of OA, the work brings new focus to the functions of the signalling pathway in maintaining joint homeostasis, may guide development of assays to detect early stages of OA and may indicate new therapeutic approaches to disease intervention.



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## INTRODUCTION

The molecular pathways that are rate-limiting in the onset and progression of osteoarthritis (OA) are unknown, consistent with the complete lack of disease-modifying drugs currently available.[1–4] Knowledge of these pathways is required for identifying individuals at risk for disease, for understanding mechanisms that trigger or amplify disease processes and for development of effective therapies. One proven approach toward identifying pathways and biological processes whose normal functions limit disease has been to identify gene variants responsible for highly penetrant familial forms of the disease. Increasing evidence demonstrates there are no/few differences between the genes contributing to 'monogenic' disease and those contributing to complex disease.[5–10] Pathways that can be mutated to have determinate effects promoting OA will also be vulnerable to the modest genetic or environmental perturbations that underlie common spontaneous forms

of OA.[5] Despite its promise, to date there have been relatively few studies of non-syndromic familial OA.[11–14] We have used a unique medical genetics resource, the Utah Population Database, to identify a large number of multigenerational families with dominantly inherited OA.[15] Here we employ genomic analyses of these families and functional analyses in mice to test the hypothesis that perturbation of the NOD/RIPK2 proinflammatory pathway is sufficient to significantly elevate susceptibility to OA.

In previous work, we identified a rare allele of the *Receptor Interacting Protein Kinase 2* (*RIPK2*) gene (p.Asn104Asp) that was associated with dominant inheritance of early-onset OA of the first metatarsophalangeal (1st MTP) joint in a single family.[11] The NOD/RIPK2 signalling pathway is a key arm of the innate immunity system, playing critical roles both in clearing bacterial infections and maintaining immune homeostasis.[16] The intracellular nucleotide-binding oligomerisation domain (NOD) receptors are activated by bacterial cell wall breakdown products and additional damage-associated molecular patterns.[16 17] Activated NOD receptors signal through the RIPK2, stimulating the MAPK and NF-κB pathways to elicit tissue-specific responses, most notably inflammatory responses.[18–21] NOD/RIPK2 signalling is tightly regulated, as mutations that either abrogate or elevate signalling are associated with chronic inflammatory diseases, including Crohn's, Blau syndrome, early-onset sarcoidosis and Behcet's disease.[16 22 23] Although chronic inflammatory diseases are often associated with arthritis, no single inflammatory pathway has yet been linked to classic non-syndromic forms of OA.[4 24]

Here we investigate the hypothesis that function of the NOD/RIPK2 signalling pathway limits susceptibility to OA. We find that individual gene variants affecting any of several components of the pathway appear sufficient to confer heightened susceptibility to OA in families. Significantly, variants affecting the NOD/RIPK2 signalling pathway are associated with divergent forms of familial OA. Last, we demonstrate that an OA-associated *RIPK2* gene variant encoding a single amino acid change in the kinase domain is sufficient to alter basal gene expression patterns in primary chondrocytes and bone marrow macrophages and confer increased susceptibility to post-traumatic OA (PTOA) when introduced into the mouse genome. Our data provide strong support for the hypothesis that modulation of the NOD/RIPK2 signalling pathway is a significant risk factor for OA.

## METHODS

### Identification of families with a dominant pattern of OA inheritance

Our study uses data drawn from the Utah Population Database (UPDB) (https://uofuhealth.utah.edu/huntsman/utah-population-database/). The UPDB provides person-based interlinked records documenting genealogy, medical records and vital statistics for over 11 million individuals from the late 18th century to the present. Medical records derive from the two largest healthcare providers in Utah (Intermountain Healthcare and University of Utah Health), Medicare claims and the Utah and Idaho Cancer Registries. Vital records include statewide birth, death and marriage certificates, as well as drivers' licenses. UPDB data are available for approved research projects. Privacy of individuals whose data are available through UPDB is strictly protected through the Utah Resource for Genetic and Epidemiological Research (https://rge.utah.edu), established by executive order of the Governor of Utah. We identified individuals with OA between 1996 and 2021 in the UPDB using the following diagnosis (ICD) and related procedure (CPT) codes: 1st MTP joint OA—ICD-9 735.2 or CPT 28289 and 28750; distal and proximal interphalangeal joint OA—ICD-9 715.14, ICD-10 M19.04x and CPT 26862, 26863, 26860, 26861, 26535 or 26536; and glenohumeral OA—ICD-9 715.11, ICD-10 M19.011, M19.012, M19.019, Z96.611, Z96.612 or M19.0x and CPT 23472. Individuals with any of the following codes were excluded: ICD-9 714.0, 714.2 or 714.3 and ICD-10 M05.xxx, M06.xx or M08.xxx. A detailed description of the ICD and CPT codes is provided in the online supplemental methods. Manual chart review was performed on affected individuals to verify our coding strategy to identify OA cases and determine if individuals had OA in additional joints. To determine if there was excess familial clustering of OA in each pedigree, we used the familial standardised incidence ratio (FSIR), with a threshold of ≥2.0. FSIR allows for the quantification of familial risk of a disease by comparing the incidence of a disease in a family to its expected incidence in the general population. See Kazmers, 2021 and Kazmers, 2020 for detailed methods.[15 25] Pedigrees segregating a dominant pattern of OA inheritance were selected for genomic analysis.

### Statistical analyses

Statistical analysis was performed using GraphPad Prism software. Tests performed and statistical significance are indicated in the figure legends. P values $<0.05$ were considered statistically significant.

## RESULTS

### Rare alleles of NOD-RIPK2 pathway genes are associated with multiple types of familial OA

We took an unbiased genetic approach to determine if the NOD/RIPK2 signalling pathway has a strong effect on OA susceptibility. We assembled a cohort of 150 independent OA families with a dominant inheritance pattern of OA. Each family is characterised by disease that primarily affects a distinct subset of joints: distal and proximal interphalangeal OA,[26] glenohumeral OA[27] or 1st MTP joint OA.[28 29] While all affected individuals have OA in the primary joint used for identification, two families contain a subset of individuals with OA in additional joints. The proband in MTP25 was diagnosed with triscaphe and thumb OA and had a total knee and hip arthroplasty. His daughter was also diagnosed with spine OA. The proband's sister in SA735 had surgery for thumb OA and bilateral total knee and hip arthroplasty (online supplemental table 1). Whole exome sequence analysis was performed on informative members of families and coding variants that invariably segregated with OA were identified.[11] Variants were prioritised using the pedigree Variant Annotation, Analysis & Search Tool (pVAAST),[30] which identifies the most likely causal variants in a pedigree based on gene tolerance to mutation, variant frequency, phylogenetic conservation and biological function. We next used the PHEnotype-driven Variant Ontological Re-ranking (PHEVOR)[31] tool with the Human Phenotype Ontology search term 'Osteoarthritis' to identify high-priority candidate genes in each family.

In addition to the previously identified OA-associated *RIPK2* allele, six novel alleles affecting five NOD/RIPK2 pathway genes were found associated with OA in the cohort of families (table 1 and online supplemental figure 1). Consistent with a dominant pattern of inheritance with strong penetrance, the variants are rare in human populations. Three variants affected the *NOD1* (FIJ744—pVAAST: p value=0.00809; LOD=0.6; PHEVOR: score 3.26, final rank=33) and *NOD2* genes

**Table 1** *NOD/RIPK2* pathway variants identified in independent osteoarthritis families

| Gene | OA phenotype (family) | Variant | Minor allele frequency | Protein domain affected by variant |
|---|---|---|---|---|
| *NOD1* | Finger interphalangeal joint OA (FIJ744) | c.G2114A:p.R705Q | 0.0008 | Leucine rich repeat domain |
| *NOD2* | 1st MTP joint OA (UUHR2) | c.C2465T:p.A822V | 0.00007 | Leucine rich repeat domain |
| *NOD2* | Finger interphalangeal joint OA (FIJ7) | c.G247A:p.A83T | 0.00008 | Caspase activation and recruitment domain |
| *IKBKB* | Glenohumeral OA (SA735) | c.G1663A:p.G555R | 0.00008 | Scaffold dimerisation domain |
| *CARD9* | Finger interphalangeal joint OA (FIJ9) | c.G722A:p.R241Q | 0.00005 | Structural maintenance of chromosomes |
| *CHUK* | 1st MTP joint OA (MTP25) | c.A376T:p.S126C | 0.0008 | Kinase domain |
| *RIPK2** | 1st MTP joint OA (UUHR1) | c.A310G:pN104D | 0.0004 | Kinase domain |

*Previously described in Jurynec *et al*.[11]

(UUHR2—pVAAST: p value=0.0592; LOD=7.195; PHEVOR: score 3.42, final rank=6 and FIJ7—pVAAST: p value=0.00333; LOD=15.556; PHEVOR: score 4.7, final rank=1), which encode intracellular receptors that function as upstream activators of RIPK2. The amino acid substitutions encoded by two of the variants reside within the autoinhibitory domain of the respective NOD protein.[32] Candidate variants in three additional families affected genes known to modify activity of the NOD/RIPK2 pathway, *CARD9* (FIJ9—pVAAST: p value=0.00106; LOD=16.838; PHEVOR: score 3.08, final rank=19), *CHUK* (MTP25—pVAAST: p value=0.00102; LOD=12.55; PHEVOR: score 4.55, final rank=3) and *IKBKB* (SA735—pVAAST: p value=0.000743; LOD=16.15; PHEVOR: score 4.23, final rank = 10).[33–36] The family studies indicate a striking correlation between inheritance of variants that alter conserved sites within proteins of the NOD/RIPK2 signalling pathway and the occurrence of disease within families exhibiting OA of the hand, 1st MTP joint or shoulder.

## Generation of the *Ripk2*104Asp mouse

*Nod1/2* and *Ripk2* are expressed in uninjured joints of mice, and the pathway is activated following injury (online supplemental figure 2). To determine whether altered pathway signalling is sufficient to confer increased susceptibility to OA, we used precise genome editing to introduce the human *RIPK2*$^{104Asp}$ variant into the C57BL/6J inbred strain of mice, thereby creating an isogenic pair of mouse lines: the parental strain, which encodes the mammalian lineage-conserved Asn at position 104 (WT), and a derived line whose *Ripk2* allele encodes Asp at position 104 (*Ripk2*$^{104Asp}$) (figure 1A). The modified allele is expressed at WT levels (figure 1B and online supplemental figure 3). Homozygous

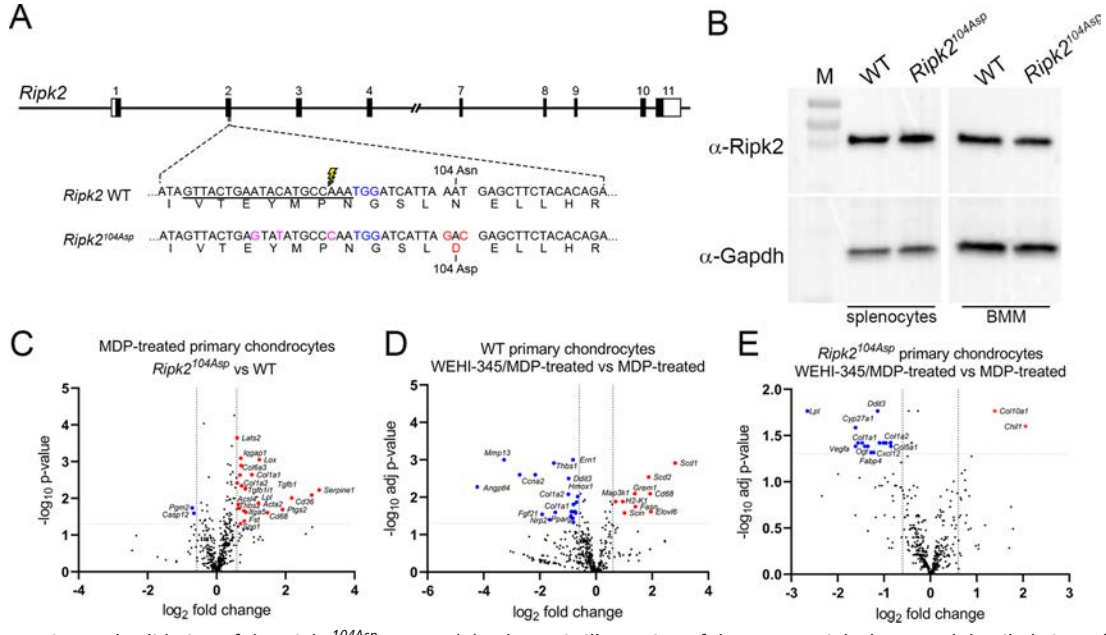


**Figure 1** Generation and validation of the *Ripk2*$^{104Asp}$ mouse. (A) Schematic illustration of the mouse *Ripk2* locus and detailed view of exon 2. CRISPR/Cas9-stimulated homology-directed repair was used to edit sequences of C57BL/6J mice (WT) encoding the *Ripk2*$^{104Asn}$ protein to generate an isogenic line that expressed the OA-associated Ripk2$^{104Asp}$ protein from the native locus. The guide RNA target sequence is underlined, the PAM site is highlighted in blue and the Cas9 cleavage site is denoted with lightning bolt. An oligonucleotide donor was used as a template to create the mutations to generate Asp 104 (red) as well as silent mutations (magenta) to prevent targeting of the modified locus. (B) Immunoblot analysis indicates similar Ripk2 protein present in WT and *Ripk2*$^{104Asp}$ splenocytes or bone marrow derived macrophages (BMM). GAPDH is used as a loading control. M=protein mass standards in kDa. (C) A single copy of *Ripk2*$^{104Asp}$ is sufficient to alter the gene expression response of primary chondrocytes to MDP treatment. Volcano plots indicate genes significantly upregulated (red) or downregulated (blue) in MDP-treated *Ripk2*$^{104Asp}$ as compared with MDP-treated WT primary chondrocytes. (D and E) Increased gene expression in response to MDP stimulation is dependent on Ripk2 activity. (D) WT or (E) *Ripk2*$^{104Asp}$ primary chondrocytes were stimulated with MDP in the presence or absence of the Ripk2 inhibitor, WEHI-345. Volcano plots indicate genes significantly upregulated (red) or downregulated (blue) on MDP-stimulation in the presence of the inhibitor. MDP, muramyldipeptide; OA, osteoarthritis.

and heterozygous mice carrying the *Ripk2*$^{104Asp}$ allele are viable and display no overt phenotypes. To recapitulate the dominant human phenotype,[11] heterozygous *Ripk2*$^{104Asp}$ mice were used for all subsequent analyses.

### A single copy of the *Ripk2*104Asp allele is sufficient to alter gene expression in primary chondrocytes

To determine if the *Ripk2*$^{104Asp}$ allele perturbed NOD/RIPK2 signalling, cultured primary chondrocytes[37] were stimulated with the Nod2 agonist, muramyldipeptide (MDP). Both WT and *Ripk2*$^{104Asp}$ chondrocytes responded to MDP by upregulating genes associated with proinflammatory signalling (online supplemental figure 4 and online supplemental table 2). However, the transcriptional response of *Ripk2*$^{104Asp}$ chondrocytes was significantly amplified as compared with that of WT controls, consistent with previous functional assays of the *Ripk2*$^{104Asp}$ allele[11] (figure 1C). MDP-stimulated gene expression was indeed dependent on Ripk2 activity as co-incubation of chondrocytes with the Ripk2 inhibitor WEHI-345[38] significantly reduced expression of many genes, including those whose expression is directly associated with OA (*Mmp13*, *Col1a1*, *Col1a2* and *Ccna2*) (figure 1D,E). These data indicate that the *Ripk2*$^{104Asp}$ allele confers heightened gene expression on activation of the NOD2 receptor.

### The *Ripk2*104Asp allele acts dominantly and is sufficient to confer increased susceptibility to OA

The joints of mature *Ripk2*$^{104Asp}$ animals appear structurally similar to those of WT mice with no histological evidence of joint degeneration (figure 2A–D, I and J). Nevertheless, *Ripk2*$^{104Asp}$ mice displayed increased sensitivity to experimentally induced OA, initiated by destabilisation of the medial meniscus (DMM) of the stifle (knee) joint.[39] Eight weeks after DMM surgery *Ripk2*$^{104Asp}$ mice exhibited a significant increase in the extent and severity of cartilage damage on the medial (both femoral condyle and tibial plateau) and lateral (femoral condyle) faces of the knee as compared with operated WT controls (figure 2A–J and online supplemental figure 5). Blinded histological scoring of the entire joint or individual quadrants revealed highly significant differences in the average and maximal Osteoarthritis Research Society International (OARSI) scores[39] of DMM-treated WT and *Ripk2*$^{104Asp}$ mice (figure 2I,J). In contrast, no significant

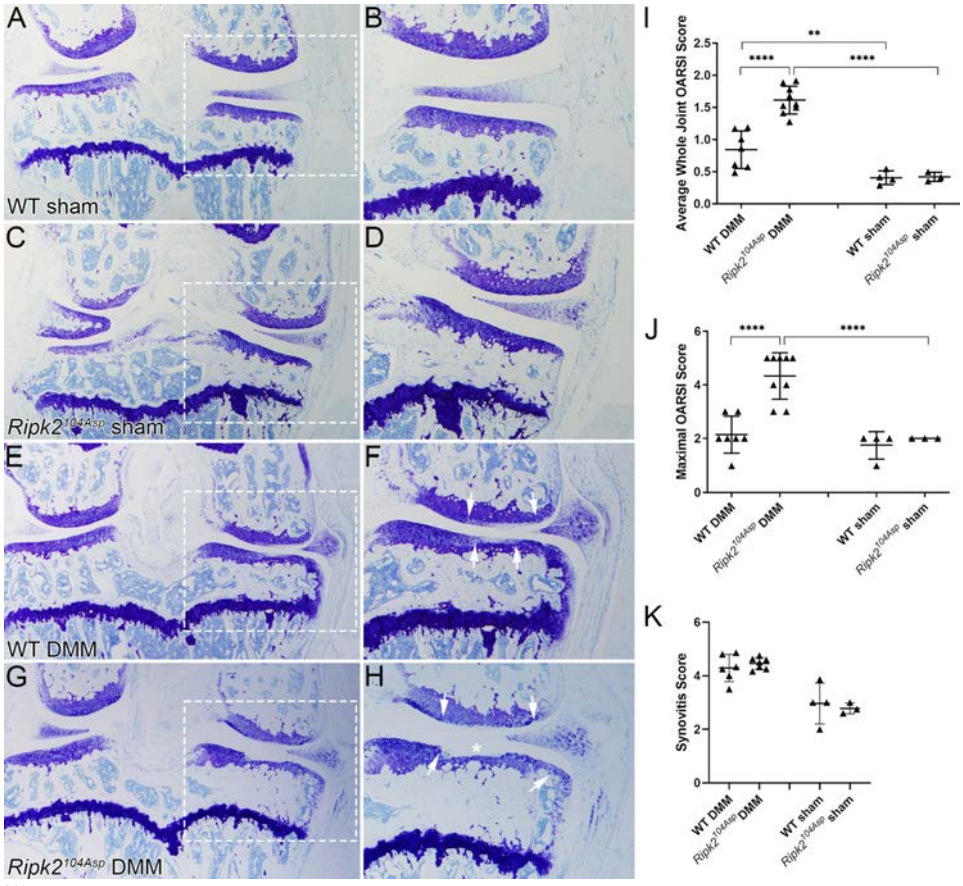


**Figure 2** The *Ripk2*$^{104Asp}$ allele acts dominantly and is sufficient to confer increased susceptibility to post-traumatic osteoarthritis. (A–D) Knee joints of WT and *Ripk2*$^{104Asp}$ mice that underwent sham surgery are similar histologically, with no indication of an OA phenotype. (E, F) Following DMM surgery, WT knees displayed mild/moderate loss of proteoglycan content in the articular cartilage on the medial side of the knee (indicated by loss of toluidine blue staining). The extent of the damage is indicated by the arrows in F, G, H. Following DMM surgery, joints of *Ripk2*$^{104Asp}$ mice displayed moderate/severe loss of proteoglycan content (arrows in H), cartilage fibrillation, and complete loss of articular cartilage in the medial tibial plateau (asterisk in H). (I) Average whole joint and (J) maximal Osteoarthritis Research Society International (OARSI) scores of sham-operated and DMM-operated knee joints. (K) There was no difference in the degree of synovitis between different genotypes. A, C, G, E are images of the entire knee joint; dashed boxes were magnified in B, D, F, H to focus on degradation on the medial side of the joint. Femur is up and medial is to the right in all images. WT sham (n=4), *Ripk2*$^{104Asp}$ sham (n=3), WT DMM (n=7), *Ripk2*$^{104Asp}$ DMM (n=9). All animals were analysed 8 weeks postsurgery. Error bars represent ±SD and statistically significant differences of p≤0.01 (**), p≤0.001 (***) and p≤0.0001 (****) were determined by two-way Analysis of Variance with Tukey's multiple comparisons test. OA, osteoarthritis.

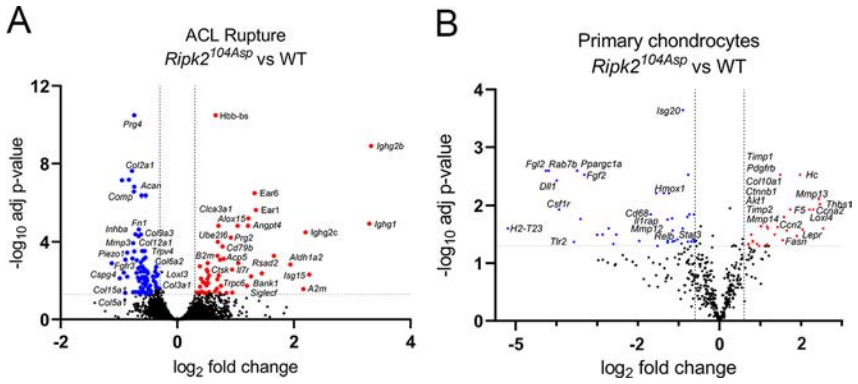


**Figure 3** *Ripk2*$^{104Asp}$ enhances expression of OA-associated markers in the surgically injured joint as well as primary chondrocytes. (A) Comparative analysis of RNA-seq performed on whole joints isolated from WT or *Ripk2*$^{104Asp}$ mice 10 days post ACL rupture. The volcano plot indicates genes significantly upregulated (red) or downregulated (blue) in *Ripk2*$^{104Asp}$ as compared with WT joints. (B) The nCounter Fibrosis panel was used to measure gene expression in AC cultured from WT or *Ripk2*$^{104Asp}$ mice. Volcano plot indicates genes significantly upregulated (red) or downregulated (blue) in *Ripk2*$^{104Asp}$ primary chondrocytes compared with WT primary chondrocytes. ACL, anterior cruciate ligament; OA, osteoarthritis.

difference was evident in the degree of synovitis observed in the operated joints of the two groups of mice (figure 2K). As a non-invasive alternate method for inducing post-traumatic OA (PTOA) in 16-week-old mice, we employed mechanical rupture of the anterior cruciate ligament (ACL).[40] Again, *Ripk2*$^{104Asp}$ mice showed a significant increase in cartilage damage compared with WT controls when examined 5 weeks post-rupture (online supplemental figure 6).

