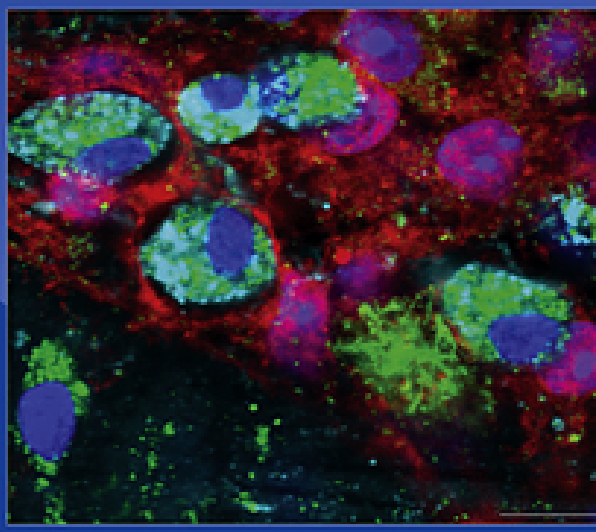
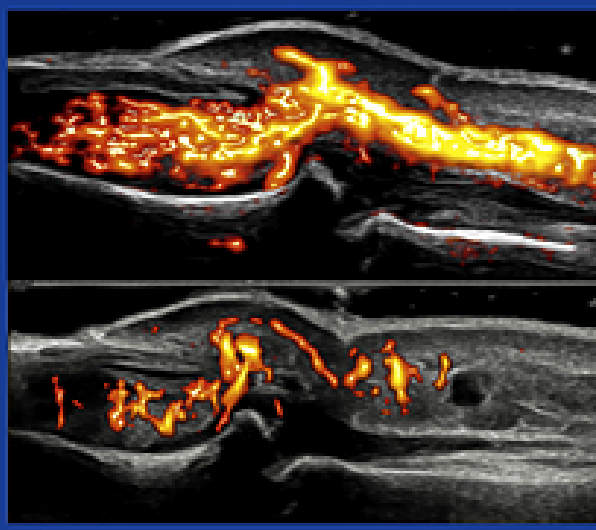
FEBRUARY 2026
VOLUME 85
ISSUE
2

ANNALS OF THE RHEUMATIC DISEASES

THE EULAR JOURNAL





ELSEVIER

eular
EUROPEAN ALLIANCE OF ASSOCIATIONS FOR RHEUMATOLOGY

The home of innovative rheumatology research

Ann Rheum Dis 85 (2026) 219–222

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Editorial

Guidance for the management of SARD-ILDs in clinical practice—impact of the ERS/EULAR recommendations

INTRODUCTION

Interstitial lung diseases (ILDs) comprise a heterogeneous group of more than 200 entities including many rheumatic diseases. ILD is a frequent complication of systemic autoimmune rheumatic diseases (SARDs), occurring in up to 50% to 70% of patients with systemic sclerosis (SSc), 40% to 70% of patients with idiopathic inflammatory myopathies (IIMs), 30% to 70% of patients with mixed connective tissue disease (MCTD), 20% to 50% of patients with rheumatoid arthritis (RA), 10% to 25% of patients with Sjögren's disease (SjD) and up to 10% of patients with systemic lupus erythematosus (SLE) [1,2]. ILD is thought to develop as an exaggerated wound healing response to repetitive cycles of injury, which triggers cascades of inflammation, fibroblast activation, and fibrotic tissue remodelling [3,4]. ILD can manifest in different patterns. Although the frequencies vary across individual diseases, nonspecific interstitial pneumonia and usual interstitial pneumonia (UIP) are most common in SARD-ILD, whereas other pattern such as organising pneumonia, diffuse alveolar damage, lymphocytic interstitial pneumonia, and desquamative interstitial pneumonia together account for less than 15% of all cases of SARD-ILD [1].

The current therapeutic paradigm aims at the prevention or treatment of patients in the early stages of the disease before accrual of damage. This is challenging in SARDs since the pathologic process started long before the patient is seen by a physician and reliable, validated biomarkers to entrust the screening of subjects at risk are lacking. Moreover, SARD-ILDs are pathophysiologically heterogeneous and follow trajectories that are highly variable not only between individual SARDs, but also in individual patients within a given disease.

ILD is the leading cause of death in SSc and a major contributor to the overall mortality in other SARDs with a high unmet need for novel diagnostic approaches and more effective therapies. SARD-ILDs are an area of very active research with successful approval of novel therapeutics such as the multityrosine kinase inhibitor nintedanib (for progressive ILD irrespective of the underlying phenotype) [5,6] and tocilizumab (for SSc-ILD and in the US only) [7], multiple ongoing clinical trials with other treatment candidates [8], and ongoing validation of novel approaches for diagnosis of ILD or assessment of disease activity such as lung ultrasound and fibroblast activation protein inhibitor-positron emission tomography/computed tomography, respectively [9,10]. These developments have been flanked by recommendations and guidelines on the management of SARD-ILD by different societies to facilitate optimal use of current diagnostic and therapeutic tools.