Injured knee joints of *Ripk2*$^{104Asp}$ mice exhibited gene expression signatures associated with classical OA, but indicative of a more advanced disease state as compared with WT. Genes upregulated in the injured *Ripk2*$^{104Asp}$ knee joints soon after ACL rupture are involved in both the innate and adaptive immune response (*Prg2*, *Trpc6*, *Bank1*, *Siglecf*, *Clca3a1*, *Isg15*, *Ighg1*, *Ighg2b* and *Rsad2*) and include genes linked to OA (*B2m*, *A2m*, *Il17r*, *Ctsk*, *Angpt4*, *Aldh1a2* and *Alox15*) (figure 3A and online supplemental table 2). Conversely, many ECM genes whose depletion is associated with OA pathogenesis were significantly downregulated in *Ripk2*$^{104Asp}$ joints, including *Acan*, *Col2a1*, *Col3a1*, *Comp*, *Prg4*, *Fn1*, *Hspg2*, *Matn2* and *Cspg4* (figure 3A and online supplemental table 2). Thus, whereas the transcriptional response of *Ripk2*$^{104Asp}$ mice to injury closely parallels that of WT, as indicated by the altered expression of OA-associated genes, it is exaggerated, with increased expression of inflammatory and catabolic factors and increased down-regulation of many ECM genes.

### The *Ripk2*104Asp allele alters the basal physiology of knees joints and the response to PTOA

In the absence of evident tissue remodelling that might indicate emergent OA in the knee joints of *Ripk2*$^{104Asp}$ mice (figure 2A–D,I,J), we asked whether the joints exhibited altered signs of gene expression and/or inflammatory state. Analysis of primary chondrocytes revealed that gene expression was significantly altered in *Ripk2*$^{104Asp}$ cells as compared with those isolated from WT mice (figure 3B). Genes upregulated included well-known markers of OA, including those associated with hypertrophic chondrocytes and ECM remodelling (*Mmp13*, *Mmp14*, *Timp1*, *Timp2*, *Loxl4* and *Col10a1*), growth factor signalling (*Ctnnb1*, *Ckap4* and *Pdgfrb*), leptin signalling (*Lepr*), PI3K/Akt/mTOR signalling (*Akt1*), as well as genes involved in inflammatory signalling (*Lpcat1*, *Rbx1*, *Ccn2*, *Fasn*, *Cfhr2*, *F5*, *Elovl6*, Hc and *Thbs1*) (figure 3B and online supplemental table 2).

The striking linkage between the altered gene expression profile of cultured *Ripk2*$^{104Asp}$ chondrocytes and markers of mature OA led us to investigate if altered marker expression presaged the response to injury in the whole joint. The joints of both sham-operated and DMM mice revealed substantive effects of the *Ripk2*$^{104Asp}$ allele on Nod/Ripk2 activity, matrix components and markers of inflammation (figure 4 and online supplemental figure 7). Although the Ripk2 protein is present at low levels in tibial chondrocytes of WT and *Ripk2*$^{104Asp}$ animals subjected to sham surgery (figure 4A), pathway activity appears elevated in the *Ripk2*$^{104Asp}$ joint, as reflected in higher levels of activated phospho-NF-κB (pNF-κB) compared with that of WT sham controls (figure 4B and online supplemental figure 7). Following DMM surgery, pathway activity differences are enhanced, as both the level of Ripk2 expression and the number of chondrocytes expressing NF-κB are considerably elevated in the operated joints of *Ripk2*$^{104Asp}$ mice relative to WT (figure 4A–C, and online supplemental figure 7). Similarly, expression of matrix markers is altered in sham-operated *Ripk2*$^{104Asp}$ joints in a manner that parallels the gene expression changes associated with overt OA. As compared with WT sham controls, knee joints of sham surgery *Ripk2*$^{104Asp}$ mice express elevated levels and have increased numbers of chondrocytes expressing Mmp13, a metalloproteinase that targets collagen for degradation (figure 4C,E). Mmp13 expression in the *Ripk2*$^{104Asp}$ mice extends beyond the superficial layer of cartilage into deeper layers relative to WT controls (brackets in figure 4C and online supplemental figure 7). Consistent with this finding, collagen deposition scored by the presence of Col2 appears relatively deficient in the joints of sham-operated *Ripk2*$^{104Asp}$ mice (figure 4C,D, and online supplemental figure 7). The differences between WT and *Ripk2*$^{104Asp}$ mice in the levels of expression and numbers of chondrocytes expressing Mmp13 and Col2 between WT and *Ripk2*$^{104Asp}$ mice become even more exaggerated following DMM surgery (figure 4C–E and online supplemental figure 7). Altogether these data indicate that the *Ripk2*$^{104Asp}$ allele alters the basal physiological state of the joint so that it expresses features normally associated with overt OA.

As *Ripk2*$^{104Asp}$ chondrocytes have elevated expression of proinflammatory genes, and as local inflammation can sensitise joints to PTOA,[41] knees were examined in situ for the expression of inflammatory markers. iNos is a major inflammatory mediator that is increased in OA. It is expressed in many tissues of the joint, including chondrocytes and macrophages,[42] both of which contribute directly to homeostatic maintenance of the joint.[43 44] Whereas sham-operated WT mice have uniformly low levels of iNos expression throughout the joint, following DMM surgery

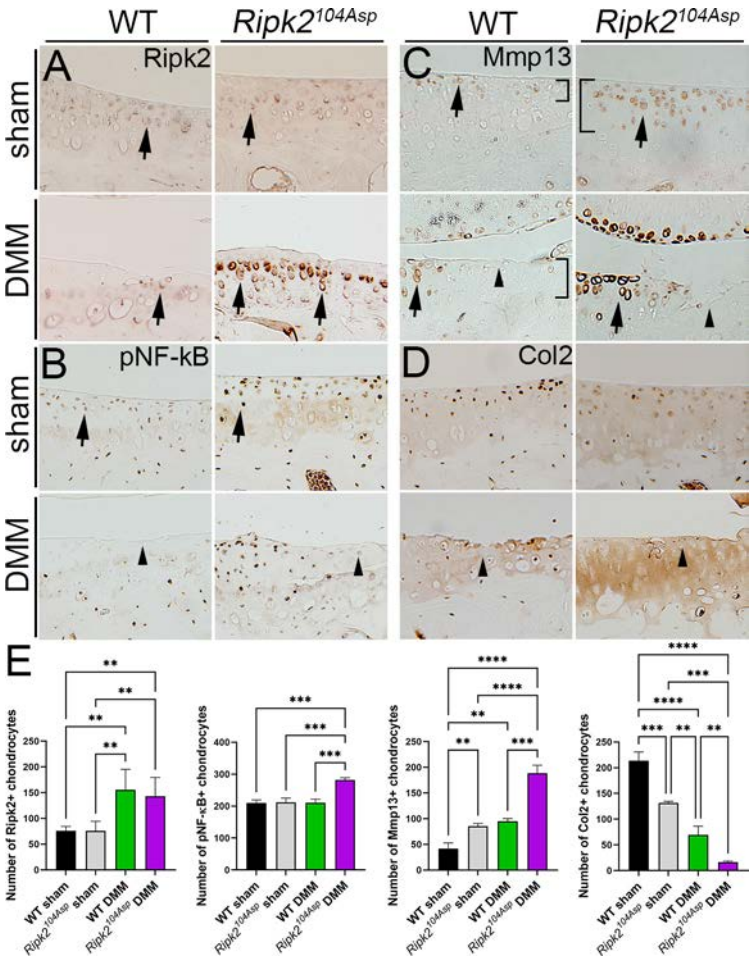


**Figure 4** *Ripk2*$^{104Asp}$ enhances NOD/RIPK2 signalling as well as expression of OA-associated markers of matrix remodelling in uninjured joints and joints with PTOA. Immunohistochemical detection of (A) Ripk2, (B) pNF-κB, (C) Mmp13 or (D) Col2 in WT and *Ripk2*$^{104Asp}$ mice 8 weeks following sham or DMM surgery. (A) Ripk2 is expressed at low levels in chondrocytes (arrows) of WT sham-, WT DMM- and *Ripk2*$^{104Asp}$ sham-operated joints. In contrast, Ripk2 expression is highly elevated in chondrocytes in *Ripk2*$^{104Asp}$ knees following DMM surgery (arrows). (B) Activated NF-κB (pNF-κB) expression levels are higher in *Ripk2*$^{104Asp}$ sham-operated joints as compared with WT controls (arrows). The relatively increased Ripk2 expression is maintained in *Ripk2*$^{104Asp}$ joints following DMM surgery. (C) Mmp13 expression is elevated in chondrocytes of sham-operated *Ripk2*$^{104Asp}$ mice as compared with WT controls (arrows); the relatively increased expression Ripk2 is maintained in *Ripk2*$^{104Asp}$ mice following DMM surgery (arrows). Furthermore, in the unoperated *Ripk2*$^{104Asp}$ joint, Mmp13 expression extends into deeper layers of cartilage, an expression domain normally only seen in WT joints following DMM surgery (brackets in C). (D) Col2 expression is reduced in *Ripk2*$^{104Asp}$ sham surgery mice relative to WT controls, and this loss is further exacerbated by DMM surgery (arrows). In regions with severe cartilage damage (arrowheads), pNF-κB, Mmp13 and Col2 expression is low in both WT and *Ripk2*$^{104Asp}$ DMM-operated joints. Images are of selected regions of the medial tibial condyle (see online supplemental figure 7). (E) Quantification of the number of Ripk2, pNF-κB, Mmp13 and Col2 positive chondrocytes in the medial knee joint of WT sham, *Ripk2*$^{Asp104}$ sham, WT DMM and *Ripk2*$^{Asp104}$ DMM mice. n=3 independent animals for each experimental condition. Error bars represent ±SD and statistically significant differences of p≤0.01 (**), p≤0.001 (***) and p≤0.0001 (****) were determined by two-way Analysis of Variance with Tukey's multiple comparisons test.

iNos is highly induced in the joints, especially in the meniscus and osteophyte (figure 5A). In contrast, even sham-operated *Ripk2*$^{104Asp}$ mice exhibit a striking elevation of proinflammatory iNos signal in cartilage, meniscus and synovial tissue (figure 5A). Following DMM surgery, elevated iNos expression is also evident in the cartilage, meniscus and osteophytes (figure 5A,B).

In the normal response to inflammation, CD206+ macrophages accrue in joints and are involved in resolving inflammation and tissue repair. Despite elevated iNos expression in the knees of sham-operated *Ripk2*$^{104Asp}$ mice, they did not have increased numbers of CD206+ cells relative to WT joints (figure 5C,D). Moreover, *Ripk2*$^{104Asp}$ mice had a clear deficit in the recruitment of anti-inflammatory CD206+ cells into the cartilage, meniscus and synovium of operated joints as compared with joints of WT mice (figure 5C,D). In sum, prior to overt injury, knee joints of *Ripk2*$^{104Asp}$ mice exhibit higher than normal levels of inflammatory gene expression; following injury, *Ripk2*$^{104Asp}$ mice are relatively poor at recruiting factors to resolve acute inflammation.

### The *Ripk2*104Asp mice do not exhibit an elevated inflammatory phenotype

The NOD/RIPK2 signalling pathway operates broadly and the heightened expression of OA-associated markers in the knee joints of *Ripk2*$^{104Asp}$ mice may reflect widespread elevation of inflammation. We examined the ability of cultured bone marrow-derived macrophages (BMM) from WT and *Ripk2*$^{104Asp}$ mice to respond to MDP. In contrast, to the effects of the *Ripk2*$^{104Asp}$ allele on the expression profile of chondrocytes, relatively few genes were differentially expressed on stimulation of the BMM

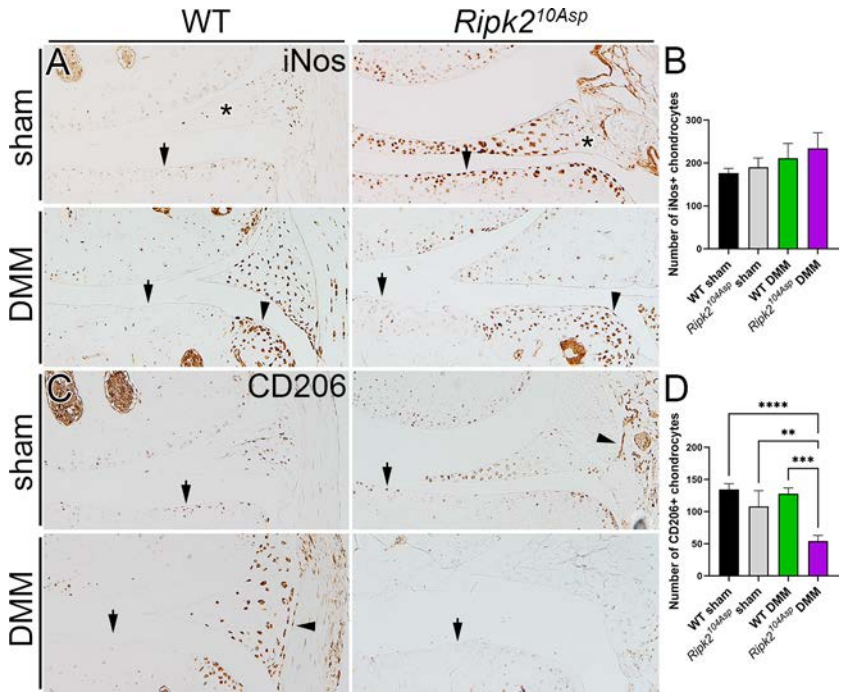


**Figure 5** *Ripk2*$^{104Asp}$ joints have elevated expression of proinflammatory markers. (A) In WT mice, the proinflammatory marker iNos is normally expressed at low levels in the joint, and is markedly elevated following DMM surgery. In contrast, knee joints of *Ripk2*$^{104Asp}$ have chronically high levels of iNos expression, independent of injury. In A arrows indicate chondrocytes, asterisks mark the meniscus and arrowheads indicate osteophytes in DMM-operated joints. (B) There is no difference in expression of the anti-inflammatory marker, CD206, between sham-operated surgery WT and *Ripk2*$^{104Asp}$ joints. Following DMM surgery, CD206 is prominently expressed in WT joints whereas it is almost absent in the joints of *Ripk2*$^{104Asp}$ mice. In B arrows indicate chondrocytes and arrowheads indicate synovium. All joints are 8 weeks postsurgery. (C) Quantification of the number of iNos and CD206 positive chondrocytes in the medial knee joint of WT sham, *Ripk2*$^{Asp104}$ sham, WT DMM and *Ripk2*$^{Asp104}$ DMM mice. n=3 independent animals for each experimental condition. Error bars represent ±SD and statistically significant differences of p≤0.01 (**), p≤0.001 (***) and p≤0.0001 (****) were determined by two-way Analysis of Variance with Tukey's multiple comparisons test.

cells (online supplemental figure 8). To assess systemic differences in the inflammatory states of the mice, serum cytokine levels pre-DMM or post-DMM surgery were measured. There was no difference in the serum concentration of any of 13 inflammatory cytokines sampled from 16 week old pre-surgery WT and *Ripk2*$^{104Asp}$ mice (figure 6), indicating unoperated *Ripk2*$^{104Asp}$ mice do not have a measurably elevated systemic phenotype. In contrast, in response to localised joint injury, *Ripk2*$^{104Asp}$ mice mount a highly augmented systemic response. At 4 weeks following DMM surgery both WT and *Ripk2*$^{104Asp}$ responded with raised serum levels of the proinflammatory cytokines IL-1β, INF-β and the anti-inflammatory IL-10, but the degree of increase and the levels of these cytokines were significantly elevated in the *Ripk2*$^{104Asp}$ mice (figure 6). The differences in serum cytokine response are transient, as they are resolved by 8 weeks postsurgery (figure 6).

## DISCUSSION

Animals carrying the single amino acid change encoded by the *Ripk2*$^{104Asp}$ variant have a magnified response to joint injury that leads to a predisposition to develop OA. The allele creates a chronically hyperactive inflammatory state in the joint with early signs of defective joint maintenance, as evidenced by

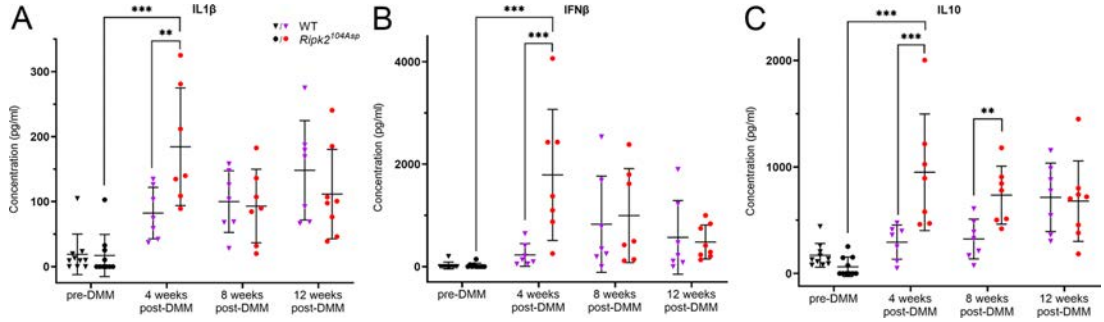


**Figure 6** DMM surgery induces an acute systemic inflammatory response in *Ripk2*$^{104Asp}$ mice. Quantification of serum (A) IL-1β, (B) IFN-β and (C) IL-10 levels from 16-week-old WT and *Ripk2*$^{104Asp}$ mice just prior to DMM surgery (black diamonds and triangles) and at 4, 8 and 12 weeks postsurgery (magenta triangles and red circles). Error bars represent ±SD and statistically significant differences of p≤0.01 (**) and p≤0.001 (***) were determined by one-way Analysis of Variance (ANOVA) with Tukey's multiple comparisons test (4 week post-DMM group) and a two-tailed unpaired t-test (8 and 12 week groups).

gene expression in chondrocytes isolated from young mice and altered expression of pNF-κB, iNos, Mmp13 and Col2 in mature animals. Nevertheless, the elevated activity of the NOD/RIPK2/NF-κB pathway caused by the variant allele has a very modest effect on tissue remodelling under normal laboratory conditions. The *Ripk2*$^{104Asp}$ allele does not alter which signalling pathways and genes are activated in response to joint injury. Rather, consistent with the elevated signalling activity of the variant protein,[11] it simply leads mice to mount an accelerated and elevated response to injury, characterised locally in the joint by amplified Ripk2 and pNF-κB expression, exaggerated changes in the expression of genes indicative of OA progression, including Mmp13 and Col2, and histologically recognisable deterioration. Both local as well as systemic inflammatory responses are amplified following acute injury, seen by the deficit of CD206+ cells in the joint, altered gene expression in BMM and the transient rise in serum cytokines.

Multiple sources of inflammation have been proposed as potential drivers of OA, including mechanosensory signalling and responses of chondrocytes or other joint resident cells to damage-associated molecular patterns (DAMPs).[4 45–47] Our work helps identify which of the potential inflammatory signalling pathways functions to limit or promote the occurrence of OA and which cells are essential for promoting the inflammatory response that leads to the tissue remodelling seen in OA.[24] Here we demonstrate the NOD/RIPK2 is an important inflammatory pathway in the development of OA. Mice that constitutively express the *Ripk2*$^{Asp104}$ allele exhibit a set of marked changes in their knee joints without chronic systemic inflammation, reflecting alteration in normal local homeostatic mechanisms in the joint. Although NOD/RIPK2 signalling is known to activate multiple downstream pathways (eg, NF-κB, p38 and so on), we do not yet know which of the targets of the activated Ripk2$^{Asp104}$ protein are particularly significant for OA susceptibility. Finally, even though our results suggest *Ripk2*$^{Asp104}$ activity functions in the knee joint, we have not defined the specific cells and tissues of the joint that require *Ripk2*$^{Asp104}$ activity for OA development. Conditional spatial and temporal activation of *Ripk2*$^{Asp104}$ will allow us to test the necessity of the NOD/RIPK2 pathway in OA development.

Understanding the ligands that activate the NOD/RIPK2 pathway is important for determining risk factors. The NODs were initially described as activated by bacterial peptidoglycan fragments,[48 49] yet there is increasing evidence they can be activated by non-pathogen associated molecules, including DAMPs.[50] One possible DAMP activator of NODs in the synovial joint is the pro-catabolic Fibronectin fragment (FN-f), which is produced by cleavage of the Fibronectin protein in response to injury.[51 52] *NOD1* and *NOD2* expression is upregulated in human chondrocytes treated with the 29 kDa amino-terminal FN-f, and activation NF-κB pathway is dependent on NOD2/RIPK2 activity.[17] Thus, local hyperactivity of the NOD/RIPK2 pathway may augment the response to FN-f release after injury to the joint and lead to increased OA susceptibility. Interestingly, two of the OA-associated NOD variants we have identified affect the Leucine Rich Repeat (LRR) domain, which functions in autoinhibition of NOD-mediated signalling activity and ligand binding. In the absence of ligand, the LLR domain interacts with the NOD domain to keep NOD1/2 in an inactive state and prevent NOD:NOD protein interactions that promote signaling.[18 32 53] Thus, the NOD variant proteins may have heightened basal activity or may be hyperresponsive to ligand binding. Functional analyses of the NOD variants will allow us determine if the variants alter signalling activity and if the altered activity is dependent on FN-f or other ligands.

Our study is focused on the NOD/RIPK2 pathway in OA of the 1st MTP joint, hand and shoulder. We selected these joints to minimise the phenotypic heterogeneity to increase our power of identifying causal variants in these families. We have excluded families with knee and hip OA from our study due to confounding factors often present in affected individuals (eg, traumatic injury, ligament tear, developmental dysplasia of the hip, Perthes disease and avascular necrosis of the hip). Given that we have identified NOD/RIPK2 pathway variants in multiple joints, we predict that alteration of the pathway is also a risk factor for knee and hip OA. It is possible the NOD/RIPK2 pathway is not a major risk factor for these joints as no NOD/RIPK2 pathway genes have been identified in GWAS studies to date.[54] Identification and analysis of knee and hip OA families free from confounding factors will allow us to test this hypothesis. Further, we only address the role of *Ripk2*$^{104Asp}$ in PTOA and do not test if the allele leads to increased OA onset or severity in aged animals. Given the *Ripk2*$^{104Asp}$ allele creates a chronically hyperactive inflammatory state in the joint, the most parsimonious hypothesis is that aged animals carrying the disease allele may have increased prevalence of OA. Alternatively, in the absence of traumatic injury to the joint, aged *Ripk2*$^{104Asp}$ mice may not have elevated OA susceptibility.

In sum, we propose modulation of the NOD/RIPK2 signalling pathway is a general vulnerability factor for OA. Supporting our hypothesis, we have found rare variants altering conserved positions in NOD/RIPK2 pathway proteins in 7 of the 151 (5%) families we have examined with non-syndromic OA. Consistent with a causal connection between pathway activity and OA, hyperactivity of the NOD/RIPK2 pathway is associated with several human inflammatory syndromes that include arthritis as a comorbidity.[22 23 55] Our data indicate that modification of the NOD/RIPK2 pathway can render multiple joints (both weight and non-weight bearing) susceptible to OA. While the initiating factor (injury, repetitive use, diabetes, obesity and so on) and activating ligand may differ between joints and individuals, our work has shown that elevated NOD/RIPK2 signalling is a predictive indicator of susceptibility to OA. Further pursuit of this signalling pathway and the spatiotemporal requirement for its activity may lead to assays for detection of early stages of disease and have therapeutic potential.

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**Contributors** MJJ is responsible for the overall content as the guarantor. MJJ and DJG conceived and designed the study, analysed data and wrote the manuscript. CMG and NHK conceived and designed the study and analysed data. MH, YM, SRV, KAN and KH analysed data and provided feedback on the manuscript.

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**Competing interests** None declared.

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

**Patient consent for publication** Consent obtained directly from patient(s)

**Ethics approval** This study involves human participants and was approved by The Institutional Review Boards of the University of Utah (IRB # 79442) and Intermountain Healthcare (IRB # 1050554), and the Resource for Genetic and Epidemiologic Research approved this study. Participants gave informed consent to

participate in the study before taking part. Mice were maintained in accordance with approved institutional protocols at the University of Utah.

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



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# Livedo racemosa and thrombotic vasculitides of scalp in systemic lupus erythematosus

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One 50-year-old systemic lupus erythematosus male patient presented to our emergency room with a 2-month history of intermittent high fever and diffuse hair loss. On admission to our hospital, spiking fever up to 40°C, pancytopenia, hyperferritinemia 9091 ng/mL (normal range: 30–400 ng/mL in male) and impaired kidney function with heavy proteinuria 8.91 gm/day (normal range: less than 0.2 gm/day) were noted. After investigation with laboratory examination and bone marrow biopsy, macrophage-activated syndrome and lupus nephritis with nephrotic syndrome were noted. Serial serology results including hepatitis B virus, hepatitis C virus, HIV and cytomegalovirus were all tested negative. Furthermore, rapidly progressive alopecia and multiple geographic-like erythematous as well as purple-coloured patches scattered over the scalp with ulcerative wounds deep into aponeurosis and covered with eschar-like tissue (figure 1A: occipital view; figure 1B: parietal view) developed. No other similar skin lesions were identified elsewhere. Skin biopsy of scalp revealed subcutaneous medium-sized blood vessels with full thickness involvement by neutrophils, eosinophils and lymphocytes with intraluminal thrombus, and leucocytoclasia. Serum levels of anticardiolipin antibody, anti-β2-glycoprotein antibody, lupus anticoagulant and cryoglobulin were all in normal limit.

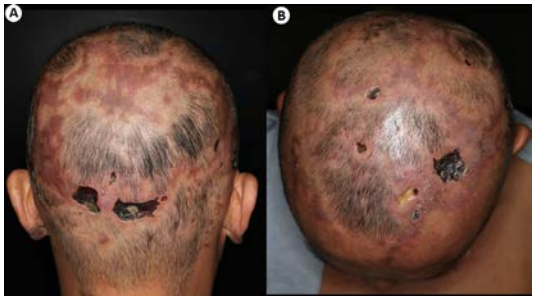


**Figure 1** Multiple geographic-like erythematous and purple-coloured patches scattered over the scalp with ulcerative wounds deep into aponeurosis and covered with eschar-like tissue (A: occipital, B: parietal).

After aggressive treatment with intravenous anti-IL-6 receptor monoclonal antibody (tocilizumab, 8 mg/kg, once), methylprednisolone pulse therapy (1 g/day for 3 days then gradually tapered, sum up to 5 g during the whole hospitalisation) and intravenous immunoglobulin injection (1 g/kg, divided in 3 days), the patient fully recovered from the skin ulcerations and the proteinuria had much improved (2.7 g/day).

## PICTURE QUIZ

A 50-year-old male patient with lupus-related macrophage-activated syndrome and lupus nephritis, who suffered from multiple geographic-like erythematous and purple-coloured patches scattered over the scalp with ulcerative wounds deep into aponeurosis and covered with eschar-like tissue.

What is the diagnosis?

1. Pyoderma gangrenosum.
2. Acute febrile neutrophilic dermatosis (Sweet's syndrome).
3. Skin lymphoma.
4. Livedo racemosa with thrombotic vasculitides.

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# Chronic glucocorticoid maintenance treatment is associated with the risk of SARS-CoV-2 infection in patients with systemic lupus erythematosus who received vaccination

SARS-CoV-2-related disease (COVID-19) constitutes an ongoing challenge for public health. Vaccination campaigns have effectively reduced COVID-19-related morbidity and mortality, leading to less restrictive preventive measures. This has, however, led to worldwide absolute increasing rates of COVID-19 cases during the last months. Patients with multiorgan autoimmune disorders, including systemic lupus erythematosus (SLE) are at increased risk of morbidity due to SARS-CoV-2 infection and pandemic-related disruptions in public health services. Vaccination also constitutes a challenge for patients with SLE and other rheumatic disorders, who might develop dysfunctional immunisation responses due to disease-related and treatment-related factors. Poor humoral and cellular immunogenicity of current anti-SARS-COV-2 vaccines has been reported in patients with SLE and other immune-mediated diseases.[1 2] However, less is known about the incidence and risk factors for breakthrough COVID-19 following vaccination.[3 4]

To address this issue, we analysed data from 452 patients with SLE (93% women) followed in seven Italian tertiary referral centres, who completed a primary vaccination cycle between January and November 2021. Data from patient history and status were collected during the last visit before and the first visit after the primary vaccination cycle. Postvaccination evaluations took place after a median (IQR) time of 3.9 (3.6–4.8) months. Clinical features of interest consisted of symptoms or signs affecting the constitutional, musculoskeletal, mucocutaneous, neuropsychiatric, renal, cardiopulmonary, gastrointestinal, ophthalmological and/or haematological domains and were attributable to SLE as defined in the British Isles Lupus Assessment Group 2004 instrument.[5] Data on previous or current history of antiphospholipid antibody syndrome were also collected. History data included clinical manifestations and laboratory features (low complement, positive anti-DNA and antiphospholipid antibodies) having consistently occurred at any time during the course of SLE until the prevaccine visit. Status data encompassed active clinical manifestations and laboratory variables (low complement, positive anti-DNA, erythrocyte sedimentation rate, C reactive protein levels) before and after vaccination. Data on ongoing treatments before vaccination were also collected. Incident COVID-19 cases until 31 March 2022 were recorded. The median (IQR) age and disease duration at the time of analysis were 48 (35–57) years and 11 (6–16) years, respectively. Three hundred and twenty-one patients were receiving corticosteroids (median dose=5 (5–5) mg/day) and 131 were receiving immunosuppressants. Four hundred and forty-five patients received mRNA-based vaccines, while seven were vaccinated with viral-vector vaccines. Thirteen patients (3%) had a history of COVID-19 before vaccination. Seventy-seven patients (17%) had postvaccine COVID-19. Univariate Cox regression analysis showed that the following demographic and clinical factors were negatively associated with postvaccine COVID-19: age (HR=0.98, 95% CI 0.97 to 1.00; p=0.028), disease duration ≥10 years (HR=0.63, 95% CI 0.40 to 0.98; p=0.041), history of constitutional symptoms (HR=0.46, 95% CI 0.27 to 0.79; p=0.005) and a history of positive antiphospholipid antibody profile (HR=0.50, 95% CI 0.29 to 0.88; p=0.016). Corticosteroid treatment before vaccination was associated with the risk of experiencing COVID-19 after vaccination (HR=3.15, 95% CI 1.62 to 6.12; p=0.001; figure 1). In a multivariate model including the abovementioned variables, corticosteroid treatment (HR=2.55, 95% CI 1.29 to 5.04; p=0.007) and absence of constitutional symptoms (HR 1.76, 95% CI 1.01 to 3.06; p=0.046) remained significantly associated with COVID-19 after vaccination. There was no corticosteroid dose-dependent effect (HR=0.99, 95% CI 0.90 to 1.07, p=0.743, n=321) nor any association with immunosuppression with one or more agents. There was no association with clinical activity nor with serological status before and after vaccination. Prior COVID-19 did also not affect the risk of postvaccine COVID-19.

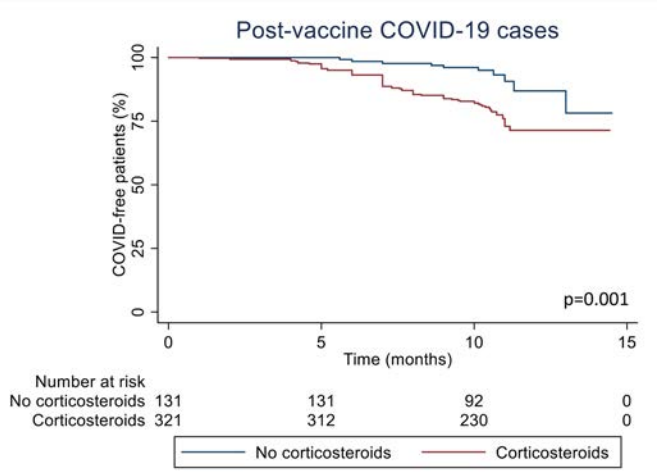


**Figure 1** Postvaccine COVID-19 cases in patients with systemic lupus erythematosus (SLE). Kaplan-Meier survival curves showing the differential rate of COVID-19 occurrence between patients receiving corticosteroids (red line) and those with other treatments (blue line).

In this multicentre study we found that constitutional symptoms might be associated with a reduced risk of postvaccine COVID-19, while corticosteroid treatment constituted a risk factor for breakthrough infections. Mechanistic explanations for the apparent protective role of constitutional symptoms are not straightforward. Systemic inflammatory manifestations might simply have masked COVID-19-related symptoms, leading to delayed or missing diagnoses. More intriguingly, constitutional symptoms might identify a subset of patients with enhanced, rather than dysfunctional,[6] interferon-alpha-related responses and relatively better performances to either vaccination and/or eventual SARS-CoV-2 exposure. Corticosteroids still constitute the mainstay of treatment for SLE, but also contribute to chronic morbidity and damage accrual even at low doses. In the setting of COVID-19, corticosteroids are used to combat acute inflammatory manifestations, but also associate with an increased risk of SARS-CoV-2 infection[7] and with poor outcomes in patients with systemic autoimmune disorders, including SLE. Furthermore, glucocorticoids have been shown to impair vaccine immunogenicity.[8] While larger translational studies are still needed to confirm that quantitation of antibody and T cell-mediated responses to SARS-CoV-2 reliably detect vaccination failures, our data consistently show that corticosteroids also associate with impaired protection from COVID-19 at a clinical level.

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# Oral antiviral treatment in patients with systemic rheumatic disease at risk for development of severe COVID-19: a case series

Significant advances have been made in the diagnosis and treatment of SARS-CoV-2 infection. Nevertheless, it seems that the virus and its mutations will be present in our lives for the years to come.[1] People living with systemic rheumatic diseases (SRDs),[2] especially those with certain characteristics and comorbidities such as coexisting lung disease, male gender and increasing age, are at increased risk for severe COVID-19.[3]

Despite vaccines have impeded the consequences of COVID-19,[4] infections with adverse outcomes can occur in patients with SRD. To further decrease COVID-19 reated morbidity and mortality in high-risk patients, two oral antiviral therapies (molnupiravir and nirmatrelvir/ritonavir combination) have been approved for the outpatient treatment of patients at risk for progression to severe COVID-19.[5]

Both drugs have been shown to reduce COVID-19 related adverse outcomes. However, SRDs were not largely represented in the approval studies. Besides, due to interference of nirmatrelvir/ritonavir with cytochrome P450 enzymes, interactions with numerous drugs used in rheumatology, such as colchicine, cyclosporine, voclosporin, sildenafil, sirolimus, tacrolimus, bosentan and anticoagulants, should be checked to avoid possibly dangerous side effects and/or decreased antiviral activity.

Herein, we describe our experience on safety and efficacy with nirmatrelvir/ritonavir and molnupilavir, in real-world SRD patients.

We retrospectively reviewed the medical files of all SRD patients, being followed-up in three tertiary rheumatology centers, who were SARS-CoV-2 infected between 15/2/22 and 30/4/22 and received as outpatients, Nirmatrelvir/Ritonavir or Molnupilavir, as per national guidelines (https://eody.gov.gr/)

**Table 1** Characteristics (demographics and disease related), outcomes and eligibility for oral antiviral treatment of the patients included in the study

| Characteristics | n=31 |
|---|---|
| **Demographics** | |
| Female gender, n (%) | 21 (67.7) |
| Age (years), mean (SD) | 55.4 (12.9) |
| BMI, mean (SD) | 29.6 (8.1) |
| Vaccination* status, doses, n (%) (0/1/2/3/4) | 2 (6.5)/0 (0.0)/0 (0.0)/25 (80.6)/4 (12.9) |
| Follow-up time, mean (SD), days | 47.0 (22.8) |
| **Type of SRD** | |
| Inflammatory arthritis, n (%) | 15 (48.4) |
| Vasculitis, n (%) | 6 (19.4) |
| Connective tissue diseases, n (%) | 10 (32.3) |
| **Treatment** | |
| Glucocorticoids, n (%) | 11 (35.5) |
| Dose of prednisolone, mean (SD) | 3.8 (6.5) |
| csDMARDs, n (%) | 17 (54.8) |
| tsDMARDs, n (%) | 2 (6.4) |
| bDMARDs, n (%) | 22 (70.1) |
| Other immunosuppressives, n (%) | 8 (25.8) |
| **Outcomes** | |
| COVID-19 outcome (cure), n (%) | 29 (93.6) |
| Other drug temporary discontinuation due to possible interaction, n (%) | 4† (9.7) |
| Adverse events, n (%) | 3 (12.9) |
| **Eligibility for oral antiviral treatment‡** | |
| **One of the following required** | |
| bDMARD/tsDMARD therapy, n (%) | 24 (77.4) |
| High/prolonged glucocorticoid use§, n (%) | 2 (6.5) |
| Organ transplantation, n (%) | 0 (0.0) |
| Cystic fibrosis, n (%) | 0 (0.0) |
| Solid or haematological malignancy | 1 (3.2) |
| HIV, n (%) | 0 (0.0) |
| Age ≥75 years old, n (%) | 4 (12.9) |
| End-stage renal disease, n (%) | 0 (0.0) |
| **Two of the following required** | |
| Age ≥65 years old, n (%) | 6 (19.4) |
| BMI ≥35, n (%) | 6 (19.4) |
| Diabetes mellitus, n (%) | 3 (9.7) |
| Chronic kidney disease, n (%) | 0 (0.0) |
| Chronic liver disease, n (%) | 0 (0.0) |
| Cardiovascular disease¶, n (%) | 11 (35.8) |
| Pulmonary fibrosis, n (%) | 5 (16.1) |
| Chronic obstructive pulmonary disease**, n (%) | 0 (0.0) |
| Thalassaemia, sickle cell disease, n (%) | 0 (0.0) |

*Vaccination against SARS-CoV-2.
†In one patient, two drugs (apixaban and sildenafil) were discontinued.
‡As per national guidelines (ref), a patient is eligible for antiviral treatment if: (A) one of the following conditions were present: organ transplantation, cystic fibrosis, solid or haematological malignancy, individuals with HIV and CD4 T cells <200/µL, age ≥75 years old, end-stage renal disease, immunocompromised subjects (primary or due to treatment with anti-CD20 regimes, bDMARDs or glucocorticoids (prolonged and/or high doses)) or (B) two of the following conditions were present: age ≥65 years old, BMI ≥35, diabetes mellitus, chronic kidney disease, chronic liver disease, cardiovascular disease (stroke, myocardial infarction, aneurysms and hypertension), pulmonary fibrosis, chronic obstructive pulmonary disease (requiring treatment with oxygen), thalassemia and sickle cell disease.
§Prednisone or equivalent equal or more than 15 mg for more than 4 weeks.
¶Stroke, myocardial infarction, aneurysms and hypertension.
**Requiring treatment with oxygen.
bDMARDs, biological DMARDs; BMI, body mass index; csDMARDs, conventional synthetic disease modifying anti-rheumatic drugs; n, number; SRD, systemic rheumatic disease; tsDMARDs, targeted synthetic DMARDs.

(table 1). Due to the anonymised and non-interventional nature of the study, ethics approval was not required. Patients were not involved in the design of this study. The following characteristics were recorded: a. demographics: age, gender, body mass index (BMI), b. disease-related characteristics: type of SRD (inflammatory arthritis (rheumatoid arthritis -RA-, psoriatic arthritis, axial spondyloarthritis), connective tissue diseases (systemic lupus erythematosus -SLE-, systemic sclerosis, antiphospholipid syndrome, dermatomyositis), vasculitis (antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis, giant-cell arteritis)), immunosuppressive/immunomodulatory treatment being received (conventional synthetic (cs) disease modyfying anti-rheumatic drugs (DMARDs), targeted synthetic (ts) DMARDs, biologic (b) DMARDs, glucocorticoids, other immunosuppressives (azathioprine, mycophenolate mofetil, cyclophosphamide)) c. Covid-19-related characteristics: Covid-19 vaccination status, adverse events from anti-virals, Covid-19 outcomes (cured, long-covid [>30days], hospitalization, death) and reasons for receiving anti-virals.

As shown in table 1, 31 patients with SRD received nirmatrelvir/ritonavir (n=29) or molnupilavir (n=2) (but no other treatment) during the first 5 days after COVID-19 diagnosis by their rheumatologist according to national guidelines. The majority (29/31, 94%) were fully vaccinated (three doses: 80.6%, four doses: 12.9%) against SARS-CoV-2 with mRNA vaccines and were on treatment with bDMARDs or tsDMARDs (24/31, 77.4%). As depicted in table 1, in addition to treatment with bDMARDs/tsDMARDs (which makes someone eligible for oral-antiviral treatment as per national guidelines), other reasons were also present in a considerable number of our patients.

During follow-up, no patient required hospitalisation for COVID-19 after receiving antiviral therapy. Three patients reported mild adverse events (gastrointestinal upset, headache and dysgeusia) that could be also related to COVID-19. In four cases, comedications had to be temporally discontinued (apixaban, pravastatin, sildenafil and bosentan) due to potential interactions with nirmatrelvir/ritonavir. Interestingly, in two cases (both vaccinated with three mRNA vaccine doses), COVID-19 relapsed within 1 month after nirmatrelvir/ritonavir initiation, both having negative antigen tests after the initial infection. The first was a 44-year-old woman being treated for SLE with rituximab and methotrexate (15 mg/week, subcutaneously) for the last 2 months, who relapsed (fever, positive antigen test) 30 days after the initial diagnosis. The second case was a 57-year-old woman with long-standing RA receiving abatacept and methotrexate (20 mg/week, subcutaneously) for the last month, who relapsed (fever, cough, positive antigen test) 20 days after the initial diagnosis and received remdesivir for 3 days. In both of them, antigen tests became again negative, and their symptomatology resolved uneventfully.

In conclusion, this is the first reported series in SRD patients treated with oral antivirals for COVID-19. Of note, clinical trials so far for both nirmatrelvir/ritonavir and molnupilavir have enrolled unvaccinated patients. Additionally, the effect of these antivirals in newer SARS-CoV-2 variants (like Omicron) has not been extensively studied.[5] These preliminary results show an excellent outcome of oral antivirals in high-risk SRD patients without any safety signals. A limitation of this study, however, is that a comparator arm with SRD patients not treated with antivirals is lacking. The early recurrence of COVID-19 after nirmatrelvir/ritonavir has not been reported in the randomised clinical trial[6] and has only been anecdotally described in the general population (https://emergency.cdc.gov/). Whether treatment with immunosuppressive/immunomodulatory drugs is somehow related requires further study.

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## Guselkumab for treating immune checkpoint inhibitor-induced psoriatic arthritis

Rheumatic immune-related adverse events (irAEs) occur in approximately 10% of patients receiving immune checkpoint inhibitors (ICIs).[1] Here, we report a case of psoriatic arthritis (PsA) that occurred during ICI treatment for lung cancer and was successfully treated with guselkumab.

A 64-year-old man with stage IIIC lung cancer was treated with definitive concurrent chemoradiotherapy followed by six cycles of weekly carboplatin and paclitaxel. He was then started on biweekly durvalumab (antiprogrammed death-ligand 1 antibody) monotherapy. After the sixth cycle of durvalumab administration, he developed shoulder pain, a skin rash, and nail changes. Due to the high possibility of irAEs, durvalumab was discontinued and prednisolone 20 mg/day was started. However, his symptoms continued to worsen; therefore, he was referred to our department of oncorheumatology.

Before starting durvalumab, he had never experienced such skin rashes or nail changes and had no family history of any autoimmune disease. On physical examination, tender entheses on the shoulders, distal interphalangeal joint arthritis of the fingers, and dactylitis of the left fourth toe were observed without any signs of axial involvement. Well-demarcated, scaly, erythematous papules and plaques were present on the trunk, hands and feet. Onycholysis, oil-drop discolouration and nail bed hyperkeratosis were also present (figure 1A–C), leading to the diagnosis of plaque psoriasis and nail psoriasis. His C reactive protein level was normal (4.53 mg/dL), while the rheumatoid factor level was elevated (115 U/mL, normal range 0–10), and the anticitrullinated peptide antibody assay revealed negative results.

The patient was diagnosed with PsA (Composite Psoriatic Disease Activity Index (CPDAI) score 9), for which celecoxib 200 mg/day in combination with calcipotriol/betamethasone dipropionate ointment was started; prednisolone 20 mg/day was continued. However, the symptoms worsened over the next 4 weeks; hence, we introduced guselkumab. His active PsA symptoms improved from week 3 of guselkumab treatment. Therefore, we discontinued celecoxib and tapered prednisolone dosage. Two months after guselkumab initiation, all symptoms except nail psoriasis disappeared, but he developed pulmonary tuberculosis despite a negative interferon-gamma release assay result before starting guselkumab. We terminated guselkumab after two doses (weeks 0 and 4) and administered antituberculosis drugs. After 8 months of guselkumab treatment, prednisolone dosage was reduced to 2 mg/day, and the symptoms of PsA remained absent except some residual toenail psoriasis (CPDAI score 0) (figure 1D–F). Treatment of pulmonary tuberculosis was successful, and there was no recurrence or metastasis of lung cancer. Durvalumab was not rechallenged.