Overview of the European Respiratory Society/European Alliance of Associations for Rheumatology recommendations for SARD-ILD

The new European Respiratory Society (ERS)/European Alliance of Associations for Rheumatology (EULAR) recommendations were drafted by a task force consisting of rheumatologists, pulmonologists, pathologists, radiologists, methodologists, and patient representatives [11]. The guidelines followed the Grading of Recommendations, Assessment, Development and interventions approach and the final results were approved by EULAR and ERS. The expert panel drafted 25 PICO (25 patients, interventions, comparison and outcomes) and 28 narrative questions that were addressed by systematic literature reviews.

The final ERS/EULAR guidelines provide 15 recommendations for screening, diagnosis, monitoring, and treatment of SARD-ILDs with specification of the strength of the recommendation and the certainty of the evidence.

The recommendations on *screening* highlight the key role of high-resolution computed tomography (HRCT) for screening and recommend against replacing HRCT with pulmonary function testing (PFT) or lung ultrasound. They also provide a graded recommendation on whom to screen with HRCT: they recommend screening in all patients with SSc and MCTD and suggest it in patients with IIMs, RA and SjD with risk factors and potentially also in patients with IIMs without risk factors.

The recommendations on *diagnosis* of SARD-ILD suggest that a global assessment of risk factors of progression should be performed in all patients with SSc, IIMs and RA, that bronchoalveolar lavage (BAL) could be performed in patients with suspicion of alternative diagnosis such as infection and that patient-reported outcome (PRO) measures and 6-minute walking testing (6MWT) could be used in patients without physical limitations.

The recommendations on *monitoring* suggest repeating PFTs every 3 to 6 months within the first years after diagnosis and every 6 to 12 months thereafter, suggest repeating the initial HRCT after 3 to 6 months in IIM-ILD and after 1-2 years in other SARD-ILDs, and suggest repeating HRCT and PFTs in all patients in case of suspected progression.

The recommendations on *treatment* suggest that treatment decisions should be guided by the inclusion criteria of randomised controlled trials in SARD-ILD. For the treatment of

https://doi.org/10.1016/j.ard.2025.11.004
Received 6 October 2025; Revised 3 November 2025; Accepted 4 November 2025

SSc-ILD, they strongly recommend tocilizumab in patients with SSc with early, inflammatory SSc and suggest the use of nintedanib, mycophenolate mofetil (MMF), rituximab and cyclophosphamide (conditional recommendations). Immunosuppressive treatment is recommended for IIM-ILD and suggested for RA-, MCTD-, SjD-, and SLE-ILD. Nintedanib is suggested in patients without SSc with progressive pulmonary fibrosis. Pirfenidone is suggested as an alternative antifibrotic treatment to nintedanib in patients with RA-ILD with a UIP pattern. The recommendations also provide guidance on the use of combination therapies. They suggest the use of the combination therapy with nintedanib and MMF specifically in SSc-ILD, combination therapy with immunosuppressants and glucocorticoids specifically in IIM-ILD and combinations of immunosuppressants, or in case of progressive ILD combinations of immunosuppressants and nintedanib in all patients with SARD-ILD.

Comparison of ERS/EULAR and American College of Rheumatology/American College of Chest Physicians guidelines on SARD-ILD

The American College of Rheumatology (ACR)/American College of Chest Physicians (CHEST) guidelines were first presented in 2023 [12]. Despite multiple areas of uncertainty driven by low or very low certainty of evidence, both the EULAR/ERS and ACR/CHEST guidelines issued strong recommendations in key clinical areas. This was particularly notable in the context of HRCT screening in appropriate patient populations and the use of immunosuppressive therapies in specific subgroups. The justification for strong recommendations, despite low certainty of evidence, rests on the potentially severe consequences of missed or delayed diagnosis of ILD, including increased morbidity and mortality.

Areas of uncertainty and research gaps

The ERS/EULAR as well as the ACR/CHEST guidelines acknowledge significant knowledge gaps that limit the strength of some recommendations. Key areas requiring further research include: identification of novel biomarkers for early detection and monitoring; establishing a more precise definition of ILD worsening and evaluating the long-term impact of early ILD screening on clinical outcomes.

Methodological similarities and differences

Both guidelines employed the GRADE methodology and reflected a multidisciplinary collaboration among rheumatology, pulmonology, and radiology experts, with patient participation integrated into the process. Each set recognised the limitations inherent in the existing evidence base and emphasised the need for high-quality future studies. However, whereas the ERS/EULAR Task Force formulated its guidance after discussions that aimed for a collective consensus, the ACR/CHEST panel reached consensus through a voting process that mandated at least 70% agreement on both the direction and strength of each recommendation.

Screening recommendations

There is broad alignment on the importance of HRCT in ILD screening, but with differences in application. The ERS/EULAR guidelines recommend universal HRCT screening in SSc and MCTD, and targeted screening for high-risk individuals with IIMs, RA, or connective tissue disease. The ACR/CHEST guidelines recommend HRCT screening only in high-risk patients across all disease categories. Differences in the use of PFTs and other testing modalities are minimal.

Monitoring and follow-up

The ERS/EULAR guidelines addressed monitoring intervals more directly, although with the caveat that these were conditional and based on low-grade evidence. Nevertheless, the recommendations offer a practical framework for clinicians. ACR/CHEST deferred firm recommendations on screening frequency, offering only expert opinion-based suggestions, such as annual rescreening for high-risk patients or re-evaluation upon new ILD signs or symptoms.

Diagnostic modalities

Both guidelines align on the core diagnostic tools again with relatively minor differences, for example, both groups emphasised the use of HRCT, PFTs, and disease-specific factors; recommend against the routine use of bronchoscopy/BAL, lung biopsy, or 6MWT unless in selected cases (eg, alternative diagnoses considered or inconclusive standard testing). Additionally, ACR/CHEST explicitly discouraged plain chest radiographs for ILD screening, monitoring, or diagnosis. EULAR/ERS included PROs, which are not addressed in the ACR/CHEST document in spite of the input from a patient panel.

Treatment recommendations

Treatment guidance is in many of the key points concordant between the 2 publications. However, the ACR/CHEST guideline structures its recommendations using an apparent hierarchical system, whereas the ERS/EULAR Task Force determined that there was insufficient evidence due to a lack of direct head-to-head clinical trials to establish a treatment hierarchy. Moreover, it should be noted that the ACR/CHEST recommendations include azathioprine as a possible first-line therapy for ILD in SARDs other than SSc. Other differences include the fact that the ACR/CHEST guideline but not the ERS/EULAR guideline generally favours immunosuppressive treatment over nintedanib and recommends against upfront combination therapy with MMF and antifibrotics without ILD progression or the recommendation of pirfenidone specifically for patients with RA-ILD with a UIP pattern in the ERS/EULAR guideline vs a conditional recommendation for pirfenidone across all types of SARD-ILDs in the ACR/CHEST guideline.

While both guideline sets are constrained by the limitations of low-certainty evidence, they provide valuable and actionable recommendations. The EULAR/ERS guidelines offer more granular direction on screening frequency and monitoring, making them especially useful as a practical tool for treating physicians. Both documents underscore the critical need for future research to refine screening strategies, define disease progression, and assess treatment impact on long-term outcomes.

Musing on the recommendations

Although the strength of many recommendations produced is *conditional,* several positive aspects should be emphasised. Not only are all the therapeutic options for each individual disease reported, but an attempt has been made on the basis of the available evidence to guide the choice of the optimal therapeutic approach for the individual patient based on assessment of the degree of severity, the risk of progression and the functional status. The inclusion of clinical risk factors with the subclassification in patients at high and low risk of progression together with the PRO are further points of merit of the present recommendations.

The role of nonpharmacological approaches to the management of ILD, such as vaccination, cessation of smoking habit, aerobic physical activity and rehabilitation, and the role of cellular therapies (eg, high-dose chemotherapy followed by stem cell transplantation) are not addressed in the current guidelines.

The strong recommendation to use HRCT and not pulmonary function tests for ILD screening in patients with SARD is important as this is not always done in clinical practice, particularly in countries with limited economic resources. The advice to screen all patients with SSc with HRCT is underlined by recent data indicating that a subset of patients develop ILD also later in the course of the disease and that patients with SSc negative for the autoantibodies associated with SSc (anti-centromere-, anti-RNA polymerase III-, and anti-topoisomerase I-antibodies) had a higher rate of progression of ILD in a post hoc analysis of the SENSCIS trial [13].

A limitation to keep in mind is that access to expensive diagnostics and medications varies in different geographical areas and that high costs can constitute a barrier [14].

To optimally translate the treatment recommendations in daily clinical practice, multidisciplinary ILD boards are required that include rheumatologists, pulmonologists, radiologists, and pathologists and eventually also patient representatives. Shared decision making can improve patient stratification in particular in challenging cases, for example, suspicion of coexisting infection, referral for lung transplantation or experimental therapies, or to palliative care.

CONCLUSIONS AND OUTLOOK

The recommendations provide a framework for screening, follow-up, and treatment of SARD-ILDs that can provide guidance in daily clinical practice with modifications and adjustments for individual SARDs. Although the recommendations convert current knowledge into a set of easy-to-follow recommendations, several areas of SARD-ILD require further studies to enable evidence-based recommendations. Major areas of future research include identification and validation of biomarkers of disease activity (eg, circulating biomarkers in serum or BAL or molecular imaging biomarker) and as well as additional markers for patient stratification, for example, genetic markers or integrated omics data. Development of PROs that are more sensitive to change and implication of those PROs in screening for and follow-up of SARD-ILD are another area of further research. Novel biomarkers and PROs may also enable a personalised approach for the management of SARD-ILD. Given the numerous ongoing clinical trials in SARD-ILD and recent positive trials that have not yet been considered in the current guidelines such as the FIBRONEER-ILD trial [15], we will likely have a broader therapeutic arsenal in future. To avoid prolonged periods of exposure to suboptimal or even ineffective therapies, upfront stratification of patients according to markers of response (eg, markers based on target expression or molecular signatures associated with activation of the targeted pathway) or markers that allow early assessment of target engagement or treatment response will become more and more important.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interests