Psoriasis and PsA develop in some patients after ICI initiation.[2] In our patient, PsA developed 4 months after ICI initiation and worsened rapidly thereafter, suggesting its development as an irAE. When using biologic agents for PsA treatment, it is necessary to exercise caution for patients with underlying ICI-treated malignancies. Tumour necrosis factor alpha (TNF-α) inhibitors can increase risk of infection, and interleukin (IL)-17 inhibitors may reduce the antitumour effect of ICIs.[3]

Guselkumab is a specific IL-23 inhibitor used to treat PsA, with a joint efficacy comparable to that of subcutaneous TNF-α and IL-17 inhibitors. It is particularly robust against skin manifestations[4] and is relatively safe without side effects such as serious infections or any malignancy.[5]

Although a similar drug, IL-12/23 inhibitor ustekinumab, was used for refractory irAE colitis,[6] this is the first case of using an IL-23 inhibitor for treating an irAE. While pulmonary tuberculosis developed after guselkumab initiation, the patient had also been receiving prednisolone 15–20 mg/day for 3–4 months, possibly making the steroid a major contributor in the development of pulmonary tuberculosis.

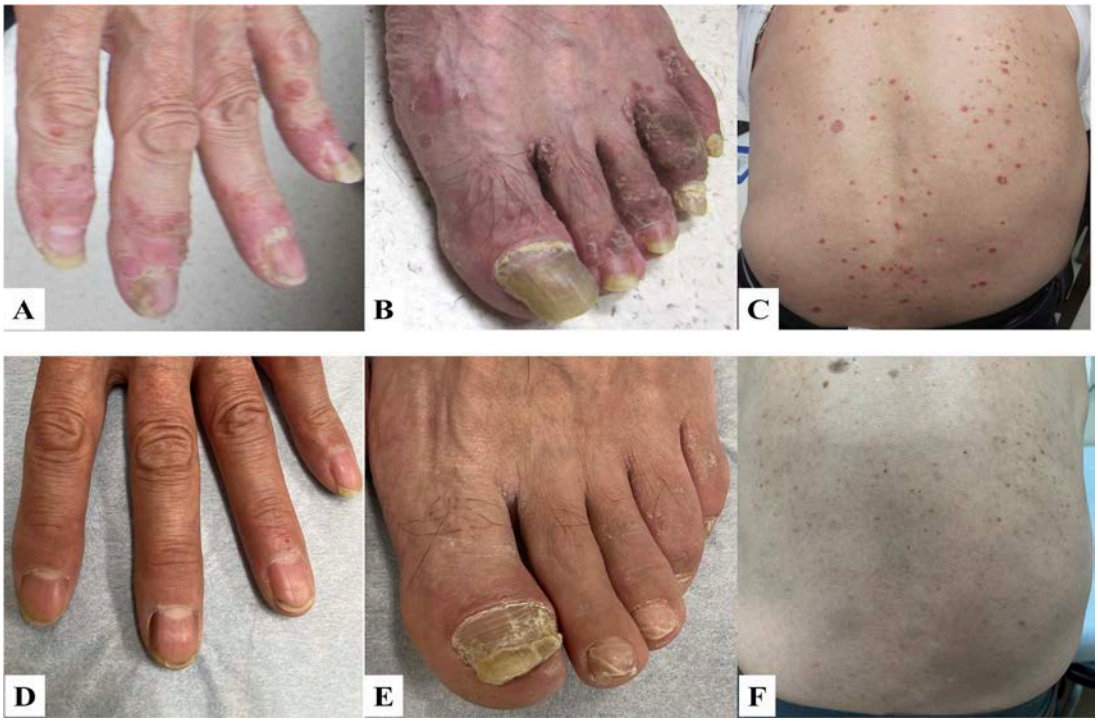


**Figure 1** Photographs before and after guselkumab initiation. (A–C) Before guselkumab initiation; plaque psoriasis of the fingers, toes and back; nail psoriasis and dactylitis of the left fourth toe. (D–F) Eight months after guselkumab initiation. Clinical signs associated with psoriatic arthritis disappeared, with some residual nail psoriasis.

In conclusion, guselkumab is a promising treatment option for ICI-induced PsA because it is effective, relatively safe and unlikely to compromise antitumour efficacy.

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## Genetically proxied IL-6 receptor inhibition and risk of polymyalgia rheumatica

Polymyalgia rheumatica (PMR) is among the commonest inflammatory rheumatic diseases for which the cornerstone of treatment is long-term glucocorticoids.[1] Adverse effects of glucocorticoids in elderly and often comorbid individuals can lead to significant additional morbidity. Steroid-sparing treatment options are limited and remain a major unmet need. Interleukin 6 (IL-6) signalling is thought to play a key role in PMR pathophysiology,

and open-label studies of IL-6 receptor inhibition (IL-6Ri) have shown promising efficacy.[2] Open-label studies are susceptible to bias, while effective blinding may be challenging since IL-6Ri reduces C-reactive protein (CRP), which can unblind participants. Natural variation in the gene that encodes a protein drug target can offer insight into the clinical effects of perturbing that target pharmacologically.[3] We leveraged large population-level data to examine the effect of genetically proxied IL-6Ri on risk of PMR.

To proxy IL-6Ri, we used seven previously identified variants (online supplemental table S1) within or near the *IL6R* gene (±300 kilobases), which encode the receptor of IL-6; these variants were uncorrelated ($r^2$ <0.1) and associated with circulating CRP levels at genome-wide significance ($p<5\times10^{-8}$) from a genome-wide association study of 204 402 European individuals.[4] These instruments were validated through associations with higher circulating IL-6 and soluble IL-6R levels.[4] We used these variants and coefficients to construct a weighted allele score among 408 654 unrelated individuals of white British ancestry from the UK Biobank, a cohort study of ~0.5 million participants who were 37–73 years of age at recruitment, which occurred 2006–2010. We used the ratio method, that is, dividing the variant–outcome association (from logistic regression of the allele score and PMR) by the variant–exposure association (regressing the allele score against CRP). Each model was adjusted for age, sex, recruitment centre and principal components. PMR was defined using International Classification of Diseases (ICD) code (M353), self-report, and/or Read codes (N20. or XE1FJ) in participants with linked primary care data. In the sensitivity analysis, we first repeated the analysis using an allele count of the missense variant rs2228145 (which increases soluble IL-6R levels through proteolytic cleavage of membrane IL-6R) as the sole instrument. Second, we restricted analyses to PMR cases defined by ICD and/or Read codes. We performed colocalisation analysis to examine the potential for genetic confounding. Lastly, we included RA (for which IL-6Ri is an approved therapeutic) as a positive control outcome. Detailed methods are provided in the online supplemental materials.

There were 4285 cases of PMR (3180 ICD, 1073 self-report, 1239 Read). Genetically proxied IL-6Ri was associated with lower risk of PMR (OR 0.74 per 1 mg/L reduction in CRP, 95% CI 0.61 to 0.88; p=0.0008). Sensitivity analysis instrumented using the missense variant rs2228145 showed similar results, as did excluding self-reported PMR (figure 1). The probability of colocalisation conditional on the presence of a causal variant for the outcome was 85%, that is, the results were unlikely attributable to genetic confounding (online supplemental table S2). Genetically proxied IL-6Ri was associated with lower risk of the positive control outcome RA (OR 0.85, 95% CI 0.75 to 0.96; p=0.006)

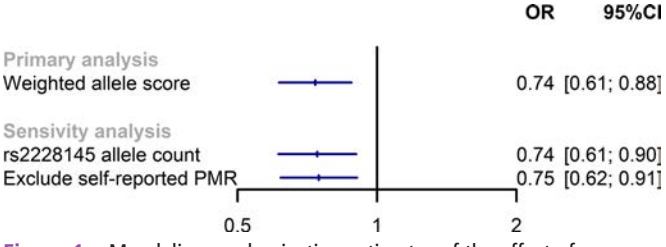


**Figure 1** Mendelian randomisation estimates of the effect of genetically proxied interleukin-6 receptor inhibition on polymyalgia rheumatica.

We provide genetic evidence to support IL-6Ri as a therapeutic target for reducing PMR risk. These findings support results of the recent PMR-SPARE trial, in which 19 patients with new-onset PMR who were randomised to tocilizumab demonstrated superior rates of glucocorticoid-free remission, compared with 17 given placebo.[5] The current results additionally provide insights into the therapeutic potential of IL-6Ri as monotherapy. Furthermore, the PMR-SPARE trial was not powered to examine related or adverse outcomes. Prior MR studies using the same instruments to proxy IL-6Ri showed results consistent with clinical experience (eg, reduced granulocyte count, reduced alkaline phosphatase, increased total cholesterol and infections) but also potential advantages over other steroid-sparing candidates; for example, genetically proxied IL-6Ri was associated with reduced risk of ischaemic heart disease and ischaemic stroke.[4] Although these findings require validation in larger randomised controlled trials (RCTs) in PMR, benefits on cardiovascular risk are appealing in the context of concurrent glucocorticoid treatment. In summary, this genetic investigation supports IL-6Ri as a therapeutic target for PMR, results of which were simultaneously confirmed by an RCT.

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UK Biobank data are available to all bona fide researchers for use in health-related research that is in the public interest. The application procedure is described at www.ukbiobank.ac.uk.

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# Correspondence on 'Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry' by Gianfrancesco *et al*. Compassionate use of tocilizumab in severe COVID-19 with hyperinflammation prior to advent of clinical trials – a real-world district general hospital experience

The coronavirus disease 2019 (COVID-19) has resulted in a global pandemic with multiple casualties. Within the UK, specific groups of patients including those with rheumatic diseases requiring significant immunosuppression were advised to shield from the public to protect themselves from COVID-19 during the heart of the pandemic.[1] In their important paper, Gianfrancesco *et al* found lower rates of hospitalisation in patients with rheumatic diseases with COVID-19 who were taking traditional synthetic and biological disease modifying antirheumatic drugs (DMARDs).[2] With regard to biologic DMARDs, most of their registry patients were taking tumour necrosis factor inhibitors but did also include other therapies including interleukin-6 (IL-6) antagonists.

They also provide an interesting suggestion of the potential benefit of biologic DMARD therapy in COVID-19 patients particularly in cases associated with a hyperinflammatory response. Indeed, it has been recognised that subsets of COVID-19 patients can develop a cytokine storm involving the uncontrolled production of cytokines such as IL-6.[3 4] Moreover, observational studies suggest the potential benefit of IL-6-antagonism using tocilizumab (TOC).[5–7] Internationally, TOC has been used in Italy, China and Ireland.[8–10]

Early during the UK pandemic, there was no access to clinical trials. Moreover, our Trust faced the second highest pressure index in the UK in relation to the number of admissions of COVID-19 patients.[11] Our intensive care unit and general medical inpatient wards had to expand within our hospital to meet the necessary demands of patient care. We also recognised that certain COVID-19 patients developed significant inflammatory responses. Based on this, published observational data elsewhere and the lack of early access to clinical trials, we proposed the compassionate use of TOC in a specific subset of COVID-19 patients.

In March 2020, we identified patients with severe COVID-19 and hyperinflammation. We defined severe COVID-19 as any patient with positive COVID-19 PCR swab and respiratory failure requiring a minimum of 40% oxygen therapy. Hyperinflammation was defined as a ferritin above 500 ng/mL with upgoing trend, and a C-reactive peptide (CRP) above 100 mg/L. The decision to initiate TOC necessitated multidisciplinary discussion between intensivists, pharmacists and both local and tertiary care rheumatologists. This was a novel approach to bedside therapeutic decision-making, reflecting the close collaboration between clinicians in secondary and tertiary care, thereby directly sharing specialist experience and knowledge at the clinical coalface. The TOC treatment regime consisted of two intravenous doses at 8 mg/kg 12 hours apart. We would consider a third dose after 24 hours if there was no significant improvement.

A total of eight patients (seven male, one female) received TOC with doses ranging between 400 and 700 mg. The mean age was 59.4 years (49–81 range) with seven belonging to the Black, Asian and Ethnic Minority (BAME) group. Three patients

**Table 1** Clinical characteristics of COVID-19 patients treated with tocilizumab (TOC)

| Patient | Comorbidities | Organ Failure | Pre-TOC Inflammation Parameters | Post-TOC Inflammation Parameters | Outcome |
|---|---|---|---|---|---|
| 1 | None | Respiratory<br>Cardiovascular | Ferritin 1933 ng/mL<br>CRP 360 mg/L | Ferritin 1759 ng/mL<br>CRP 154 mg/L | Deceased |
| 2* | Asthma<br>Diabetes mellitus<br>Hypertension<br>Obesity | Respiratory<br>Cardiovascular<br>Renal | Ferritin 3504 ng/mL<br>CRP 412 mg/L | N/A | Deceased |
| 3 | Splenectomy<br>Hypertension | Respiratory | Ferritin 24, 203 ng/mL<br>CRP 168 mg/L | Ferritin 3202 ng/mL<br>CRP 40 mg/L | Alive |
| 4 | Diabetes Mellitus<br>Hypertension<br>Chronic Kidney Disease<br>Obesity | Respiratory<br>Renal | Ferritin 1730 ng/mL<br>CRP 355 mg/L | Ferritin 356 ng/mL<br>CRP 113 mg/L | Deceased |
| 5 | Hypertension | Respiratory<br>Renal<br>Cardiovascular | Ferritin 2006 ng/mL<br>CRP 390 mg/L | Ferritin 933 ng/mL<br>CRP 51 mg/L | Deceased |
| 6 | None | Respiratory<br>Cardiovascular | Ferritin 1449 ng/mL<br>CRP 376 mg/L | Ferritin 1353 ng/mL<br>CRP 94 mg/L | Alive |
| 7† | Hypertension | Respiratory<br>Cardiovascular | Ferritin 1386 ng/mL<br>CRP 233 mg/L | Ferritin 1317 ng/mL<br>CRP 75 mg/L | Alive |
| 8 | None | Respiratory<br>Renal<br>Cardiovascular | Ferritin 17, 810 ng/mL<br>CRP 442 mg/L | Ferritin 18, 777 ng/mL<br>CRP 164 mg/L | Deceased |

Organ failure listed is prior to TOC. Post-TOC inflammation parameters are after 72 hours unless otherwise stated.
*Patient only received one dose of tocilizumab as passed away prior to second dose.
†Patient received four doses of tocilizumab due to administrative error.
TOC, tocilizumab; I & V, intubation & ventilated; CRP, C-reactive peptide.

had no comorbidities. Seven patients required intensive care. Three patients improved following TOC administration and were discharged home. Two of these patients received TOC in intensive care within 24 hours of hospital presentation. The third patient avoided intensive care. The five deceased patients were all of BAME ethnicity and died of COVID-19-related complications. They were all in multiorgan failure at the time of TOC administration, receiving it 3–4 days following hospital presentation. All patients except one had improvements in CRP, and six had improvements in ferritin, triglycerides or D-dimer following TOC. Five patients had worsening transaminitis following TOC administration which was of no clinical significance. One patient was readmitted with pyelonephritis, acute kidney injury, ureteric stone and hydronephrosis requiring a ureteric stent and high-dependency care. This patient by administrative error received four doses of TOC at his initial admission. The patient made a full recovery. The clinical characteristics of the eight patients are summarised in table 1.

In this unprecedented time, with limited treatment options available for rapidly deteriorating patients, we explored if IL-6 blockade with TOC may benefit a specific, defined subgroup of patients with evidence of hyperinflammation. One significant difference we note between our practice and published observational studies was the early administration of TOC in the latter. Therefore, we raise the question of whether TOC administration at an earlier disease course prior to the development of non-respiratory organ failure would be a more suitable therapeutic window.

The rapid evolution of conventional randomised controlled clinical trials meant that continuation of our compassionate approach could not be ethically justified or sustained. However, our real-world experience describes a clinical model of how newer therapeutic approaches can be rapidly implemented in the midst of a hitherto unprecedented pandemic. We also encourage clinicians to develop strong links with tertiary care experts so that patients can be channelled into appropriate trials at an earlier disease course. Furthermore, we encourage rheumatologists to continue to record characteristics of rheumatic patients with COVID-19 onto the Global Rheumatology Alliance registry as highlighted by Gianfrancesco *et al*.

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# Comment on 'Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 global rheumatology alliance physician-reported registry' by Gianfrancesco M *et al*

We read with interest the publication on COVID-19 outcomes related to hospitalisation of people with chronic inflammatory rheumatic diseases (CIRD) by Gianfrancesco *et al*.[1] In our centre, we have taken a different approach by contacting 1495 patients with CIRD by telephone to ask for COVID-19 tests and symptoms. A total of 917 patients who agreed to participate (61%) was interviewed between 15 April and 15 June 2020: about 60% women, mean age 54, mean disease duration 12 years. Most had spondyloarthritis (SpA) including psoriatic arthritis (51%), 41% rheumatoid arthritis (RA) and 7% connective tissue diseases (CTD), mainly lupus. In RA, rheumatoid factor was found in 88%, anti-citrullinated protein antibodies (ACPA) in 77% and human leukocyte antigen (HLA) B27 in 73% of patients with axSpA, while 92% with CTD had antinuclear antibodies. Less than half of patients were vaccinated against *pneumococci* (43%) and *influenca* (47%).

The German government started a national shutdown on 22 March 2020. To give some guidance to rheumatologists, the German Society of Rheumatology (DGRh) released recommendations on 29 April 2020.[2] Our survey started about 2 weeks earlier.

The care of our patients with CIRD is largely based on the 'treat to target' approach. Most patients with RA were on cDMARDs with methotrexate (50%), while 47% took glucocorticoids (GC). Patients with CTD were mostly on GC (48%) and hydroxychloroquine (77%). In addition, 63% of patients were on bDMARDs, mostly on antitumour necrosis factor agents (60%), 12% on anti-interleukin 17 agents and on antibodies targeting B-cells. Of interest, about 30% of patients had recently changed medication in a shared decision process, about half due to the pandemic with significantly more patients changing bDMARDs versus cDMARDs.

Only 62 patients from our cohort (6.8%) told to have been tested against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with only 3 (4.8%) being PCR+ (all with mild disease), and 1.4% (out of 352 tested) had anti-SARS-CoV-2 IgG antibodies. The region our hospital is mainly serving is North Rhine-Westphalia with 17.9 million inhabitants. On 19 June 2020, 40 153 reports of confirmed SARS-CoV-2 tests had been registered corresponding to 0.22% of the population.[3] The median age of infected subjects was 49 years with 50% women, 15% were hospitalised and 9% had severe disease. Thus, the infection rates in our region were not as high as in other countries.[4] In our cohort, the cumulative prevalence of SARS-CoV-2 infections corresponds well with the SARS-CoV-2 IgG antibody seroprevalence of 1.42%, which is similar to the reported seroprevalence of 1.6% among healthcare workers in a hospital nearby[5] and consistent with the low rate of infections in our federal state. This seroprevalence indicates a dark figure factor of about 5 that seems to be considerably higher in other regions.[6]

**Table 1** Characteristics of patients related to change of medication

| Item | Total cohort | RA | AxSpA | PsA | CTD | P value |
|---|---|---|---|---|---|---|
| N | 917 | 378 | 292 | 179 | 68 | |
| Changed medication | 292 (31.8) | 139 (36.8) | 84 (28.8) | 61 (34.1) | 8 (11.8) | <0.001 |
| Changed DMARDs | 243 (83.2) | 109 (78.4) | 80 (95.2) | 48 (78.7) | 6 (75.0) | 0.003 |
| Stopped | 41 (16.9) | 18 (16.5) | 9 (11.3) | 13 (27.1) | 1 (16.7) | |
| Net dose reduction | 164 (67.5) | 73 (67.0) | 63 (78.8) | 26 (54.2) | 2 (33.3) | |
| Net dose increase/start of new therapy | 26 (10.7) | 13 (11.9) | 5 (6.3) | 6 (12.5) | 2 (33.3) | |
| No net change or change of drug | 12 (4.9) | 5 (4.6) | 3 (3.8) | 3 (6.3) | 1 (16.7) | |
| Additional GC change | 31 (12.8) | 22 (20.2) | 2 (2.5) | 3 (6.3) | 4 (66.7) | <0.001 |
| Changed GC medication | 80 (27.4) | 52 (37.4) | 6 (7.1) | 16 (26.2) | 6 (75.0) | <0.001 |
| Stopped | 21 (26.2) | 12 (23.1) | 4 (66.7) | 5 (31.3) | 0 | |
| Dose reduction | 27 (33.8) | 21 (40.4) | 0 | 2 (12.5) | 4 (66.7) | |
| Dose increase/start | 32 (40.0) | 19 (36.5) | 2 (33.3) | 9 (56.3) | 2 (33.3) | |
| Time point of change | [71] (N=219) | [42] (N=97) | [8] (N=76) | [19] (N=42) | [4] (N=4) | |
| After 30 April 2020 | 4 (1.8) | 3 (3.1) | 1 (1.3) | 0 | 0 | 0.608 |
| After 15 March 2020 (cumulative) | 144 (65.8) | 71 (73.2) | 47 (61.8) | 25 (59.5) | 1 (25.0) | 0.087 |
| Reason for change | [19] (N=273) | [17] (N=122) | [2] (N=82) | (N=61) | (N=8) | |
| Corona pandemic | 138 (47.3) | 56 (40.3) | 52 (61.9) | 28 (45.9) | 2 (25.0) | 0.009 |
| Activity of rheumatic disease | 63 (21.6) | 31 (22.3) | 10 (11.9) | 17 (27.9) | 5 (62.5) | 0.003 |
| Inactivity of rheumatic disease | 77 (26.4) | 38 (27.3) | 22 (26.2) | 16 (26.2) | 1 (12.5) | 0.835 |
| Other | 66 (22.6) | 30 (21.6) | 19 (22.6) | 15 (24.6) | 2 (25.0) | 0.97 |
| Responsible for change | [11] (N=281) | [8] (N=131) | [1] (N=83) | [2] (N=59) | (N=8) | |
| Patient alone | 29 (10.3) | 11 (8.4) | 9 (10.8) | 8 (13.6) | 1 (12.5) | 0.739 |
| Physician alone | 27 (9.6) | 16 (12.2) | 4 (4.8) | 7 (11.9) | 0 | 0.22 |
| Shared decision patient/physician | 225 (80.1) | 104 (79.4) | 70 (84.3) | 44 (74.6) | 7 (87.5) | 0.498 |
| Using b/ts DMARDs | 182 (78.1) [10] | 78 (74.3) [4] | 69 (87.3) [1] | 34 (73.9) [2] | 1 (33.3) [3] | 0.032 |
| Not using b/ts DMARDs | 36 (15.5) [10] | 18 (17.1) [4] | 7 (8.9) [1] | 9 (19.6) [2] | 2 (66.7) [3] | 0.024 |

Numbers are N (%). Numbers in square brackets indicate the number of missing values and/or unknown state.
CTD, connective tissue diseases; GC, glucocorticoids; PsA, psoriatic arthritis; RA, rheumatoid arthritis.

The prevalence is similar to Veneto in Italy[7] but Spanish patients with CIRD had 1.32-fold higher prevalence of SARS-CoV-2 infections than the reference population.[8] In another study from Northern Italy, 10% of SARS-CoV-2 infected patients with CIRD died.[9] In contrast, from Wuhan where the pandemic started[10] and New York,[11] different outcomes were reported. However, two German patients treated with rituximab had normal IgG levels but a fatal course of COVID-19,[12] and two patients with lymphoma on rituximab developed SARS-CoV-2 viraemia and died.[13] We did not observe problems with rituximab to date. Thus, whether patients with CIRD on immunosuppressants are at risk for SARS-CoV-2 infections is not clear to date.