JHWD has consultancy relationships with Active Biotech, Anamar, ARXX, AstraZeneca, Bayer Pharma, Boehringer Ingelheim, Callidatas, Calluna, Galapagos, GSK, Janssen, Kyverna, Novartis, Pfizer, Quell Therapeutics and UCB. JHWD has received research funding from Anamar, ARXX, BMS, Boehringer Ingelheim, Cantargia, Celgene, CSL Behring, Exo Therapeutics, Galapagos, GSK, Incyte, Inventiva, Kiniksa, Kyverna, Lassen Therapeutics, Mestag, Sanofi-Aventis, SpicaTx, RedX, UCB, and ZenasBio. JHWD is the CEO of 4D Science and scientific lead of FibroCure. AG did not disclose competing interests. MDM has received research funding from Prometheus/Merck, Mitsubishi Tanabe, Boehringer Ingelheim, AstraZeneca, ATYR, Horizon/Amgen, BMS. MDM has consultancy relationships with Cabaletta, GSK, AstraZeneca, EICOS, and Novartis.

Patient consent for publication

N/A

Ethics approval

N/A

Provenance and peer review

Invited manuscript with external peer review.

Orcid

Jörg H.W. Distler: http://orcid.org/0000-0001-7408-9333

Jörg H.W. Distler[1,2,*], Armando Gabrielli[1,2,3], Maureen D Mayes[4]

[1] *Department of Rheumatology, University Hospital Düsseldorf, Medical Faculty of Heinrich Heine University, Düsseldorf, Germany*
[2] *Hiller Research Center, University Hospital Düsseldorf, Medical Faculty of Heinrich Heine University, Düsseldorf, Germany*
[3] *Foundation of Molecular Medicine and Cellular Therapy Polytechnic University of Marche, Ancona, Italy*
[4] *Department of Internal Medicine, Division of Rheumatology, The University of Texas Health -Houston, Houston, TX, USA*

*__Correspondence to__ Dr Jörg H. W. Distler, Department of Rheumatology, University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany.
E-mail address: joerg.distler@med.uni-duesseldorf.de (J.H.W. Distler).

REFERENCES

[1] Maher TM. Interstitial lung disease: a review. JAMA 2024;331(19):1655–65 May 21.
[2] Joy GM, Arbiv OA, Wong CK, Lok SD, Adderley NA, Dobosz KM, et al. Prevalence, imaging patterns and risk factors of interstitial lung disease in connective tissue disease: a systematic review and meta-analysis. Eur Respir Rev 2023;32(167):220210 Mar 31.
[3] Rieder F, Nagy LE, Maher TM, Distler JHW, Kramann R, Hinz B, et al. Fibrosis: cross-organ biology and pathways to development of innovative drugs. Nat Rev Drug Discov 2025;24(7):543–69 Jul.
[4] Spagnolo P, Distler O, Ryerson CJ, Tzouvelekis A, Lee JS, Bonella F, et al. Mechanisms of progressive fibrosis in connective tissue disease (CTD)-associated interstitial lung diseases (ILDs). Ann Rheum Dis 2021;80(2):143–50 Feb.
[5] Distler O, Highland KB, Gahlemann M, Azuma A, Fischer A, Mayes MD, et al. Nintedanib for systemic sclerosis-associated interstitial lung disease. N Engl J Med 2019;380(26):2518–28 Jun 27.

[6] Flaherty KR, Wells AU, Cottin V, Devaraj A, Walsh SLF, Inoue Y, et al. Nintedanib in progressive fibrosing interstitial lung diseases. N Engl J Med 2019;381(18):1718–27 Oct 31.
[7] Khanna D, Lin CJF, Furst DE, Goldin J, Kim G, Kuwana M, et al. Tocilizumab in systemic sclerosis: a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Respir Med 2020;8(10):963–74 Oct.
[8] Distler JHW, Kuwana M, Assassi S, Denton CP. Emerging therapies for the treatment of systemic sclerosis. Nat Rev Rheumatol 2025;21(10):612–25 Oct.
[9] Bergmann C, Distler JHW, Treutlein C, Tascilar K, Müller AT, Atzinger A, et al. ^{68}Ga-FAPI-04 PET-CT for molecular assessment of fibroblast activation and risk evaluation in systemic sclerosis-associated interstitial lung disease: a single-centre, pilot study. Lancet Rheumatol 2021;3(3):e185–94 Mar.
[10] Gomes Guerra M, Machado Pinto T, Águeda A, Rodrigues J, Marona J, Violante A, et al. The role of lung ultrasound in systemic sclerosis: a systematic review. J Clin Rheumatol 2023;29(4):e32–9 Jun 1.
[11] Antoniou KM, Distler O, Gheorghiu AM, Moor CC, Vikse J, Bizymi N, et al. ERS/EULAR clinical practice guidelines for connective tissue diseases associated interstitial lung disease. Ann Rheum Dis 2025 Sep 3.
[12] Johnson SR, Bernstein EJ, Bolster MB, Chung JH, Danoff SK, George MD, et al. 2023 American College of Rheumatology (ACR)/American College of Chest Physicians (CHEST) Guideline for the Treatment of Interstitial Lung Disease in People with Systemic Autoimmune Rheumatic Diseases. Arthritis Care Res (Hoboken) 2024;76(8):1051–69 Aug.
[13] Volkmann ER, Assassi S, Denton CP, Simonovska R, Sambevski S, Alves M, et al. Outcomes in systemic sclerosis-associated interstitial lung disease based on serological profiles with a focus on anticentromere and anti-RNA polymerase III antibodies. J Rheumatol 2025;52(9):914–8 Sep 1.
[14] Assayag D. Broad adoption of antifibrotics in idiopathic pulmonary fibrosis: still a long way to go. Ann Am Thorac Soc 2021;18(7):1115–6 Jul.
[15] Maher TM, Assassi S, Azuma A, Cottin V, Hoffmann-Vold AM, Kreuter M, et al. Nerandomilast in patients with progressive pulmonary fibrosis. N Engl J Med 2025;392(22):2203–14 Jun 12.

Ann Rheum Dis 85 (2026) 223–226

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Editorial

Implications of population ageing for the burden and cost of musculoskeletal conditions: insights from a Global Burden of Disease decomposition and economic analysis, 1990-2021

AGEING AS A MEGATREND

Population ageing from declining mortality and reduced fertility is recognised as a global 'megatrend', alongside climate change, urbanisation, emerging and frontier technologies, and inequalities for populations across the globe [1]. While each of these megatrends is relevant to the incidence, prevalence, and impact of musculoskeletal (MSK) conditions, population ageing has profound implications for the health and well-being of populations globally, particularly in the context of the increasing health burden related to the noncommunicable diseases (NCDs) including MSK conditions, also known as rheumatic and musculoskeletal disorders (osteoarthritis, low back pain, neck pain, gout, rheumatoid arthritis, and others). By 2050, 16% of the global population is expected to be aged 65 years or older (1.6 billion) [2]. Increased longevity does not guarantee healthy life expectancy (HALE). Indeed, a widening gap is surfacing between life expectancy and HALE, where HALE varies according to national development and aggregate healthcare quality [3]. Importantly, the rate of ageing is not uniform across the globe. While the upper-middle and high-income nations are predicted to have the largest *proportions* of older people by 2050, the *rate* and *size* of population ageing disproportionately impact the developing countries, increasing from 652 million older people in 2017 to 1.7 billion by 2050, whereas more developed countries will see an increase from 310 million to 427 million [4]. This context will have profound implications for the health and prosperity of individuals, families, and communities, coupled with major service, cost, and policy implications for health and social care systems [5].

AGEING DRIVES THE INCREASING BURDEN OF MUSCULOSKELETAL CONDITIONS

The relationship between ageing and MSK conditions (and indeed, most other NCDs) has been well characterised in the Global Burden of Disease (GBD) capstone and MSK series publications [6–12]. With larger populations of older people predicted globally, the absolute number of cases of MSK conditions will soar alongside their attributed disease burden. For example, earlier GBD publications have forecast that by 2050, osteoarthritis will affect 984 million people, low back pain 843 million [9], neck pain 269 million [12], gout 96 million [8], rheumatoid arthritis 32 million [7], and the remaining other MSK conditions 1.1 billion people (Fig 1) [7–12].

Growth projections for MSK conditions have been attributed to both population expansion and ageing, along with a smaller proportion to potentially modifiable risk factors. Yet, communicating evidence of the net contribution of population ageing to burden projections for MSK conditions has been disparate and not well integrated across conditions and publications. Guan et al bridged this gap with their recent paper published in the *Annals of the Rheumatic Diseases* [13]. The authors eloquently highlight the rising global burden of MSK conditions using the publicly available GBD trend data from 1990 to 2021 (available at https://www.healthdata.org/research-analysis/gbd-data). They calculated the net contribution of ageing to the burden estimates, independent of population expansion and epidemiological changes (incidence and prevalence). The authors also modelled total healthcare costs for MSK conditions attributed to ageing. While some might argue their contribution is simply another publication that replicates GBD data, we suggest that the work is timely, important, and offers critical insights for advocacy efforts in raising attention and priority to MSK health within the global ageing agenda. However, the estimates should be interpreted in the context of prevailing uncertainty in burden estimates where primary incidence and prevalence data are lacking, especially in the low- and middle-income countries (LMICs), the inability to disentangle the relative contributions of declining mortality and declining fertility to ageing, the absence of uncertainty intervals around the modelled estimates, and that the estimated healthcare costs are extrapolated to all countries based on healthcare costs for MSK conditions in the USA.