How did our patients handle the pandemic? Asked about their behaviour, patients told to have been rather careful and more than 90% of patients with CIRD announced to follow the advice not to change therapy because of the pandemic.[14] However, our results tell a different story (table 1).

In the early days of the pandemic, before 30 April 2020, about 30% of our patients had already changed their medication with about 80% reducing DMARDs and about 30% changing GC, and significantly more changed bDMARDs and tsDMARDs as compared with cDMARDs. The majority reduced the dose or even discontinued but some active patients also increased the dose. Importantly, about 80% of patients declared that this was a shared decision-making with their rheumatologist. Currently, we do not know about the outcome of these decisions but follow-ups are planned. The recommendations of DGRh released early[2] may have guided to stop the tendency of reducing medication.

More than 10 million cases of SARS-CoV-2 infections and over 500 000 deaths have been globally reported until 10 July 2020. Our data are—as several others—also not consistent with an increased risk of COVID-19 in patients with CIRD. However, patients may have protected themselves well. A high number of patients changed their medication due to the pandemic, mostly those on biologics. Most patients reduced but some also increased the dose due to disease activity. Although the data are reassuring, caution is still mandatory. The low vaccination rate in patients with CIRD is not acceptable. Timely expert recommendations are important in such a situation.

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# COVID-19 outcomes in patients with systemic autoimmune diseases treated with immunomodulatory drugs

We read with great interest the paper published by Gianfrancesco and colleagues in *Annals of the Rheumatic Diseases* in 2020.[1] They examined demographic and clinical factors associated with COVID-19 hospitalisation status in people with rheumatic disease using 600 cases from 40 countries. In their multivariable model, it was found that prednisone dose ≥10 mg/day (OR: 2.05, 95% CI 1.06 to 3.96) and anti-tumour necrosis factor inhibitor use (OR: 0.40, 95% CI 0.19 to 0.81) were associated with odds of hospitalisation.[1]

Patients with autoimmune diseases (AD) are at an increased risk of infectious diseases due to the effects of the disease on the immune system function, much comorbidity caused by various comorbidities such as kidney and lung damage, diabetes mellitus and hypertension, as well as the chronic use of immunomodulatory drugs.[2 3] Patients treated with immunomodulatory drugs are vulnerable to viral infections,[3 4] and worse prognosis of COVID-19 is probable in patients with ADs[5] that need to be studied. Here, we would like to share our study results that were conducted on patients with ADs treated with immunomodulatory drugs.

In our single-centre retrospective study, charts of patients diagnosed with COVID-19 who were admitted to Imam Reza Hospital and were discharged or deceased were reviewed. Imam Reza Hospital is a referral centre for COVID-19 in the East Azerbaijan province, which is one of the high-risk areas in Iran.

In this centre, patients with symptoms suggestive of COVID-19 who had oxygen saturation lower than 90% were admitted. Diagnosis was made using positive PCR or findings consistent with COVID-19 pneumonia based on chest CT scan and ruling out other causes of pneumonia. Disease outcomes were assessed based on the level of care, the time interval between the onset of symptoms and intubation, duration of intubation, duration of admission in intensive care unit (ICU) and the number of patients who died.

For statistical analysis, we used SPSS V.16 software. Continuous variables with normal distribution were reported as mean±SD and non-normally distributed continuous variables were reported as median (25%–75% IQR). Categorical variables were reported as frequency and percentage. $\chi^2$ and independent samples t-test/Mann-Whitney U test were used to assess differences between groups of patients treated with or without immunomodulatory drugs.

Four hundred and eleven patients who were diagnosed with COVID-19 pneumonia were included in this study. Thirty of these patients had ADs (figure 1). In the immunomodulatory drugs-naïve and treated with immunomodulatory drugs groups 69.9% and 62.5% of patients were PCR positive for COVID-19, respectively (p=0.615). The frequency of some clinical manifestations such as malaise, dyspnoea, myalgia, anosmia and taste loss was significantly higher in patients with ADs treated with immunomodulatory drugs compared with immunomodulatory drugs-naïve patients (p<0.05) (table 1). In addition, lymphopenia was found to be less prevalent in patients treated with immunomodulatory drugs (p=0.015).

No significant differences were observed in the admission level, time interval between the onset of symptoms and intubation, duration of intubation, duration of admission in ICU and number of deceased patients in the two groups (table 1).

Pablos *et al* reported 1.3-fold higher prevalence of hospital PCR+COVID-19 in patients with rheumatic diseases.[6] Grasselli *et al* reported inflammatory diseases and suppression of immune system as the most common comorbidities in patients younger than 40 years with COVID-19 admitted to the ICU.[7]

To the best of our knowledge, no study has been conducted to assess the outcomes of COVID-19 in patients with ADs treated with immunomodulatory drugs in comparison with other patients. Our preliminary findings suggest that the severity and mortality of COVID-19 in patients with ADs treated with immunomodulatory drugs are probably not significantly different from the general population.

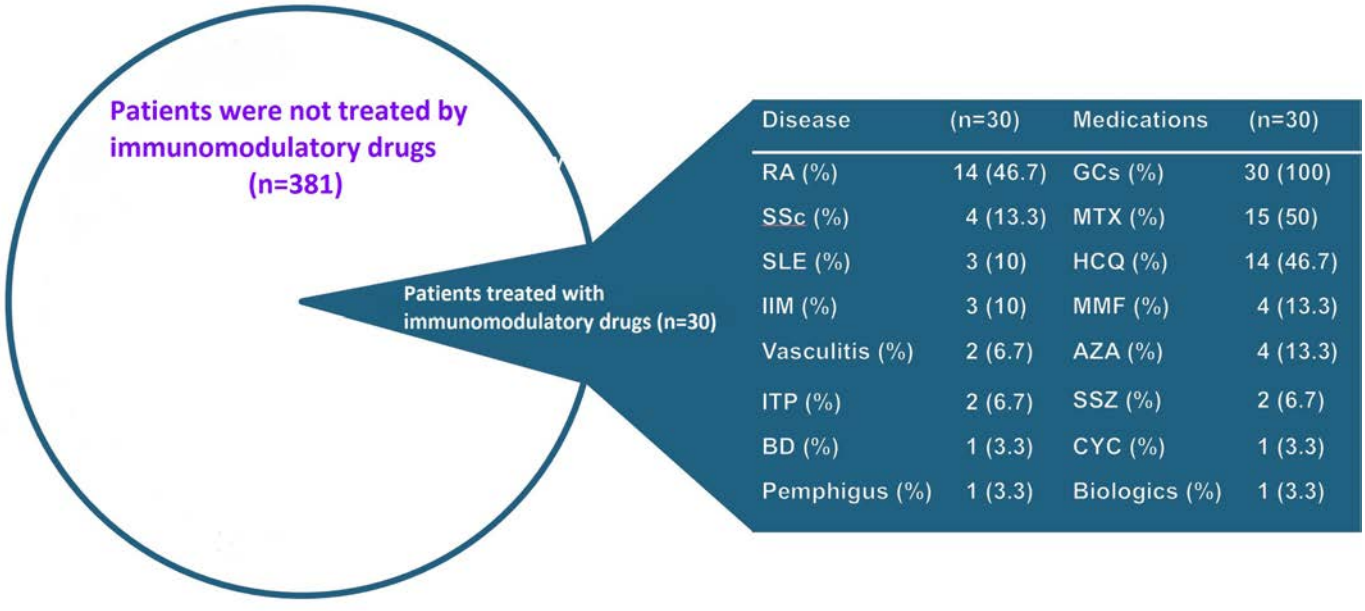


**Figure 1** Patients admitted with diagnosis of COVID-19. AZA, azathioprine; BD, Behcet's disease; CYC, cyclophosphamide; GC, glucocorticoid; HCQ, hydroxychloroquine; IIM, idiopathic inflammatory myopathy; ITP, idiopathic thrombocytopenia; MMF, mycophenolate mofetil; MTX, methotrexate; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; SSZ, sulfasalazine.

**Table 1** Demographic, clinical and laboratory characteristics and outcomes of studied groups

| | Immunomodulatory drugs-naïve patients (n=381) | Patients with ADs treated with immunomodulatory drugs (n=30) | P value |
|---|---|---|---|
| Age (mean±SD), years | 62.6±17.1 | 55.1±13.6 | 0.020 |
| Gender (female/male) | 0.64 | 3.28 | 0.001 |
| Clinical and laboratory manifestations | | | |
| Fever (%) | 85 (22.3) | 8 (26.7) | 0.519 |
| Dyspnoea (%) | 274 (71.9) | 24 (80.0) | 0.036 |
| Cough (%) | 223 (58.57) | 16 (53.3) | 0.129 |
| Myalgia (%) | 124 (32.5) | 17 (56.7) | 0.006 |
| Malaise (%) | 117 (30.7) | 15 (50.0) | 0.001 |
| Nausea/vomiting/ diarrhoea (%) | 63 (16.5) | 7 (23.3) | 0.085 |
| Anorexia (%) | 57 (14.9) | 10 (33.3) | 0.050 |
| Taste loss (%) | 38 (10) | 6 (20.0) | 0.001 |
| Anosmia (%) | 32 (8.4) | 7 (23.3) | 0.001 |
| Sore throat (%) | 30 (7.9) | 4 (13.3) | 0.080 |
| Lymphopenia (%) | 278 (72.9) | 13 (43.3) | 0.015 |
| High C-reactive protein (%) | 328 (86.1) | 21 (70.0) | 0.078 |
| Level of care | | | |
| Admitted in ward (%) | 155 (40.7) | 11 (36.7) | 0.889 |
| Admitted in ICU (%) | 89 (23.4) | 7 (23.3) | |
| Intubated and mechanically ventilated (%) | 137 (36.0) | 12 (40.0) | |
| The time interval from the onset of symptoms to admission, median (IQR) days | 7 (3, 10) | 6 (3.5, 11) | 0.912 |
| The time interval from the onset of symptoms to mechanical ventilations, median (IQR) days | 0 (0, 2) | 2.5 (0, 6.75) | 0.096 |
| Duration of admission in ICU, median (IQR) days | 9 (5, 16) | 12.5 (4, 18) | 0.711 |
| Duration of intubation, median (IQR) days | 4 (2, 10) | 5 (1.5, 13.5) | 0.889 |
| Death (%) | 95 (24.9) | 8 (26.7) | 0.491 |

AD, autoimmune disease; ICU, intensive care unit.;

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# COVID-19 Global Rheumatology Alliance Registry, anti-IL-6 therapy, shared decision-making and patient outcomes. Response to: 'Correspondence on 'Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry' by Gianfrancesco *et al*. Compassionate use of tocilizumab in severe COVID-19 with hyperinflammation prior to advent of clinical trials – a real-world district general hospital experience' by Khan *et al,* 'Comment on 'Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 global rheumatology alliance physician-reported registry' by Gianfrancesco M *et al'* by Andreica *et al* and 'COVID-19 outcomes in patients with systemic autoimmune diseases treated with immunomodulatory drugs' by Ansarin *et al*

We thank Dr Khan and colleagues, Dr Ansarin and colleagues and Dr Andreica and colleagues for their correspondence in relation to our paper.[1–4] The reported experience of Dr Khan and colleagues[2] in using anti-interleukin (IL)-6 therapy in the treatment of COVID-19 is interesting, and although there has been much positive observational data reported on the value of anti-IL-6 therapy in COVID-19, including in this journal,[5] preliminary reports from two randomised trials have not shown benefit.[6 7] When further data are published on anti-IL-6 therapy in treating COVID-19, we can hopefully understand better if this therapy will have a place. Detailed cytokine analysis of 1484 patients with COVID-19 found that IL-6 and tumour necrosis factor (TNF) were independent and significant predictors of poor outcome.[8] Therefore, TNF also seems to play an important role in severe COVID-19 and our paper reported a reduced odds of hospitalisation in those taking anti-TNF therapy compared with those not receiving any disease modifiying anti-rheumatic drug s (DMARDs).[1] Efforts are underway to assess whether anti-TNF treatment is an effective therapy in COVID-19 in the form of the UK-based CATALYST trial (ISRCTN40580903).[9]

Dr Andreica and colleagues report that 80% of patients undertook a shared decision with their physician about the management of their rheumatic disease during the early part of the pandemic.[3] This provides reassurance that patients are choosing to consult their doctor about potential changes to their rheumatic therapy. Changes to therapy may or may not reduce risk of poor outcomes from COVID-19 and may expose the patient to risks of disease flare, new disease complications or Addisonian crisis; therefore, doing this in conjunction with a doctor is the best way to ensure that all the consequences of altering therapy are considered and weighted appropriately.

Dr Ansarin and colleagues report the outcome of 30 patients with autoimmune disease treated with immunomodulatory medications.[4] It is reassuring that their outcomes do not differ from the comparison group of 381 patients also detailed in the report. D'Silva and colleagues also recently examined 52 patients with rheumatic disease and 104 comparison patients and found no difference in hospitalisation, length of stay or death. However, they did find an increased rate of intensive care admission/mechanical ventilation in patients with rheumatic disease.[10] As further data are published, we can further understand the risk profile of patients with rheumatic disease with COVID-19.

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# Similarities and differences between severe COVID-19 pneumonia and anti-MDA-5-positive dermatomyositis-associated rapidly progressive interstitial lung diseases: a challenge for the future

We read with great interest the article by Megremis *et al*,[1] who identified three immunogenic linear epitopes with high sequence identity to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins in patients with dermatomyositis (DM). Speculatively, this finding could indicate that latent exposure to the Coronaviridae family might contribute to musculoskeletal autoimmune disease development.[1] Consequently, SARS-CoV-2 infection might mimic myositis and could also lead to catastrophic results in patients with DM with prior interstitial lung disease (ILD) manifestation.

COVID-19, caused by SARS-CoV-2, has rapidly spread to the whole world. Lung involvement is the hallmark of the disease, significantly associated with worse prognosis and higher mortality. The mechanism leading to acute lung injury in COVID-19 has not yet been completely elucidated. Nevertheless, immune dysfunction and cytokine dysregulation seem to play a pivotal role in this process. It is speculated that SARS-CoV-2 binds to target host cells through ACE 2, which is expressed in the airway and on type 2 pneumocytes in the lung. Subsequently, the virus triggers a storm of innate and adaptive immune response, resulting in the aberrant release of a large number of cytokines, including interleukin (IL)-1, IL-6, IL-10, granulocyte-macrophage colony stimulating factor (GM-CSF), monocyte chemotactic protein-1 and interferon gamma (IFN-γ), called 'cytokine storm' by some.[2] Abnormally high levels of these cytokines/chemokines are considered to lead to acute pulmonary interstitial tissue and alveolar damage, accounting for respiratory failure. The major high-resolution CT (HRCT) features of COVID-19 pneumonia encompass multifocal bilateral peripheral ground glass area associated with subsegmental patchy consolidations, mostly subpleural and predominantly involving the lower lung lobes and posterior segments, similar to ILD. Pathological findings in severe cases of COVID-19 pneumonia showed pneumocyte desquamation and pulmonary oedema with hyaline membrane formation, and interstitial lymphocyte infiltration.[3] Growing evidence, although uncontrolled and anecdotal, supports the prompt use of an anticytokine regimen, including IL-6 inhibitors, IL-1 blockade, GM-CSF receptor antagonist, antitumour necrosis factor alpha (anti-TNF-α), glucocorticoid and Januskinase (JAK) inhibitors, to treat this cytokine storm. If any of these medications are used during the initial time window of pulmonary involvement, they appear to dampen the inflammation, prevent the 'cytokine storm' and improve clinical outcome.[4]

Patients with antimelanoma differentiation-associated gene 5 (MDA-5) antibody-positive DM are prone to present with life-threatening, rapidly progressive ILD (RP-ILD), contributing to significant mortality. The pathogenesis of this clinical scenario is not fully understood. Given the critical role of MDA-5 in the innate immune defence against viruses by driving the production of large amounts of type I IFN, one hypothesis is that viral infection and subsequent immune response induces the manufacture of anti-MDA-5 antibodies, which in turn leads to RP-ILD.[5] The role of anti-MDA-5 antibody in ILD is supported by the finding that anti-MDA-5 concentrations correlate with RP-ILD activity as well as relapse.[6] The macrophage activation markers, ferritin and IL-18, increased in anti-MDA-5-positive RP-ILD and were associated with severity and poor outcomes. In addition, high-titre soluble macrophage-mannose receptor, sCD206, a serum marker for M2 polarisation, correlated with worse prognosis in this subset of patients.[7] These findings imply that macrophages play an important role in the pathogenesis of RP-ILD. Recent studies further revealed that multiple cytokines were involved in the pathogenesis of RP-ILD, such as IFN-α, interferon-inducible protein-10 (IP-10), IL-6, IL-8, IL-10, IL-15 and TNF-α,[8] in many ways similar to the cytokine storm of COVID-19. Organising pneumonia and non-specific interstitial pneumonia are major patterns of DM-ILD, and lower zone consolidation on HRCT correlated with RP-ILD (also similar to COVID-19 lung involvement). Although no standard treatment regimen for anti-MDA-5 antibody-positive DM-RP-ILD has been established, aggressive combination with high-dose glucocorticoid, calcineurin inhibitors and intravenous cyclophosphamide has been proposed. Plasmapheresis has been used for additional effect in intractable disease.[9] Among the many extrapulmonary manifestations of SARS-CoV-2, myalgia is prominent, although only one acute autoimmune myositis case (confirmed by muscle MRI) induced by SARS-CoV-2 has been described.[10]

**Table 1** Comparison of severe COVID-19 pneumonia and anti-MDA-5 antibody-positive DM-RP-ILD

| | Severe COVID-19 pneumonia | Anti-MDA5 antibody-positive DM-RP-ILD |
|---|---|---|
| Clinical behaviour | Acute. | Rapidly progressive. |
| Trigger | SARS-CoV-2. | Possible virus infection. |
| Ethnic and/or geographical differences | All ethnicities are susceptible and vulnerable. | More severe in Asian populations. |
| Typical rash | No. | Gottron's rash, skin ulceration, palmar papule. |
| Muscle involvement | Myalgia and myositis. | Amyopathy or hypomyopathy. |
| Predictive factors | Older age, male sex, comorbidities, high levels of proinflammatory cytokine. | High titre of anti-MAD5 antibody, hyperferritinaemia, high levels of proinflammatory cytokine. |
| Cytokine/chemokine profile | IL-1, IL-2, IL-6, IL-10, IL-18, IP-10, MCP-1, GM-CSF, IFN-γ, TNF-α. | IL-1β, IL-4, IL-6, IL-8, IL-10, IL-18, IP-10, IFN-α, IFN-γ, TNF-α. |
| HRCT pattern | GGO, consolidation, AIP. | NSIP, OP. |
| Treatment | | |
| Glucocorticoid | Possible benefit. | Benefit. |
| Immunosuppressant | No data. | Benefit. |
| Anticytokine therapy | Possible benefit. | Benefit. |
| Antifibrotic agents | No data. | Probable benefit. |
| Plasmapheresis | Possible benefit | Probable benefit. |

AIP, acute interstitial pneumonia; DM-RP-ILD, dermatomyositis-associated rapidly progressive interstitial lung disease; GGO, ground glass opacity; GM-CSF, granulocyte-macrophage colony stimulating factor; HRCT, high-resolution CT; IFN, interferon; IL, interleukin; IP-10, interferon-inducible protein-10; MCP-1, monocyte chemotactic protein-1; MDA-5, melanoma differentiation-associated gene 5; NSIP, non-specific interstitial pneumonia; OP, organising pneumonia; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ;TNF-α, tumour necrosis factor alpha.

The clinical similarities and differences between the two entities are summarised in table 1.

Since the association of muscle inflammation with interstitial pneumonia can be encountered in either COVID-19 or autoimmune myositis, it would be very important to be able to separate these two or three circumstances. One can only speculate as to how to do this, but our suggestions include consideration of the non-pulmonary differences between COVID-19 and DM-RP-ILD. Thus, marked change in creatine kinase (CPK) or swallowing points towards worsening DM. Marked lymphopaenia, anosmia and positive SARS-CoV-2 PCR point to COVID-19. Classic signs of infection such as changing pulmonary infiltrates, marking increase in white blood cell count, urine

with signs of infection, positive cultures and so on would point to infection. This does not mean one cannot have all of COVID-19, worsening DM and infection, but the above may be hints to help rheumatologists in a difficult position.

In summary, we wish to point out that muscles and lungs are two vulnerable target organs attacked by SARS-CoV-2 and that this virus may worsen MDA-5-related DM-ILD. Thus, rheumatologists need to be particularly vigilant in MDA-5-positive patients with DM-ILD and use all laboratory resources plus good clinical judgement to separate overlapping clinical scenarios.

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# Response to: 'Similarities and differences between severe COVID-19 pneumonia and anti-MDA-5 positive dermatomyositis associated rapidly progressive interstitial lung diseases: a challenge for the future' by Wang *et al*

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We thank Wang *et al* for their interest in our letter. In this study, we investigated pre-COVID-19 adult-onset anti-TIF1 autoantibody positive dermatomyositis (DM) patients, and identified antibodies against immunogenic epitopes with high sequence identity to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] We speculated that latent lifetime microbial exposure to the Coronaviridae family might contribute to future musculoskeletal autoimmune disease development.

In their correspondence,[2] Wang *et al* review the features of severe COVID-19 pneumonia and anti-MDA-5 (melanoma differentiation-associated gene 5) autoantibody positive DM presenting with rapidly progressive interstitial lung disease (RP-ILD). The authors compare the clinical signs and symptoms, demographics, likely pathogenesis, cytokine and chemokine profiles and pharmacological treatment of these two clinical presentations. Based on the clinical similarities identified, Wang *et al* suggest 'SARS-CoV-2 infection might mimic myositis and could also lead to catastrophic results in DM patients with prior ILD'. Therefore, it is important to be able to separate the muscle inflammation with interstitial pneumonia encountered in COVID-19 from that of autoimmune myositis.

We concur with the authors' suggestions to be able to distinguish between COVID-19 and autoimmune myositis and the need for clinicians to be vigilant to ensure differential diagnosis and treatment. Tests for myositis-specific autoantibodies should be carried out where clinically indicated, for example, in the presence of a DM rash. It is intriguing that the cytokine storm reaction bears similarities between the two conditions, where aggressive anti-cytokine treatment regimens have been suggested for both. At the same time, we note that autoimmune myositis is a rare disorder, with an incidence of up to 20 per million per year,[3] of whom only a proportion are MDA-5 autoantibody positive. However, as Wang *et al* observe, intriguing geographical differences exist in the prevalence of anti-MDA-5 autoantibodies and RP-ILD in individuals with DM of different ethnicity, particularly in the Japanese population, suggesting that genetic and/or environmental (eg, viruses) factors might play a role. Our experimental approach[1] might therefore be informative in anti-MDA-5 positive DM. Lung disease is also a well-established extra-muscular symptom of other autoimmune myositis subgroups, such as anti-synthetase syndrome.[4] Data on COVID-19 in myositis-specific autoantibody defined patient subgroups has not yet been reported. Notably, recent data from the COVID-19 Global Rheumatology Alliance physician-reported registry shows that the frequency of comorbid lung disease (chronic obstructive pulmonary disease, asthma, interstitial lung disease or other not specified) is higher in hospitalised than non-hospitalised COVID-19 rheumatic disease patients.[5]

Molecular mimicry of SARS-CoV-2 with epitopes of self-proteins is a possible scenario underlying COVID-19 heterogeneity,[6] but to our knowledge has not been experimentally explored. Several recent reports have suggested that SARS-CoV-2 infection could lead to various autoimmune and auto-inflammatory diseases in both children and adults.[6] In our opinion, there is a need to establish registries, epidemiological and molecular studies within both rheumatic disease cohorts and at the population level to explore the long-term sequelae of SARS-CoV-2 infection.