Guan et al adopted a decomposition analysis to model their projections [13]. This approach disentangles the contribution of ageing from other drivers of burden attributed to the MSK conditions (i.e., incident cases, prevalence, and disability-adjusted life years [DALYs]), including (i) population expansion and (ii) age-specific prevalence and incidence rates. The decomposition model identifies the contribution of each driver independently and their interactions, enabling the unique contribution of ageing to be estimated for each burden metric. While methodologically sound [14], the decomposition analysis can only disaggregate variables available in the GBD data set. The modelling suggests a decline in incidence rates, yet these are not well captured for MSK conditions. Despite this, total cases will continue to increase due to population ageing and population

https://doi.org/10.1016/j.ard.2025.10.022
Received 6 October 2025; Revised 19 October 2025; Accepted 21 October 2025

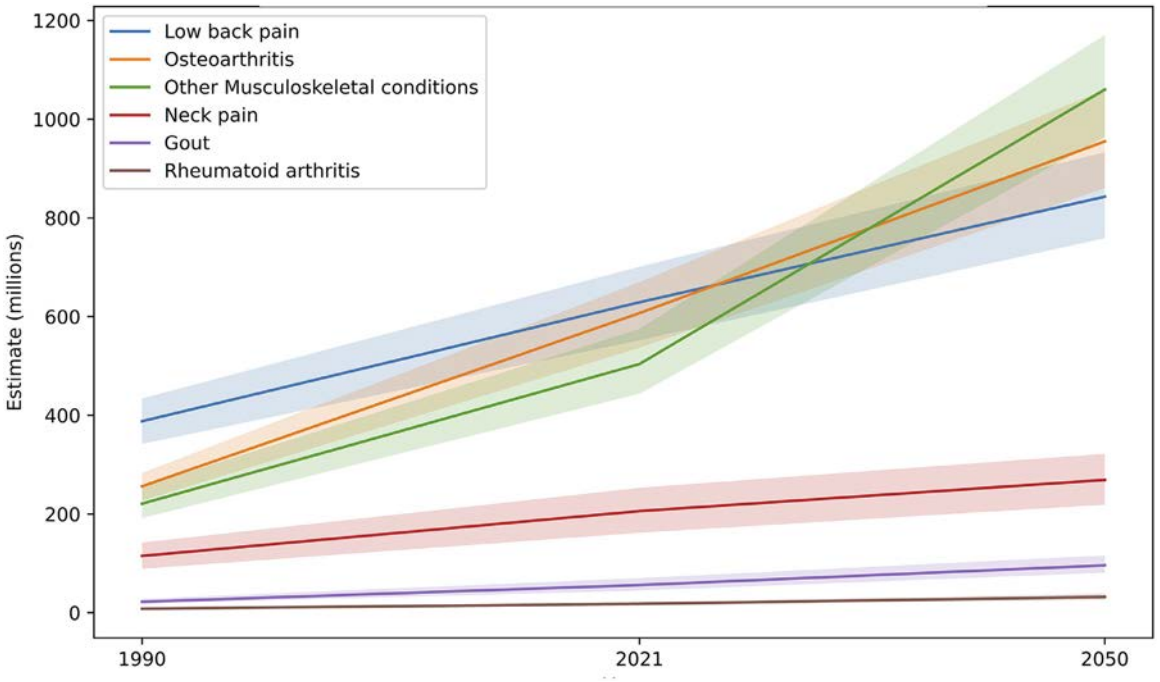


Figure. Cases of musculoskeletal conditions (in millions) in 1990 and 2021 with forecasts to 2050, including low back pain, osteoarthritis, neck pain, gout, rheumatoid arthritis, and other musculoskeletal conditions. Shading represents the 95% uncertainty interval. Data points for 1990 and 2021 were derived from the Institute for Health Metrics and Evaluation (IHME) GBD Results. Seattle, WA: IHME, University of Washington, 2024. Available from https://vizhub.healthdata.org/gbd-results/ (Accessed 25 September 2025). Forecast data points to 2050 were derived from published projections for low back pain, neck pain, gout, rheumatoid arthritis, and other musculoskeletal conditions [7–10,12]. The aggregated 2050 osteoarthritis estimate was derived from the IHME (personal communication), while the sum of OA subtypes is 1.1 billion based on published projections [11].

expansion and the increasing age-standardised epidemiological rates that capture additional potentially preventable drivers of burden of MSK conditions, such as the risk factors of smoking, obesity, injury, physical (in)activity, low bone mineral density, and occupational exposures (some of which are available in GBD). The authors identified that over the 3 decades, at a global level, *population expansion* was the main driver of increasing incident cases, prevalent cases, and DALYs for MSK conditions. Nonetheless, the contribution of *population ageing* was substantial, to 28% of the growth in incident cases, 35% of prevalent cases, and 32% of DALYs, and the relative contribution of *population ageing* to the burden of MSK conditions increased each year.

Critically, the devil is in the detail. The contribution of ageing to the MSK conditions burden parameters is not uniform—it varies by geography, sociodemographic index (SDI; a GBD composite measure of a country's development based on wealth, education and fertility [6]), sex, and condition. From a national development perspective, population ageing contributed to the highest proportion of burden growth in the middle SDI countries. For geographic regions, population ageing had the greatest contribution to increasing burden indices in East Asia, Central and Eastern Europe, and high-income Asia-Pacific. At the country level, in about one third of countries, population ageing was identified as the largest contributor to increases in MSK conditions burden estimates, most notably for the United Arab Emirates, Qatar, and the Maldives. Here, the implication is that among these countries and the middle SDI countries (eg, those in the regions of North Africa and the Middle East, South-East Asia, Central Asia, Latin America, and Southern Sub-Saharan Africa), population ageing is the principal driver of the increasing burden of MSK conditions. However, in countries like Denmark, a decrease in epidemiological rates of MSK conditions has been observed, offsetting the attributable burden due to ageing, despite population ageing. The authors suggest that there may be some policy approaches implemented in Denmark that could be transferable to other nations.

The attributed burden of MSK conditions due to population ageing varied by sex. At a global level, and in higher SDI countries, a slightly higher proportion of burden was estimated in males, while this trend was reversed in the low and middle SDI nations, where females experienced a higher proportion of burden. While sex differences were modest in the ageing-attributed burden of MSK conditions, these data signal that some sex-specific considerations for prevention and management strategies may be warranted. From the perspective of subcategories of MSK conditions, the relative contribution of population ageing was greatest for osteoarthritis burden at a global level, where between 49% and 61% of the attributable increase in burden indices was driven by ageing, followed by gout and rheumatoid arthritis. However, the contribution of population ageing to disease burden varied by region. Therefore, positioning prevention and management of osteoarthritis in integrated service models for healthy ageing will become increasingly important in the global 'healthy ageing' agenda, while individual countries and regions may prioritise other MSK conditions within healthy ageing policy and programmes (eg, neck pain in low SDI countries).

HEALTHCARE COSTS FOR MUSCULOSKELETAL CONDITIONS ATTRIBUTED TO AGEING ARE SUBSTANTIAL

A unique aspect of this GBD analysis is the inclusion of direct healthcare expenditure for MSK conditions attributed to population ageing. Healthcare expenditure by country was derived using US expenditure as a reference, adjusting by the ratio of each individual country's per capita healthcare expenditure to that of the USA to estimate the unit cost of each MSK case. While

an accepted approach for cost modelling for other NCDs, the extrapolation model does not account for national variation in how MSK healthcare interventions are resourced and delivered in countries outside the USA. Further, the cost estimates do not capture personal out-of-pocket costs borne by patients or indirect costs related to lost work productivity and lost wealth due to early retirement, which often are far greater than direct costs. Global expenditure on direct healthcare costs for MSK conditions was estimated at US$96 billion (0.10% of global gross domestic product [GDP]), eclipsing the financial impacts of risk factors such as high body mass index [15]. The highest cost burden was borne by the high SDI nations (US$65.6 billion; 0.12% GDP), in particular, North America (US$37.6 billion; 0.14% GDP), highlighting the urgency of disinvesting in low-value care and resourcing high-value care for MSK conditions in these nations.

IMPLICATIONS AND ACTIONS FOR GLOBAL HEALTH

The contribution of population ageing to the increasing burden of disease attributed to MSK conditions is well recognised. MSK conditions were identified a decade ago as a key driver to healthy ageing in the WHO World Report on Ageing and Health [5,16]. More recently, flagship technical products from 2 current global health priorities—the UN Decade of Healthy Ageing 2021-2030 and the WHO Rehabilitation 2030 agenda—provide guidance to countries on improving MSK well-being [17–22]. These agendas provide entry points to strengthen health systems for the prevention and control of MSK conditions. Indeed, the analysis by Guan et al [13] strengthens the case for genuine integration of MSK health within the global population ageing agenda, such as low back pain, where a specific WHO guideline has been created as a Public Health Goods Technical Product [17]. The data point to an urgent need for action in the disproportionately impacted middle SDI countries, where the burden of MSK conditions attributed to population ageing is greatest and where rates of population ageing exceed those of high-income countries, highlighting the need for region-specific responses. For example, the WHO South-East Asia Regional Strategy on Healthy Ageing 2024-2030 sets a roadmap for the region to respond to population ageing, recognising the contribution of MSK conditions to the threat imposed by NCDs on the health and well-being of older people in the region [23]. While not specific to MSK conditions, the strategic priority areas and actions are relevant to the care of MSK conditions, such as combating ageism, fostering age-friendly environments, and integrated care for older people [23].