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# Correspondence on 'Interleukin-6 receptor blockade with subcutaneous tocilizumab in severe COVID-19 pneumonia and hyperinflammation: a case–control study' by Potere *et al*

I read with interest the recent case–control study by Potere *et al*, which describes the potential efficacy and safety of tocilizumab in patients with severe COVID-19 pneumonia and hyperinflammation.[1] Since publication of this analysis, new information on anti-inflammatory therapies in COVID-19 has become available. The RECOVERY trial randomised 2104 patients with COVID-19 to receive dexamethasone 6 mg daily or usual care for up to 10 days.[2] In the overall cohort, dexamethasone significantly reduced the incidence of 28-day mortality from 26% to 23%. The benefits of dexamethasone on mortality were greatest in those patients undergoing mechanical ventilation (41% vs 29%) or receiving supplemental oxygen without mechanical ventilation (26% vs 23%) at baseline. A clinical trial of sarilumab, another interleukin-6 receptor blocker, failed to meet its primary and key secondary endpoints.[3] The lack of efficacy in the sarilumab clinical trial contrasts with the reported efficacy in the Potere *et al* study. It would be of great interest to analyse the results of the analysis by Potere *et al* in the context of these findings. In particular, an analysis according to concurrent use of systemic corticosteroids would allow for estimation of whether the benefits in this observational analysis may be attributable to background corticosteroid use.

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# Response to: 'Correspondence on 'Interleukin-6 blockade with subcutaneous tocilizumab in severe COVID-19 pneumonia and hyperinflammation: a case–control study' by Potere *et al*' by Buckley

We thank Dr Buckley[1] for the interest in our report on interleukin-6 receptor blockade with subcutaneous tocilizumab in patients with severe COVID-19 pneumonia receiving supplemental oxygen without mechanical ventilation and hyperinflammation.[2] We acknowledge that the recently published results from the RECOVERY trial showed reduced mortality in patients treated with dexamethasone (6 mg daily up to 10 days) in addition to usual care, with the benefits being greater in critically ill patients receiving mechanical ventilation (41% vs 29%), while considerably reduced in severe patients on supplemental oxygen without mechanical ventilation (18% vs 14%).[3] We also read with interest the results of the CHIC study showing that high-dose intravenous tocilizumab (8 mg/kg body weight, single infusion) may increase the benefits of high-dose methylprednisolone (250 mg on day 1, followed by 80 mg on days 2–5) in patients with severe COVID-19 pneumonia and cytokine storm syndrome requiring supplemental oxygen, mostly through nasal cannulas or mask.[4] It is therefore of utmost importance to determine whether the combination of systemic corticosteroids and another anti-inflammatory drug such as tocilizumab may further improve outcomes in selected patients with COVID-19.

Although answering this clinical question was beyond the purpose of our study, we conducted a post hoc analysis to assess whether the benefits observed with subcutaneous tocilizumab (324 mg, given as two simultaneous 162 mg doses) may be attributable to concurrent corticosteroid treatment in the subgroup of patients with severe COVID-19 and hyperinflammation receiving both drugs.[2] In our study, corticosteroid use was defined as intravenous administration of methylprednisolone 20 mg or 40 mg twice daily for ≥1 day during hospitalisation. Corticosteroids were prescribed after tocilizumab in all patients who received both drugs in the tocilizumab plus standard of care group. A total of 49 (61.25%) patients received corticosteroid treatment, 26 (65.0%) in the tocilizumab plus standard of care group and 23 (57.5%) in the standard of care only group. Overall, corticosteroid therapy was associated with an increased mortality (log-rank Mantel-Cox $\chi^2$ 5.918, p=0.015). When stratifying patients according to corticosteroid use, subcutaneous tocilizumab was associated with reduced mortality in the stratum on corticosteroid therapy (log-rank Mantel-Cox $\chi^2$ 8.445, p=0.004) (figure 1A), and not in patients who did not receive corticosteroids (figure 1B), suggesting that the combination of corticosteroids and tocilizumab may increase the clinical benefits observed in the tocilizumab plus standard of care group.

It is however worth pointing out that corticosteroids were administered at higher dosage in the CHIC study[4] as compared with the RECOVERY[3] trial and our case–control study,[2] and a high-dose corticosteroid administration may exert different clinical effects in patients with COVID-19, including an immunosuppressive rather than anti-inflammatory only activity. Furthermore, in contrast with our study, tocilizumab was administered intravenously at higher dosage in the CHIC study,[4] with a different pharmacokinetic and pharmacodynamic profile and possibly different clinical response. Whether the difference in the route of administration and dosage of tocilizumab is clinically relevant is still unclear, and randomised controlled trials exploring both therapeutic regimens are ongoing.

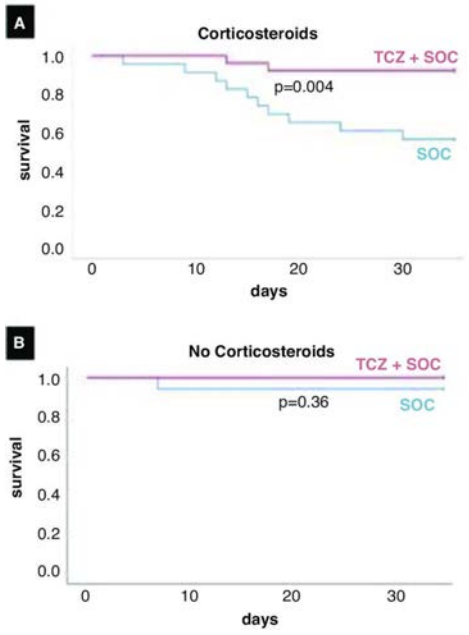


**Figure 1** Survival in patients treated with tocilizumab stratified according to corticosteroid use. Patients receiving tocilizumab (TCZ) on top of standard of care (SOC) were significantly less likely to die than patients treated with SOC only matched for sex, age and severity of illness in the stratum on corticosteroids (A), log-rank Mantel-Cox $\chi^2$ 8.445, p=0.004), and not in patients who did not receive corticosteroids (B).

Notwithstanding the many limitations of our study including the small sample size, the non-random allocation of comparisons, the heterogeneous dose and timing of concomitant corticosteroid treatment, our data, consistently with other reports,[4–6] suggest that subcutaneous tocilizumab may be considered a safe and beneficial therapeutic option for selected subgroup of patients with COVID-19 pneumonia and hyperinflammation in combination with corticosteroids.

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# Correspondence on: 'Interleukin-6 blockade with sarilumab in severe COVID-19 pneumonia with systemic hyperinflammation—an open-label cohort study' by Della-Torre *et al*

We read with deep interest the article by Della-Torre *et al*,[1] which was aimed at assessing the safety and efficacy of interleukin (IL)-6 blockade with sarilumab in patients with severe COVID-19 pneumonia and systemic hyperinflammation. The results indicated that at day 28, overall clinical improvement and mortality were not significantly different between sarilumab and standard of care. Sarilumab was associated with faster recovery in a subset of patients who showed minor lung consolidation at baseline. This conclusion might be of great significance for alleviating the current COVID-19 pandemic.

However, we noticed that most of the patients in this study met the diagnostic criteria for acute respiratory distress syndrome (ARDS,[2] according to the baseline demographic and clinical characteristics of the patients' cohort, there were 22 patients with a $PaO_2/FiO_2$ ratio of 100–200 and 30 patients with a $PaO_2/FiO_2$ ratio <100; the duration of symptoms before enrolment (days) was 7 days; and bilateral pneumonia was radiologically documented, although there were no respiratory distress data and no cardiogenic pulmonary oedema data). The pathological findings of COVID-19 also confirmed that it was associated with ARDS,[3] but most patients did not receive invasive mechanical ventilation (MV) or the authors did not consider MV as the main observation target and, therefore, did not show the data of the use of MV. However, in our opinion, it is important to understand how to reduce the use of MV in patients with severe COVID-19 pneumonia.

ARDS is a life-threatening form of respiratory failure characterised by inflammatory pulmonary oedema resulting in severe hypoxaemia.[4] Non-invasive ventilation (NIV) improves pulmonary hypoventilation as a result of persistent strong spontaneous inspiratory efforts, which simultaneously increases tissue stress. This leads to an increase in pulmonary transvascular pressure, vascular flow and fluid leakage, resulting in rapid deterioration of lung function.[5] The guidelines on the management of critically ill adults with COVID-19 recommend the use of MV in case of ARDS as early as possible; in mechanically ventilated adults with COVID-19 and ARDS, the guidelines recommend the use of low tidal volume (Vt) ventilation (Vt 4–8 mL/kg of predicted body weight),[6] higher PEEP (<15 cm $H_2O$) and prone positioning while minimising oxygen consumption and possible hypercapnia.[7] In mechanically ventilated adults with COVID-19 and ARDS, the guidelines suggest the use of systemic corticosteroids, as opposed to not using corticosteroids.[6]

Furthermore, ventilator-assisted breathing, regardless of whether it is MV or NIV, has an adverse effect on blood pressure. For NIV to work normally, good cooperation is required between the patient and the ventilator, which means that it is difficult for patients with consciousness weakness to receive NIV; however, in the demographic and clinical characteristics baseline of the patients' cohort, there were no such key data, including the patient's basic blood pressure, state of consciousness (Glasgow score), respiratory rate, arterial blood gas (pH, $PaCO_2$, $PaO_2$), peripheral oxygen saturation ($SpO_2$), and the number of patients who changed to MV during the course of treatment. Additionally, the data did not indicate whether the patients had to use vasoactive drugs after using the ventilator.

More details regarding how the authors used NIV to help patients to go through such severe hypoxia will be of great help to COVID-19 epidemic areas, particularly those facing a shortage of medical devices.

We respect the significant contributions of the authors and look forward to the follow-up results of this study.

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# Impact of sarilumab on mechanical ventilation in patients with COVID-19. Response to: 'Correspondence on: 'Interleukin-6 blockade with sarilumab in severe COVID-19 pneumonia with systemic hyperinflammation–an open-label cohort study' by Della-Torre *et al*' by Cheng and Zhang

We thank Dr Zhang and colleagues for appreciating our work and for raising relevant comments on the use of non-invasive ventilation (NIV) and mechanical ventilation (MV) in our population of critical patients.[1] The authors correctly noticed that the vast majority of patients (52/58, 93%) fulfilled the criteria for acute respiratory distress syndrome (ARDS) but they did not undergo MV or glucocorticoids as per the 'Surviving Sepsis Campaign Guidelines for Critically Ill Adults with COVID-19'.[2 3] Indeed, the standard therapeutic approach adopted in our institution during the pandemic wave that struck Northern Italy varied in light of the practical experience that we rapidly accumulated and of the scientific data that progressively became available.

Between 24 February and 22 May 2020, San Raffaele Hospital (Milan, Italy) admitted more than 1000 patients with COVID-19, and our study was carried out in March, when shortcomings of MV in this specific clinical setting were increasingly being reported.[4 5] Mounting evidence on invasively ventilated patients with COVID-19, in fact, was pointing at an extremely high mortality rate, ranging from 86% to 97%, and available guidelines at that time were not recommending early MV in case of ARDS.[3 6 7] We therefore applied continuous positive airway pressure (CPAP) whenever possible outside intensive care units (ICUs) based on its established efficacy in patients with hypoxaemia and on our historical positive experience with this approach.[8–10] In particular, 13 (23%) patients in our cohort ultimately required intubation and MV in ICU, while the remaining patients were managed outside the ICU, either with high-flow oxygen support or with NIV, some of them with pronation.[11] CPAP (positive end-expiratory pressure=10 cm $H_2O$, $FiO_2$=0.6) was introduced when oxygen saturation was <94% despite high-flow oxygen therapy, starting with four daily cycles of 3 hours each and then personalised according to the patient's need. At baseline, no patient had cardiogenic pulmonary oedema or altered state of consciousness. The mean peripheral oxygen saturation was 94% (±3.3), and the mean $PaO_2$ on arterial blood gas was 75 mm Hg (±13.5), with all patients being on respiratory distress while not on oxygen support. No patients reported hypotension either before or after NIV, and none required vasoactive drugs during follow-up observation outside the ICU. MV-free survival at 28 days was similar between patients treated with sarilumab and with standard of care, with a median time to MV of 5 and 3 days, respectively.[1]

Dr Zhang and colleagues also asked about the use of glucocorticoids in our cohort. Indeed, a recent observational Dutch study reported that a short course of high-dose methylprednisolone alone or combined with anti-interleukin (IL)-6 treatment improved survival and reduced the need for MV in hospitalised patients with COVID-19 compared with a retrospective cohort of subjects treated with standard of care.[12] Yet, significantly higher body mass index, incidence of diabetes and requirement of MV at baseline were observed in controls, introducing a major bias in patient selection and outcome interpretation.[12] As far as our experience is concerned, at the time when we conducted our study, clinical evidence did not univocally support corticosteroid treatment for COVID-19-associated pneumonia.[13] As opposed to septic shock, in fact, shock during severe hypoxaemic respiratory failure is often a consequence of increased intrathoracic pressure (during invasive ventilation) impeding cardiac filling, a context where steroid treatment is unlikely to provide a benefit.[13] In addition, available observational data from influenza, SARS-CoV and Middle East respiratory syndrome coronavirus infections suggested increased mortality, impaired viral clearance and complications of corticosteroid therapy in survivors, further arguing against the use of glucocorticoids in critical COVID-19.[13] Considering the aforementioned data, also currently available guidelines recommend against the routine use of systemic corticosteroids for respiratory failure in patients with COVID-19.[3]

This last observation—namely, the lack of efficacy of anti-inflammatory therapy with glucocorticoid in advanced stages of COVID-19—is, indeed, in agreement with the disappointing results obtained in our study as well as in randomised controlled trials with IL-6 blocking agents in patients with severe hyperinflamed COVID-19 pneumonia (http://www.sanofi.com/en/media-room/press-releases/2020/2020-07-02-22-30-00; https://www.roche.com/investors/updates/inv-update-2020-07-29.htm).[1 14] More recent evidence seem to indicate that other immunosuppressive agents might be more effective in this setting and that early administration of anti-inflammatory molecules, such as colchicine or even steroids, might represent the optimal strategy to intercept rampant inflammation before the establishment of irreversible lung damage in COVID-19.[15–17] While awaiting for definitive confirmation by ongoing randomised controlled trials, early domiciliary treatment with anti-inflammatory therapies might be of great help to COVID-19 epidemic areas, particularly those facing a shortage of medical devices, such as South American countries, South Africa and India.

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# Colchicine treatment in community healthcare setting to prevent severe COVID-19

We read with interest the article from Scarsi and colleagues about the efficacy of colchicine in hospitalised patients with severe COVID-19.[1] Colchicine is an 'old drug' originally approved for the treatment of gout and subsequently repositioned in numerous disease settings characterised by systemic inflammation and uncontrolled activation of the innate immune response.[2 3] During the recent outbreak of SARS-CoV-2 infection, uncontrolled release of major proinflammatory cytokines such as interleukin (IL)-1 and IL-6 soon emerged as a major pathological feature of COVID-19 as well as a predictor of patients' morbidity and mortality.[4 5]

Based on this evidence, hospitalised patients with severe COVID-19 are currently being treated with anti-cytokine biological drugs including the IL-1 receptor antagonist anakinra, the anti-granulocyte-macrophage colony-stimulating factor mavrilimumab and the IL-6 receptor blockers tocilizumab and sarilumab.[6–9] Yet, although these targeted approaches have provided encouraging results in preliminary retrospective cohorts, they do not seem to induce a prompt recovery as optimistically expected, likely because administered at a later stage of the disease when irreversible organ damage is already established.[6–10] Hence, if there is any rationale to use anti-inflammatory therapies in COVID-19, timely treatment before the establishment of full-blown systemic inflammation becomes imperative in order to prevent respiratory failure, to relieve pressure on healthcare infrastructures and ultimately to impact disease mortality.

Scarsi *et al* report a 20% improvement in the survival rate at 21 days in patients treated with colchicine compared with the local standard of care.[1] Despite potential caveats related to a more frequent use of glucocorticoids in patients treated with colchicine and to an unusually high overall mortality rate (37%) compared with other international cohorts, the authors provide important evidence on the ability of colchicine to interfere with established COVID-19-related inflammation.[11–14] In this sense, in order to gain clues about the optimal window for therapeutic success, it would have been informative to report the time from symptoms onset to colchicine administration and to correlate it with patient outcome. In a recent experience on domiciliary patients, for instance, we successfully administered colchicine after a median of 8 days of influenza-like symptoms and after 3 to 5 days of spiking fever despite acetaminophen or antibiotic treatment.[15] In our study, colchicine was used in patients with a hyper-inflammatory phenotype clinically characterised by persistent high fever in order to intercept 'cytokine storm' early in its rampant phase, to prevent establishment of lung damage, and to avoid hospitalisation due to COVID-19 progression.[15] Identifying the right therapeutic window where anti-inflammatory treatment might perform better is, indeed, not a trivial concern since early colchicine administration could impair physiological immune response to SARS-CoV-2 while late administration might not be as effective on established acute respiratory distress syndrome. Patients included in the active arm by Scarsi and colleagues, in fact, showed advanced respiratory impairment (mean PaO2/FiO2 ratio of 176.6 mm Hg/%) and their mortality rate was still considerable when compared with other cohorts even if treated with colchicine (16%).[1 11–14] Hence, based on their prime experience, we would be grateful if the authors could share more details about the timing of colchicine administration in their study and insights into the best hypothetical window of opportunity for this promising therapeutic approach.

While Italy is slowly getting out of the pandemic's grip, given its safety profile, widespread use and affordable costs, colchicine may provide an additional therapeutic option in areas where SARS-CoV-2 infection is rapidly spreading and healthcare systems are overwhelmed such as in South America, Africa and India. In the absence of effective antivirals and vaccines for SARS-CoV-2, results of large randomised controlled trials in both inpatient and outpatient settings are eagerly awaited to confirm the use of colchicine in the current and future COVID-19 outbreaks.

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# Anti-inflammatory action of colchicine in hospitalised patients with COVID-19. Response to: 'Colchicine treatment in community healthcare setting to prevent severe COVID-19' by Della-Torre *et al*

We thank Della-Torre *et al* for their interest on our report on the retrospective, case-control observational study with colchicine in patients hospitalised for severe COVID-19,[1] and for rising the really crucial issue of the timing of the therapeutic intervention with anti-inflammatory therapies in this disease.[2]

Our observations should be interpreted in the scenario of the uncontrolled epidemic that, during March and April 2020, overwhelmed the health system in Lombardy, Italy, with rapid shortage of intensive care unit beds. As pointed out by the authors in other papers, after this period, the severity of the COVID-19 progressively decreased, in parallel with the exhaustion of the epidemic.[3 4] The COVID-19 related mortality observed in our study (27.5% in the overall cohort of 262 consecutive cases; 36.4% in the standard of care group, and 15.8% in patients treated with colchicine), although much higher than that observed in the previous first reports from China, was very similar to those reported by the group of Della-Torre himself[4–8] (for a comment: see[9]) and by others[10 11] who described patients hospitalised for COVID-19 in Lombardy during this period of time, and cannot therefore be considered unexpected.

The intervals (mean (SD)) between the onset of respiratory symptoms (cough and/or dyspnoea), or of spiking fever, and the start of therapy with colchicine in our patients were of 7 (5) and 7 (6) days, respectively. Notably, the interval was not shorter in patients who survived after treatment, as compared with those who died (respiratory symptoms: 7 (5) vs 8 (4) ; p=0.3; fever: 8 (6) vs 6 (6) : p=0.3, respectively).

In their interesting study, Della-Torre *et al* reported the efficacy of colchicine treatment in nine domiciliary patients with COVID-19, in which this drug was started after a shorter interval of symptoms (3–5 days of fever)[12]; they observed rapid defervescence within 3 days in all nine patients, suggesting that the drug might be effective in dampening the rise of the inflammatory response in its first phases. Our experience in hospitalised patients (table 1) might support this hypothesis. In fact, we observed a marked decrease of the C-reactive protein (CRP) serum levels, and an improvement of the $PaO_2/FiO_2$ ratio after 6 days of treatment with colchicine, whereas in patients treated with standard of care only, the CRP remained highly elevated and $PaO_2/FiO_2$ ratio worsened. A trend for the reduction of serum ferritin was also observed in the colchicine group, and not in the control group. The longer half-life of ferritin (30 hours)[13] might account for the less clear evidence of this results.

The rationale for, and the potential advantages of the use of colchicine in COVID-19 were recently elucidated by others and us.[14 15] These few first observational studies seem to lend support to this approach. We agree that the use in the settings of outpatients appears very promising. Only controlled randomised trial will demonstrate the real utility of colchicine in the care of COVID-19, and the optimal time of therapeutic intervention.

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**Table 1** Comparison of clinical and laboratory features at baseline and after 6 days of therapy in patients treated with standard-of-care (SoC) or colchicine plus (+) SoC

| | SoC | | | Colchicine + SoC | | |
|---|---|---|---|---|---|---|
| Features | Day 0 | Day 6 | P value* | Day 0 | Day 6 | P value* |
| C-reactive protein (mg/L) | 112 (83) | 114 (100) | 0.75 | 159 (53) | 42 (53) | <0.0001 |
| Ferritin (ng/mL) | 1129 (1105) | 1313 (974) | 0.76 | 1987 (1983) | 1185 (1011) | 0.36 |
| Neutrophil count (cell/µL) | 5844 (3786) | 7428 (2875) | 0.51 | 6859 (4070) | 7665 (3674) | 0.20 |
| Lymphocyte count (cell/µL) | 1016 (660) | 883 (498) | 0.92 | 921 (427) | 983 (406) | 0.21 |
| $PaO_2/FiO_2$ (mm Hg/%) | 245 (106) | 215 (128) | 0.04 | 177 (81) | 201 (103) | 0.005 |

Data are expressed as the mean (SD).
*Wilcoxon signed-rank test.

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# Adherence to medication in patients with rheumatic diseases during COVID-19 pandemic

With great interest, we read the Pineda-Sic *et al*'s report on treatment adherence behaviours in rheumatic diseases during COVID-19 pandemic in Latin America.[1] They reported that 15.1% of patients with rheumatic disease suspend their medications during COVID-19 crisis.[1] Lack of availability (48%) and fear of the immunosuppressive effect of medications (25%) were the most common reasons. To address medication non-adherence in our population, we conducted a study about medication adherence in patients with rheumatic diseases in the East Azarbaijan province, which is one of the provinces of Iran with a high prevalence of COVID-19. The study was conducted in accordance with the Helsinki humanity research declaration (2008). For a period of 2 weeks from 10 to 24 July 2020, information about adherence to medication behaviours of patients after COVID-19 outbreak was obtained by telephone interview in patients with various rheumatic diseases treated with non-steroidal anti-inflammatory drugs (NSAIDs), colchicine, glucocorticoids, synthetic disease-modifying antirheumatic drugs (DMARDs) and biologic DMARDs (bDMARDs). Patients under the age of 16, patients on remission who did not take medication, patients who refused to answer the questions and patients who did not respond to three phone calls were excluded. We defined non-adherence as ≥20% change in the dose or frequency of the mentioned medications.[2]

After a telephone interview with 1324 patients with various rheumatic diseases, 591 females and 267 males with a mean age of 48.8±13.4 and median (IQR) disease duration of 5 (2, 10) years were enrolled in this study (table 1). Non-adherence was observed in 56 (6.5%) patients after the COVID-19 outbreak. Thirty-nine (6.6%) females and 17 (6.4%) males were non-adherent (p=0.448). Mean age of adherent and non-adherent patients was 49.3±13.6 and 45.3±13.4, respectively (p=0.095). Complete discontinuation of medications was the most common pattern of non-adherence (table 1). Fear of the immunosuppressive effects of medications was the most common reason for medication non-adherence (table 1). bDMARDs, NSAIDs and methotrexate were the medications that patients had the highest percentage of non-adherence to. Non-adherence in patients with seronegative spondyloarthritis was more common than other groups of diseases. The main reason was the higher rate of treatment with bDMARDs in this group of patients in our clinic. Non-adherence leads to exacerbation of symptoms in 9.6% of patients. COVID-19 was developed in 7 (0.8%) patients.

The data from this study showed that medication non-adherence was not common within 6 months after the issue of COVID-19 is widely discussed in the media. In agreement with our study, Schmeiser *et al* reported 10% non-adherence in the patients receiving antirheumatic medications.[3] Fragoulis *et al* reported non-adherence to medications in 14.6% of patients with rheumatic diseases in Greece.[4] Lack of resources/shortage of drug (3.8%), symptoms suggestive of COVID-19 (2.6%)

**Table 1** Demographic and non-adherence characteristics of patients with rheumatic diseases (n=858)

| | | | Pattern of non-adherence | | | Aetiology of non-adherence | | |
|---|---|---|---|---|---|---|---|---|
| | n | Non-adherence (%) | Dose reduction or increase in frequency (%) | Irregular consumption (%) | Complete discontinuation (%) | Fear of the IS effect of medications (%) | Fear of the referring to clinics and hospitals (%) | Symptoms suggestive of COVID-19 (%) |
| Total number of patients | 858 | 56 (6.5) | 17 (30.4) | 6 (10.7) | 33 (58.9) | 35 (62.5) | 5 (8.9) | 16 (28.5) |
| Diseases | | | | | | | | |
| RA (%) | 396 (46.2) | 11 (2.8) | 1 (9.1) | 1 (9.1) | 9 (81.8) | 4 (36.4) | 1 (9.1) | 6 (54.5) |
| SpA (%) | 139 (16.1) | 23 (16.5) | 7 (30.4) | 2 (8.7) | 14 (60.9) | 12 (52.2) | 3 (13) | 8 (34.8) |
| SLE and APS (%) | 70 (8.2) | 2 (2.9) | 1 (50) | 0 | 1 (50) | 2 (100) | 0 | 0 |
| BD (%) | 64 (7.5) | 3 (4.7) | 0 | 1 (33.3) | 2 (66.7) | 3 (100) | 0 | 0 |
| Vasculitis (%) | 53 (6.2) | 1 (1.9) | 1 (100) | 0 | 0 | 1 (100) | 0 | 0 |
| UIA (%) | 51 (5.9) | 8 (15.7) | 2 (25) | 1 (12.5) | 5 (62.6) | 6 (75) | 1 (12.5) | 1 (12.5) |
| IIM, SSc, SS and others (%) | 44 (5.1) | 4 (9.1) | 3 (75) | 0 | 1 (25) | 3 (75) | 0 | 1 (25) |
| Others (%) | 41 (4.8) | 4 (9.8) | 2 (50) | 1 (25) | 1 (25) | 4 (100) | 0 | 0 |
| Medications | | | | | | | | |
| NSAIDs (%) | 92 (10.7) | 12 (13) | 4 (33.3) | 3 (25) | 5 (41.7) | 6 (50) | 2 (16.7) | 4 (33.3) |
| Colchicine (%) | 7 (0.8) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GCs (%) | 469 (54.7) | 38 (8.1) | 10 (26.3) | 3 (7.9) | 25 (65.8) | 23 (60.5) | 2 (5.3) | 13 (34.2) |
| Hydroxychloroquine (%) | 254 (36.7) | 12 (4.7) | 2 (16.7) | 2 (16.7) | 8 (66.7) | 8 (66.7) | 2 (16.7) | 2 (16.7) |
| Sulfasalazine (%) | 72 (8.4) | 4 (5.6) | 0 | 2 (50) | 2 (50) | 4 (100) | 0 | 0 |
| Methotrexate (%) | 327 (38.1) | 33 (10.1) | 5 (15.2) | 0 | 28 (84.8) | 27 (81.8) | 2 (6.1) | 4 (12.1) |
| Leflunomide (%) | 19 (2.2) | 1 (5.3) | 0 | 0 | 1 (100) | 1 (100) | 0 | 0 |
| Azathioprine (%) | 44 (5.1) | 2 (9.1) | 2 (100) | 0 | 0 | 2 (100) | 0 | 0 |
| Calcineurin inhibitors (%) | 11 (1.3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mycophenolate mofetil (%) | 42 (4.9) | 4 (9.5) | 0 | 0 | 4 (100) | 4 (100) | 0 | 0 |
| Cyclophosphamide (%) | 13 (1.5) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| bDMARDs (%) | 82 (9.6) | 31 (37.8) | 5 (16.1) | 0 | 26 (83.9) | 13 (41.9) | 6 (19.4) | 12 (38.7) |

APS, antiphospholipid syndrome; BD, Behcet's disease; bDMARDs, biologic disease-modifying antirheumatic drugs; GCs, glucocorticoids; IIM, idiopathic inflammatory myopathies; IS, immunosuppressive; NSAIDs, non-steroidal anti-inflammatory drugs; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SpA, seronegative spondyloarthritis; SS, Sjogren's syndrome; SSc, systemic sclerosis; UIA, undifferentiated inflammatory arthritis.

and fear of immunosuppressive effects of medications (2.2%) were the main reasons for non-adherence. However, it should be noted that this pandemic may last until the end of the year and possibly longer, and with cross-sectional studies, it is not possible to give a definitive opinion on the overall impact of the COVID-19 on the medication adherence of patients with rheumatic diseases for a longer period of time.

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# Education and treatment adherence during the COVID-19 pandemic. Response to: 'Adherence to medication in patients with rheumatic diseases during COVID-19 pandemic' by Khabbazi *et al*

With great interest, we read the study of Dr Khabbazi *et al*[1] regarding treatment adherence in patients with inflammatory rheumatic diseases during the COVID-19 pandemic in the East Azarbaijan province of Iran. They conducted telephone interviews to 1324 patients and inquired about treatment adherence behaviours during 2 weeks from July 2020. Of the 858 patients included in the final analysis, non-adherence was reported by 6.5% of the patients (defined by the group as ≥20% change in the dose or frequency of medications). In accordance to previous studies,[2] this work demonstrates that a small percentage of patients were non-adherent to their treatment and with a lower frequency than the one reported in our Latin American sample population (15.1%).[3] The principal pattern of non-adherence was the complete discontinuation of medications (58.9%), and the most common reason (62.5%, n=35) was the fear of the immunosuppressive effects of therapy.

Treatment adherence in rheumatic diseases encompasses a complex relationship between patients, healthcare team/system, community and economy.[4] The COVID-19 pandemic has importantly impacted all of factors making treatment adherence during the current times a difficult challenge. While cross-sectional studies are limited to draw solid conclusions or design adequate strategies, they provide an important general overview of the impact of COVID-19 and adherence in rheumatic diseases in different populations. The evaluation of medication persistence and longitudinal evaluation are necessary to determine the real impact of COVID-19 on adherence behaviours. Nonetheless, strategies to diminish non-adherence should not wait for the evidence to accumulate. Education regarding the relationship between medications, rheumatic diseases and COVID-19 are key to improve adherence and dissipate patients' fear and unfounded beliefs. Educational strategies should be promptly established worldwide to possibly limit unnecessary morbidity and mortality due to medication non-adherence.

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# High dosage of methylprednisolone as a rescue, second-line treatment in COVID-19 patients who failed to respond to tocilizumab

We read with interest the article by Ramiro *et al*[1] about a cohort of patients affected by severe COVID-19 pneumonia. The authors evidenced the efficacy of 5 days of methylprednisolone (MP) (a single bolus of 250 mg, followed by 80 mg on days 2–5) plus, in case of insufficient response, tocilizumab (TCZ) 8 mg/kg, in reducing mortality and preventing invasive ventilation (IV).

As the massive lung damage during COVID-19 pneumonia is thought to be caused by an aberrant inflammatory response mediated by a massive release of inflammatory cytokines and chemokines, the use of biological immunosuppressants has been widely proposed. The rationale supporting their use is not only an antiviral effect,[2] but the selective anti-inflammatory role and the capability of interrupting the cytokine cascade eventually responsible for lung failure.

TCZ, a monoclonal antibody directed against interleukin 6 (IL-6) receptor, was the first biological drug administered in a Chinese cohort of patients. Despite preliminary promising data, recent reviews and meta-analysis did not find statistically significant differences, in terms of mortality, intensive care unit (ICU) admission and requiring of IV, between patients treated with TCZ and the control group.[3 4] Moreover, to our knowledge, no paper has evaluated the possibility of second-line treatment after a lack of response to TCZ.

We evaluated five patients affected by moderate to severe COVID-19 pneumonia, who failed to respond to azithromycin, hydroxychloroquine and two doses of TCZ. When admitted to the hospital, they all had chest X-rays evidence of bilateral interstitial pneumonia with diffuse consolidations. All patients had fever, cough and dyspnoea, while one reported also diarrhoea. Symptoms dated from 1 to 10 days before hospitalisation.

Blood examinations revealed elevated inflammatory markers, D-dimer, fibrinogen and ferritin and lymphopenia. All subjects required ventilatory support, ranging from venti-mask to IV.

In all five patients, hydroxychloroquine and azithromycin were immediately administered at diagnosis, whereas intravenous TCZ, 8 mg/kg, within 72 hours from hospitalisation, and then repeated after 24 hours. In two patients, TCZ was administered in ICU. None of them reported substantial benefit after anti-IL-6 treatment and one patient required ICU admission and IV.

From 3 to 5 days after the first administration of TCZ, all subjects were treated with intravenous MP 1.5 mg/kg, slowly tapered after 5 days. All five patients evidenced a prompt and remarkable improvement: within 7 days, all three subjects in ICU did not require IV anymore and were awakened (table 1).

Steroid therapy during acute respiratory distress syndrome (ARDS) is still a matter of debate, and the immunosuppressive effect often represents an obstacle for its administration in fragile patients. Nevertheless, a growing body of evidence supports its use in this condition, particularly in compromised patients.[5]

If the rationale of the use of TCZ and other biological drugs is the immunomodulation of the exaggerated immune response leading to ARDS, then glucocorticoids (GCs) s should not be rapidly neglected as an obsolete tool: immunosuppressive role of steroids is wide and embrace neutrophils, lymphocytes, macrophages and monocytes.[6] The aspects themselves which do not make GCs suitable for a chronic condition (lack of specificity, as well as the long-term side effects) may be the point of strength in such a hyperacute condition. As a matter of example, if a targeted and superselective action is the mainstay of the long-term treatment of any chronic inflammatory disease, no physician, in the clinical practice, treats a life-threatening condition with biological drugs only. Boluses of intravenous GCs and intra-articular injections are still recommended in case of severe autoimmune disease flares, as well as in many inflammatory conditions affecting upper and lower airways. In contrast, biological drugs, despite selective and with a good safety profile, have often a not negligible latency time.

Moreover, ARDS is mediated by a large number of ILs and growth factors: the selective inhibition of just one of them may be not sufficient to halt the cytokine cascade triggered by viral invasion. Finally, GCs raise concerns for their long-term administration, while their side effects are considerably minor in such a short treatment.

Our data, although limited by the small sample, confirm the evidence reported by Ramiro *et al*[1] about a possible synergic role of TCZ and MP in limiting the exaggerating autoimmune response leading to ARDS. The added value of our experience is that MP could be used also as a rescue therapy after TCZ administration and not only before. No significant differences emerge from Ramiro's shorter schedule, preceded by a small bolus, and ours, but we recommend an adequate dosage of GCs in order to fully take advantage of its action on nuclear gene transcription.

**Table 1** Patients features

| | |
|---|---|
| Males/Females | 5/0 |
| Mean age (SD) | 54 (±6.69) |
| Comorbidities | Obesity (1),previous colorectal cancer (1), hemiplegia (1), none (2) |
| Mean P/F at TCZ administration | 176.2 (±42.97) |
| Ventilation support at TCZ administration | VM (3), IV(2) |
| Mean P/F at MP administration | 204.4 (±29.97) |
| Ventilation support at MP administration | IV (3), VM (2) |
| Mean P/F 7 days after MP administration | 327.8 (±59.86) * |
| Ventilation support 7 days after MP administration | VM (4), AA (1) |

*P<0.01.
AA, ambient air; IV, invasive ventilation; MP, methylprednisolone; P/F, $PaO_2/FiO_2$ ratio; TCZ, tocilizumab; VM, ventimask.

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# Response to: 'High dosage of Methylprednisolone as a rescue, second-line treatment in COVID-19 patients who failed to respond to Tocilizumab' by Conticini *et al*

Conticini *et al* share with us their positive experience with immunosuppressive treatment in patients with severe COVID-19 pneumonia.[1] From the description we infer that the described patients suffered from COVID-19-associated cytokine storm syndrome (CSS). We would like to thank our colleagues for sharing their experience which aligns with ours[2] and for their insightful comments.

In our COVID-19 High-intensity Immunosuppression in Cytokine storm syndrome (CHIC) study we have used an immunosuppressive strategy composed by glucocorticoids in first-line treatment, followed, in case of insufficient response, by tocilizumab. Conticini *et al* have in turn used glucocorticoids in patients with insufficient response to tocilizumab.[1]

We share thus the experience of a positive effect of immunosuppressive treatment for COVID-19-associated CSS. The exact contribution of each specific part of the immunosuppressive strategy to the positive outcomes in patients with COVID-19-associated CSS is at the moment difficult to disentangle. The positive effect of glucocorticoids has also been recently shown in the RECOVERY trial.[3] The effect of tocilizumab is still under research with some reports of positive and negative results from trials, publications still have to follow.[4 5] Of note, the negative results are from a trial with unselected COVID-19 patients, meaning not specifically patients with CSS. We believe that the patient selection is crucial and the rationale for immunosuppressive treatment applies in patients with CSS and not so much in patients without CSS. Conticini *et al* describe several of the advantages of glucocorticoids as their wide spectrum of action and the parallel made with other life-threatening inflammatory conditions treated with glucocorticoids in the acute phase and only eventually later with other more selective cytokine inhibitors. Additionally, the safety of glucocorticoids, particularly in short-term use, their wide availability and low cost, make them an attractive first-line treatment for COVID-19-associated CSS. Still, future studies and ideally trials should inform us on the best immunosuppressive strategy for these patients. Head-to-head trials with different immunosuppressive strategies are, in our opinion, a next logic and relevant step. Nevertheless, early identification and intervention for patients with CSS ('window of opportunity hypothesis') may play a relevant role next to the selection of the immunosuppressive strategy.

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# Tocilizumab for the treatment of polyarteritis nodosa: a systematic literature review. Correspondence on 'Tofacitinib for polyarteritis nodosa: a tailored therapy' by Rimar *et al*

We read the paper by Rimar *et al*[1] in your journal with great interest. They reported the case with refractory polyarteritis nodosa treated with tofacitinib, a janus kinase inhibitor, successfully. As shown in their paper, recent advances in the era of biologic agents have improved the management of difficult-to-treat cases dramatically. Considering that tofacitinib blocks interleukin (IL)-6-mediated signalling pathway through inhibiting janus kinase 1, inhibiting IL-6 cascade may also be effective in polyarteritis nodosa. In this regard, tocilizumab, a biologic agent targeting IL-6 receptor, has shown its efficacy in a variety of diseases such as rheumatoid arthritis, adult-onset Still's disease, large-vessel vasculitis and Behcet's disease.[2–5] Although the precise pathogenesis of polyarteritis nodosa remains unclear, serum IL-6 levels correlate with disease severity, suggesting the involvement of IL-6 in the disease process.[6] Therefore, we assume that tocilizumab may benefit polyarteritis nodosa as a therapeutic option.

To investigate the effectiveness and safety profile of tocilizumab in patients with polyarteritis nodosa, we performed a systematic literature review from the inception dates until 23 July 2020. We used the PubMed database to identify all English publications using the Medical Subject Heading 'polyarteritis nodosa' and 'tocilizumab' and identified 13 potentially relevant articles. Among them, seven were excluded due to the following: four reviews, two duplicates and one non-related topic. Eventually, a total of 11 cases with polyarteritis nodosa treated with tocilizumab were identified from six articles (table 1).[7–12] The median age of the cases was 35 years old (range: 3–70), with equal sex distribution. The median disease duration at tocilizumab treatment was 38 months (range: 3–120). Clinical symptoms varied through the cases (online supplementary table 1). All patients showed high levels of serum C reactive protein (median, 19.7 mg/dL). As shown in table 1, the reasons for initiating tocilizumab were the following: nine cases with refractory to and/or relapsing clinical course by prior immunosuppressive treatments such as cyclophosphamide (n=6), methotrexate (n=4), mycophenolate mofetil (n=2), azathioprine (n=2), tacrolimus (n=1), anti-tumour necrosis factor agents (n=2), rituximab (n=1) and anakinra (n=1). In the other two cases, tocilizumab was initiated as a primary induction therapy. Tocilizumab was used as the intravenous administration at a dose of 8 mg/kg every 4 weeks in seven cases and every 2 weeks in one case, at a dose of 10 mg/kg every 4 weeks in one case and subcutaneous administration at a dose of 162 mg weekly in two cases. In seven cases, tocilizumab was used in combination with high-dose glucocorticoids (table 1). The median observation period after tocilizumab treatment was 12 months (range: 6–37). In all cases, tocilizumab rapidly improved clinical manifestations, mostly within a week, and glucocorticoids could be successfully tapered. All cases achieved asymptomatic condition at last visit. Glucocorticoids were completely stopped in three cases, while eight cases were receiving only low dose (≤5 mg/day) at last visit (table 1). No new safety signal or adverse event was reported.

As this literature review was based on case reports, in which positive results are inclined to be published, potential publication bias can exist. Further, the number of case reports was small due to the rarity of the disease. Nevertheless, tocilizumab is effective in cases of refractory/relapsing polyarteritis nodosa and showed its glucocorticoid-sparing effect. It could even achieve glucocorticoid-free in some cases. Generally, the prognosis of polyarteritis nodosa is poor, showing the 5-year survival rate as 13% if untreated and as 80% even if treated.[13] Our study along with the one by Rimar *et al* would shed light on the management of this rare disease by biologic agents to improve their prognosis. Future prospective randomised controlled trials are desired to confirm our results.

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**Table 1** Characteristics and outcome of 11 patients with polyarteritis nodosa treated with tocilizumab

| No | Age (years) | Sex | Disease duration (months) | Treatments before TCZ | CRP (mg/dL) | The reason for TCZ | Dose/frequency | Concomitant treatment | Observation (months) | PSL at last visit |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 11 | F | 43 | GC, AZA, MMF, TAC, CyA, IvIg, ETN, IFX, ADA | Elevated | R | 8 mg/kg IV every 2 weeks | PSL, MMF | 7 | Off |
| 2 | 23 | M | 38 | GC, CYC, MTX, RTX, ANA, IvIg | 29.1 | R | 8 mg/kg IV every 4 weeks | PSL 80 mg/day | 37 | 4 mg/day |
| 3 | 24 | M | NA | GC, CYC, IvIg | 29.8 | R | 8 mg/kg IV every 4 weeks | mPSL 250 mg IV | 11 | 5 mg/day |
| 4 | 63 | F | NA | GC | 17.4 | P | 162 mg SC every week | PSL 50 mg/day | 6 | 5 mg/day |
| 5 | 70 | F | NA | GC, MTX | 9.3 | R | 8 mg/kg IV every 4 weeks | mPSL 500 mg IV | 13 | 5 mg/day |
| 6 | 67 | M | 6 | GC, CYC | 2.03 | R | 162 mg SC every week | PSL 16 mg/day, MTX | 15 | 4 mg/day |
| 7 | 39 | F | 120 | GC, CYC, MTX, MMF, IFX | 5.9–12.6 | R | 8 mg/kg IV every 4 weeks | PSL 50 mg/day | 12 | 5 mg/day |
| 8 | 52 | F | 96 | GC, CYC, MTX, AZA, dapsone | Not mentioned | R | 8 mg/kg IV every 4 weeks | PSL 35 mg/day | 12 | 5 mg/day |
| 9 | 35 | M | 3 | GC, IvIg | 39.3 | R | 8 mg/kg IV every 4 weeks | PSL 60 mg/day | 10 | Off |
| 10 | 33 | M | NA | GC, CYC | 16.9 | R | 8 mg/kg IV every 4 weeks | PSL 4 mg/day | 50 | Off |
| 11 | 3 | M | 9 | None | 21.9 | P | 10 mg/kg IV every 4 weeks | PSL 1 mg/kg/day, CYC | 7 | Tapered |

ADA, adalimumab; ANA, anakinra; AZA, azathioprine; CRP, C reactive protein; CyA, cyclosporine A; CYC, cyclophosphamide; ETN, etanercept; GC, glucocorticoid; IFX, infliximab; IV , intravenous; IvIg, intravenous immunoglobulin; MMF, mycophenolate mofetil; MTX, methotrexate; NA, not assessed; P, primary induction; PSL, prednisolone; R, refractory/relapsing; RTX, rituximab; SC, subcutaneous; TAC, tacrolimus; TCZ, tocilizumab.

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# Response to: 'Tofacitinib for the treatment of polyarteritis nodosa: a literature review'. Correspondence on 'Tofacitinib for polyarteritis nodosa: a tailored therapy' by Rimar *et al*

We appreciate the interest of Akiyama *et al* in our report and thank them for the data presented in their letter.[1 2] Akiyama *et al* have presented a thorough literature review and have described a positive and efficacious effect of tocilizumab in 11 cases of refractory polyarteritis nodosa (PAN) described in 6 case series. Indeed, the use of tocilizumab, an interleukin (IL)-6 inhibitor, in vasculitis is gaining evidence in the literature, specifically in large vessel vasculitis including giant cell arteritis and Takayasu arteritis.[3 4] Nevertheless, it should be noted that although the IL-6 pathway is the major inducer of STAT 3, and therefore was used by us in vitro to stimulate this pathway in order to evaluate STAT 3 activation, clinically blocking the IL-6 pathway in our patient, using tocilizumab, was not beneficial, contrasting with our positive result with tofacitinib. We hypothesised that redundancy or other stimulators of the STAT 3 pathway like IL-23 may explain this discrepancy. Furthermore, we did not find tocilizumab clinically or radiologically beneficial in another patient with severe refractory PAN involving skin and coronary arteries—evidenced by positron emission tomography-CT scan revealing active inflammation in an aneurism within a coronary artery and by MRI demonstrating active deep skin involvement while under treatment with tocilizumab (unpublished data).

Thus, in considering the review by Akiyama *et al*, publication bias should always be considered when evaluating case reports, as we all tend to prefer publishing our positive experience.

Recently, we have also reported our positive experience with another agent, infliximab, for refractory PAN and reviewed the literature.[5] The ever-expanding spectrum of biological treatments is confusing for the practitioner and although case reports and case series are important to guide us in rare refractory patients, we should still follow guidelines and use evidence-based treatments that were evaluated in large randomised controlled clinical trials as first-line therapy.

New technologies may help us diagnose and treat refractory patients. Whole-exome sequencing studies may reveal novel mimickers, the monogenic vasculitides, as deficiency of adenosine deaminase 2, stimulator of interferon genes-associated vasculopathy with onset in infancy, and haploinsufficiency of A20 that should be considered in refractory cases. Finally, precision medicine, as we suggested in our report, may guide us in the future, helping us to find the right fit for each patient.[6 7]

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# Correspondence to: 'Combination of human umbilical cord mesenchymal (stromal) stem cell transplantation with IFN-γ treatment synergistically improves the clinical outcomes of patients with rheumatoid arthritis'

We read with great interest the article by He *et al*, which the authors reported that mesenchymal stem cell (MSC) transplantation (MSCT) plus interferon-γ (IFN-γ) combination therapy may realise clinical efficacy featuring good or moderate EULAR responses in patients with rheumatoid arthritis (RA) who responded poorly to conventional therapeutics including disease-modifying antirheumatic drugs.[1] MSC-based therapies have become novel therapeutic approaches for RA through immunomodulation, including the induction of T regulatory cells (Treg).[2] We agree with the authors that IFN-γ-primed MSCs may bring about improved immunomodulation in vitro (as suggested in Figure 2 by He *et al*[1]), and propose that the results based on studies in patients could be juxtaposed with further evidence on the protocol of IFN-γ treatment, serum levels of IFN-γ in patients and gating strategies for flow cytometry (FC) analysis.

As the therapeutic effect of MSCT for RA is regulated by endogenous IFN-γ level,[3] the clinical protocol of IFN-γ treatment in this study involved intramuscular infusion of IFN-γ.[1] On the contrary, the protocol adopted in vitro was IFN-γ priming using MSCs pretreated with IFN-γ for 24 hours (as mentioned in the MSC and T cell co-culture section in Supplementary Materials by He *et al*[1]), which would allow for potentiated immunomodulatory functions of MSCs. However, compared with transplanting MSCs primed with IFN-γ, the intramuscular infusion of IFN-γ may initiate further immune reactions[4 5] that have yet been pinpointed in this study.[1] From a translational standpoint, interpreting the safety of IFN-γ-primed MSC protocols as that of recombinant IFN-γ monotherapy, should be not appropriate. As a result, the discrepancy between these protocols could attribute to underestimated complications of the combination therapy.

Although IFN-γ has been proven as a foremost mediator for the inflammatory responses in RA,[6] which has also been evidenced by its interactions with MSCT in the murine model (as suggested in Figure S2 by He *et al*[1]), whether MSCT plus intramuscular infusion of IFN-γ would further modulate serum levels of IFN-γ in patients remains unclear. While the authors observed alleviated inflammatory responses in terms of serum levels of erythrocyte sedimentation rate, C reactive protein, anti-cyclic citrullinated peptide and rheumatoid factor among patients (as suggested in Figure 3 by He *et al*[1]), serum level monitoring of IFN-γ, or other proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1α or IL-1β, may facilitate our understanding of the effect of IFN-γ infusion, as well as whether their concomitant presence induced the immunosuppressive functions of MSCs.

Furthermore, gating as a data reduction technique for FC analytics, often involves certain controls to ensure proper interpretation. For instance, the Fluorescence Minus One control technique, has been used to recognise cells presenting as data spread arise from multiple fluorochromes.[7 8] Likewise, isotype controls allow for identification of the background binding caused by antibody isotypes.[7 8] It is possible that the gauged percentages of CD3+ and IFN-γ+ MSCs, and the estimated baseline for groups without IFN-γ treatment (as suggested in Figure 2 by He *et al*[1]), could differ among different control techniques for gating.

For the above reasons, we propose that the conversations between bench side and bedside considerations are necessary for developing translational models and cell therapies for RA. For instance, following up the serum levels of IFN-γ and adopting control techniques for FC gating may improve our knowledge on the effectiveness of the treatment protocol and infusion of IFN-γ in MSCT.

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# Response to: 'Correspondence to: 'Combination of human umbilical cord mesenchymal stem cell transplantation with IFN-γ treatment synergistically improves the clinical outcomes of patients with rheumatoid arthritis" by Ma *et al*

We thank Ma *et al*[1] for their interest in our recent report titled 'Combination of human umbilical cord mesenchymal stem (stromal) cell transplantation with IFN-γ treatment synergistically improves the clinical outcomes of patients with rheumatoid arthritis'.[2] Ma *et al*[1] brought up an important issue regarding the safety profile of intramuscular infusion of interferon (IFN)-γ, which may initiate further immune reactions.[3 4] As previously described, recombinant human IFN-γ monotherapy is known to be safe but ineffective in treating rheumatoid arthritis.[5 6] Furthermore, the safety of IFN-γ-primed mesenchymal stem (stromal) cells (MSCs) remains unknown, as there has been no such clinical research report addressing this issue. Therefore, for the subject's maximum safety considerations, the clinical protocol was MSC transplantation (MSCT) plus intramuscular infusion of IFN-γ, instead of IFN-γ-primed MSCs, and as we have anticipated no new or unexpected safety issues were reported for either treatment group for up to 1 year. Indeed, in future studies, interpreting the safety of the IFN-γ-primed MSC protocols would be more appropriate than that of recombinant IFN-γ monotherapy from a translational standpoint.

As to the question of whether MSCT plus intramuscular infusion of IFN-γ would further modulate serum levels of IFN-γ in patients, all patients who received intramuscular infusion of IFN-γ had a transient increase in serum IFN-γ level within 24 hours after infusion, which gradually decreased during the subsequent follow-up. However, as we described in our previous study that there are huge individual variations in the baseline serum IFN-γ levels,[7] we did not list the IFN-γ data. In addition, with regard to the serum level of proinflammatory cytokines, consistent with our previous study,[7] there was a significant decrease in the serum levels of tumour necrosis factor-α and interleukin (IL)-6 among patients of the MSCT plus IFN-γ group, while no significant changes in IL-1β, IL-2R and IL-8 levels were observed. Unfortunately, we did not find that such a proinflammatory cytokine combination affected the immunosuppressive functions of MSCs, as observed in the in vitro study.

Finally, we agreed that it is possible that the flow cytometry (FC) gauged percentages of CD3+ and IFN-γ+ MSCs could differ using different control techniques for gating. In our study, isotype controls were used for identification of the background binding caused by antibody isotypes. As there are only two kinds of fluorochromes involved in the FC experiment, a single positive control technique was more adequate as a compensation control technique than the fluorescence minus one control technique, which is suitable for multiple fluorochromes (≥3) FC study.[8 9]

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# Tapering antirheumatic drugs in a resource-poor setting: real-world evidence

Introduction of biological agents has undoubtedly revolutionised the management of different inflammatory rheumatic conditions; however, for Pakistani patients, it comes with a significant cost burden. We read with interest the article by van Mulligen *et al*,[1] and we concur with their conclusion that 'financial arguments may influence the decision to taper tumour necrosis factor-inhibitors first'. Being in a resource-poor country, the access to biological therapies is limited in our part of the world, and we would like to share our experience of tapering antirheumatic drugs.

After achieving remission or low disease activity (LDA), dosage reduction of biological disease-modifying antirheumatic drugs (bDMARDs) is an important topic in light of, not only the significant economic burden, but also as increasing number of patients who reach remission or LDA along with carrying the risk of unnecessary adverse events due to overtreatment. Hence, in our practice, we are inclined to keep these patients on the maximum tolerated doses/number of DMARDs and we plan the tapering of bDMARDs soon after achieving remission/LDA. Patients with inflammatory rheumatic disease who are given bDMARDs are followed up with a protocol on 4–6 weekly basis, and validated disease activity assessments are made (Disease Activity Score 28 (DAS 28) for rheumatoid arthritis (RA)[2]; disease activity in psoriatic arthritis and minimal disease activity for psoriatic arthritis (PsA)[3]; and Bath Ankylosing Spondylitis Disease Activity Index, Bath Ankylosing Spondylitis Functional Index, Ankylosing Spondylitis Disease Activity Score[4] for ankylosing spondylitis (AS)). The protocol involves: continuation of baseline DMARDs and/or non-steroidal anti-inflammatory drugs (NSAIDs) on the maximum tolerated doses; after 3 months of bDMARDs introduction, if patient achieves remission or LDA, then 30% reduction in bDMARDs dose or about 30% prolongation of dosing interval is made; if patient remains in remission or LDA after 4 months of bDMARDs introduction, then 50% reduction in its dosage or dosing interval is made. In our country, the commonly available and licensed subcutanous bDMARDs include: etanercept, tocilizumab and secukinumab.

In last 1 year, 47 patients were given bDMARDs (RA=26, spondyloarthritis (SpA) 21 (AS=12, PsA=9)) under our rheumatology services. By June 2020, 45 out of 47 patients have completed at least 3 months of bDMARDs therapy. For this study, only those patients who have completed 3 months of bDMARDs therapy was included (n=45). Among these 45 patients on bDMARDs, 12 patients were using etanercept, 18 patients were on tocilizumab and 15 patients used secukinumab. The median age of these patients was 34 years and a median disease duration of 9 years. Sixty per cent of the cohort was male. It was reassuring to note that 73% (n=33) of patients have successfully managed to reduce their bDMARDs without any significant flare and without any need for bDMARDs dose escalation (among them, five patients have completely stopped bDMARDs without any flare). All these patients have been maintained on the baseline DMARDs (NSAIDs in the case of AS). Interestingly, patients with SpA (AS and PsA) were noted to be more successful in reducing their bDMARDs than patients with RA (18 out of 20 patients, 90%, vs 15 out of 25 patients, 60%; p=0.04). We also examined whether any particular biological drug has more favourable outcome as regards dose reduction, but no statistical difference was found (p=0.26). Within 1 year of commencing bDMARDs, we managed to reduce the overall exposure of bDMARDs by 41% in our cohort, reflecting in significantly less cost burden for our patients. Moreover, we plan to use these data to discuss with patients requiring bDMARDs.

We conclude that in our resource-poor clinical setting, a protocol-driven stepwise reduction of bDMARDs was successful as the first choice for tapering towards DMARD-free remission, and these data can potentially be used to help alleviate the anxiety of cost implications associated with bDMARDs.

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# Response to: 'Tapering antirheumatic drugs in a resource-poor setting: real-world evidence' by Haroon *et al*

We appreciate the interest in our paper by Haroon, *et al*. We presented the 2-year results of the TARA trial, in which we concluded that 'financial arguments may influence the decision to taper tumour necrosis factor-inhibitors first'.[1] Based on this conclusion, Haroon, *et al* decided to respond to that with their real-world data from a resource-poor country.[2]

Ideally, if patients with rheumatoid arthritis (RA) are in sustained remission, then medication is quickly tapered and possibly stopped to reduce healthcare costs. Disease modifying anti-rheumatic drug (DMARD)-free remission is suggested as a preferred ultimate target in a treat-to-target management approach; however, we previously showed, in a systematic literature review, that this outcome is achievable in only 10%–20% of the RA population.[3] Within the TARA trial, we showed that DMARD-free remission was achievable in 15% of the included patients with established RA. Haroon *et al* reported that 5 of 45 (11%) patients with RA and spondyloarthritis were able to completely stop their biological (b)DMARDs. This together confirms that DMARD-free remission is reachable for a minority of patients.

Although DMARD-free remission occurs less frequently, most of the patients with RA with a well-controlled disease can lower their DMARD dosage. To illustrate, 83% of the TARA patients were able to reduce their medication dosage, which is similar to the real-world data of Haroon *et al*. Another benefit of gradual tapering with a treat-to-target approach, which includes close monitoring, is that (severe) disease flares could possibly be prevented due to slower tapering and earlier detection. In our opinion, the aforementioned approach is currently the best way to taper treatment. Especially, as we have previously shown that a disease flare has a significant impact on patients' lives, which outlast the effect of a flare on disease activity.[4] Noteworthy is the fact that although most patients reach low disease activity within 6 months after a flare, most of them have a higher disease activity postflare compared with preflare.[4]

Unfortunately, current tapering strategies are still based on a trial-and-error approach that leads to high flare rates and, therefore, a tailor-made tapering approach is preferred. Moreover, no consensus had been reached on how to taper medication because cohorts/trials directly comparing different tapering strategies are sparse.[5] Haroon, *et al* showed that 60% of patients with RA were able to reduce their bDMARD dosage when a 2-step tapering protocol was used, consisting of dose reductions every 4 months of 30% followed by 50%. Comparing this with our results from the TARA trial, in which we showed that 83% of the patients were able to reduce their DMARD dosages with 50% every 3 months, leads to our advice to gradually taper DMARDs with 30%–50%every 3–4 months in patients with RA with a well-controlled disease.

To summarise, by using a gradual tapering approach, almost all patients with RA with a well-controlled disease can reduce their DMARD dosages. The real-world data of Haroon *et al* underline the fact that the majority of patients with RA are able to gradually taper DMARDs.

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# Imaging in immune checkpoint inhibitor-induced polymyalgia rheumatica

We read with great interest the article 'Addressing immune-related adverse events of cancer immunotherapy: how prepared are rheumatologists?' by Kostine *et al*.[1] The introduction of immune checkpoint inhibitor (ICI) therapy has been a major breakthrough in the management of metastatic cancer. On the downside, ICI therapy may induce unwanted autoimmune effects, the so-called immune-related adverse effects (irAEs). Various irAEs have been described that resemble a regular rheumatic disease, including polymyalgia rheumatica (ICI-PMR).[2 3] The authors report that rheumatologists may lack confidence in diagnosing irAEs. Therefore, recommendations for the diagnosis of rheumatic irAEs are needed. Based on our experience with ICI-PMR, we propose that imaging could be an important part of such recommendations.

We investigated six consecutive patients with ICI-PMR by ultrasonography, and five of these patients also by [18F]-fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT) scan. Five patients fulfilled the provisional American College of Rheumatology/European League Against Rheumatism classification criteria for PMR.[4] A normal C-reactive protein level in the absence of an erythrocyte sedimentation rate (ESR) test precluded PMR classification in one patient. However, this patient fulfilled both the clinical and ultrasound criteria for PMR,[4] and showed findings suggestive of PMR on the FDG-PET/CT scan.[5] The median age was 73 years (range 59–83; online supplementary table 1). Patients received anti-programmed cell death protein 1 (PD-1) treatment, that is, nivolumab or pembrolizumab. ICI therapy resulted in near-complete cancer remission (n=3) or a partial response (n=3). Following the start of ICI therapy, the first symptoms suggestive of ICI-PMR developed after a median of 70 days (range 1–86).

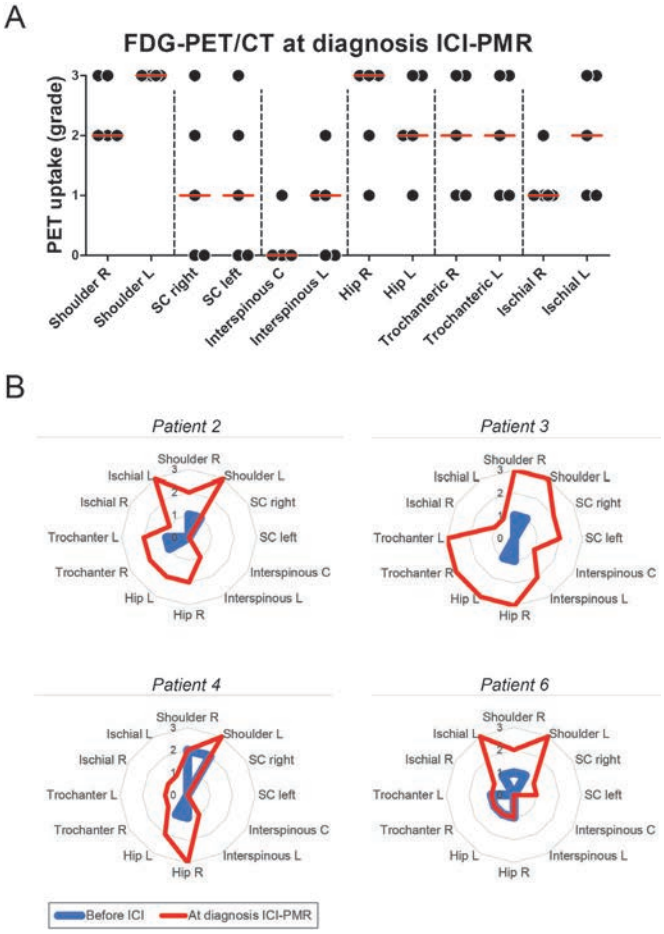


**Figure 1** Grading of PET uptake at distinct sites in patients with immune checkpoint inhibitor-induced polymyalgia rheumatica (ICI-PMR). PET uptake was graded at the shoulders, sternoclavicular (SC) joints, cervical and lumbar interspinous bursae, hip joints, hip trochanters and ischial tuberosities (n=5). Grading was performed as previously described[7]: 0, no uptake; 1, uptake lower than liver; 2 uptake equal to liver; 3, uptake higher than liver. (A) PET uptake in five patients (ie, patient 1–4, and patient 6) at diagnosis of ICI-PMR. (B) PET uptake in four patients at diagnosis of ICI-PMR and prior to ICI therapy and onset of ICI-PMR.

Ultrasonography of patients with ICI-PMR demonstrated findings consistent with PMR.[6] Shoulder examination revealed biceps tenosynovitis in five patients and subacromial–subdeltoid bursitis in three patients (online supplementary table 1, online supplementary figure 1A). Glenohumeral synovitis was not detected. Hip ultrasound was performed in three patients, but revealed no coxofemoral synovitis or trochanteric bursitis. One patient received a glucocorticoid injection of the shoulder 14 weeks before ultrasonography, while another patients used a prednisolone equivalent of 7.5 mg/day for 5 weeks due to hypophysitis and adrenal insufficiency. The other patients received no glucocorticoids prior to ultrasonography.

FDG-PET/CT scans of patients with regular PMR may demonstrate FDG uptake at the shoulders, hip joints, greater trochanters, ischial tuberosities, sternoclavicular joints and cervical/lumbar interspinous bursae.[5] FDG-PET/CT scans of patients with ICI-PMR showed inflammation at these exact sites (online supplementary figure 1B). All FDG-PET/CT scans were obtained prior to initiation of any glucocorticoids. Scoring of FDG uptake was performed: 0, no uptake; 1, uptake lower than liver; 2 uptake equal to liver; 3, uptake higher than liver.[7] All patients showed grade 2–3 uptake at the shoulders, and grade 1–3 uptake at the hip joints, greater trochanters and ischial tuberosities (figure 1A). FDG uptake at the sternoclavicular joints and cervical/lumbar interspinous bursa was present in part of the patients. In accordance with studies in regular PMR,[5] part of the patients with ICI-PMR showed FDG uptake at the elbows (n=2) and hands/wrists (n=3; online supplementary figure 2). This was associated with mild synovitis of the hands/wrists on physical examination in one patient only. Recently, Calabrese *et al* also reported peripheral synovitis in patients with ICI-PMR.[2] No evidence of giant cell arteritis was found in any of the patients.

Four patients underwent a FDG-PET/CT scan prior to ICI therapy. These scans showed grade 1–2 FDG uptake at the shoulders and hips (figure 1B). Although this mild metabolic activity may also be seen in non-inflammatory conditions, it could suggests that low-grade, subclinical inflammation was already present at these sites before ICI therapy. The checkpoint molecule PD-1 might have initially prevented the development of full-blown inflammation in these patients.

In conclusion, FDG-PET/CT and ultrasound findings in ICI-PMR are comparable to those seen in regular PMR.[5 6] Imaging may thus help to confidently diagnose ICI-PMR. Low-grade FDG uptake was already observed on the FDG-PET/CT scan prior to ICI therapy, and progressed towards the full-blown PMR pattern after initiation of ICI therapy. It remains to be elucidated whether or not baseline imaging before ICI therapy may help to predict the development of rheumatic irAEs.

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# Public/private partnerships, at what price?

From a scientific viewpoint, Huizenga and colleagues are completely right to endorse 'disruption' by public-private partnerships as a way to 'accelerate innovation and enable translation of the rapidly expanding cellular and molecular understanding of disease pathogenesis into the development of new therapeutic agents'.[1] However, one of their examples (monoclonal antibodies leading to the development of anti-tumour necrosis factor (TNF) treatment) highlights an aspect they did not discuss: publicly funded science leading to drugs marketed at prices society cannot afford.

In my opinion, the current model of drug development is broke and also needs to be disrupted. I say this in full awareness of the many great advances for example the field of rheumatology has enjoyed. A recent study by SOMO, an independent organisation that researches multinationals, suggests that pharmaceutical companies have switched from innovation to financial instruments in order to sustain themselves and increase their profitability.[2] Also, drug development is mostly targeted at areas of high potential profit, including diseases where orphan status can be obtained, rather than areas of high societal need. From the company's and shareholder's viewpoint, this is entirely legitimate.

But from the societal viewpoint, it is highly suboptimal. We need to fundamentally rethink how we want to progress science and drug development, so that new treatments can become available at affordable prices. As an aside, this includes a rethink on the outrageously complex, slow and expensive process of drug approval.

For now, scientists and society should become less naive and wary of partnerships in which they offer knowledge 'for free', with nothing in return except the opportunity to buy back the results of their knowledge at premium prices.

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# Correction: *Granulocyte colony stimulating factor exacerbates antineutrophil cytoplasmic antibody vasculitis*

Freeley SJ, Coughlan AM, Popat RJ, *et al*. Granulocyte colony stimulating factor exacerbates antineutrophil cytoplasmic antibody vasculitis. *Ann Rheum Dis* 2013;72:1053–1058. doi:10.1136/annrheumdis-2012-202160

In Figure 3 A, C, E, the y axis should be Median Fluorescence Intensity and not Mean Fluorescence Intensity.



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