From the perspective of integrating MSK conditions within the global NCD agenda, a major rate limiter is the misalignment between indicators of the health-related Sustainable Development Goal (SDG) 3.4, which focuses action on reducing premature mortality from 4 NCDs (cancer, diabetes, cardiovascular disease, chronic lung disease), and the increasing global morbidity burden, driven substantially by the MSK conditions [6]. SDG 3.4 targets are fixed to 2030, and global progress on achieving them is slow and recognised as offtrack [24]. This same context applies to the SDG target 3.8 for Universal Health Coverage [25]. Therefore, the reality is that countries and the World Health Organization have limited appetite and capacity to direct health system strengthening resources towards prevention and management of MSK conditions. This situation is reflected in the recent Political Declaration endorsed by Member States at the fourth high-level meeting of the UN General Assembly on the prevention and control of NCDs and promotion of mental well-being [26]. The declaration reaffirmed countries' commitments to act on a range of NCDs, yet failed to mention MSK conditions or chronic primary pain syndromes, despite these conditions being the leading contributors to years lived with disability globally and in most countries. Is the situation futile? No, it isn't. This context highlights the critical need for the global MSK health community to unite around an advocacy agenda to prioritise MSK conditions in global health targets beyond 2030 and in countries' evolving public health and NCD policies and strategies. There are already examples of national leadership for MSK-specific policy and integration of MSK health within broader NCD policies, although these are largely limited to high-income nations [27,28]. While some of these examples and systems in Denmark may be transferable to other countries, it is likely that transferability to the LMICs will be limited, where the evolution of policy to include MSK health remains nascent [29]. Yet, cocreated strategies and actions for countries on 'what' to do and 'how' to do it have been developed, including for LMICs [30]. Harnessing these resources and entry points from other global health agendas will be critical to catalysing global action to arrest the rising burden of MSK conditions—if not now, when?

Funding

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

Competing interests

Andrew M Briggs reports a relationship with the World Health Organization that includes consulting or advisory, employment, and travel reimbursement. Andrew M Briggs, Marita Cross, Anthony Woolf, and Lyn March report a relationship with the Global Alliance for Musculoskeletal Health that includes nonfinancial support.

Patient consent for publication

Not applicable.

Ethics approval

Not applicable.

Provenance and peer review

Not commissioned; externally peeer reviewed.

Orcid

Andrew M. Briggs: http://orcid.org/0000-0002-6736-3098
Marita Cross: http://orcid.org/0000-0003-2717-8960
Anthony D. Woolf: http://orcid.org/0000-0001-8482-8056
Lyn March: http://orcid.org/0000-0003-1736-8111

Andrew M. Briggs [1,2,*], Marita Cross [2,4], Anthony D. Woolf [2,3], Lyn March [2,4]
[1] *Curtin School of Allied Health, Faculty of Health Sciences, Curtin University, Perth, Australia*
[2] *Global Alliance for Musculoskeletal Health, Institute of Bone and Joint Research, Kolling Institute, The University of Sydney Faculty of Medicine and Health, Sydney, Australia*

[3] *Department of Clinical Sciences, Orthopedics, Lund University, Malmö, Sweden*
[4] *Florance and Cope Professorial Department of Rheumatology, University of Sydney Faculty of Medicine and Health, Kolling Institute, Royal North Shore Hospital, Sydney, New South Wales, Australia*

*Correspondence to Dr Andrew M. Briggs, Curtin School of Allied Health, Faculty of Health Sciences, Curtin University, GPO Box U1987, Perth, WA 6845, Australia.
E-mail address: A.Briggs@curtin.edu.au (A.M. Briggs).

REFERENCES

[1] United Nations. Report of the UN Economist Network for the UN 75th Anniversary Shaping the Trends of Our Time. New York: United Nations Department of Economic and Social Affairs, Population Division; 2020.
[2] United Nations Department of Economic and Social Affairs, Population Division. World Population Prospects 2022: Summary of Results. New York: Population Division, United Nations; 2022.
[3] GBD 2019 Ageing Collaborators. Global, regional, and national burden of diseases and injuries for adults 70 years and older: systematic analysis for the Global Burden of Disease 2019 Study. BMJ 2022;376:e068208.
[4] World Health Organization. UN Decade of Healthy Ageing: Plan of Action 2021-2030. Geneva: World Health Organization; 2020.
[5] Briggs AM, Cross MJ, Hoy DG, Sànchez-Riera L, Blyth FM, Woolf AD, et al. Musculoskeletal health conditions represent a global threat to healthy aging: a report for the 2015 World Health Organisation World Report on Ageing and Health. Gerontologist 2016;56(S2):S234–S55.
[6] Hay SI, Ong KL, Santomauro DF, Bhoomadevi A, Aalipour MA, Aalruz H, et al. Burden of 375 diseases and injuries, risk-attributable burden of 88 risk factors, and healthy life expectancy in 204 countries and territories, including 660 subnational locations, 1990-2023: a systematic analysis for the Global Burden of Disease Study 2023. Lancet 2025;406(10513):1873–922.
[7] Black RJ, Cross M, Haile LM, Culbreth GT, Steinmetz JD, Hagins H, et al. Global, regional, and national burden of rheumatoid arthritis, 1990-2020, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. Lancet Rheumatol 2023;5(10):e594–610.
[8] Cross M, Ong KL, Culbreth GT, Steinmetz JD, Cousin E, Lenox H, et al. Global, regional, and national burden of gout, 1990-2020, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. Lancet Rheumatol 2024;6(8):e507–e17.
[9] Ferreira ML, de Luca K, Haile LM, Steinmetz JD, Culbreth GT, Cross M, et al. Global, regional, and national burden of low back pain, 1990-2020, its attributable risk factors, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. Lancet Rheumatol 2023;5(6):e316–e29.
[10] Gill TK, Mittinty MM, March LM, Steinmetz JD, Culbreth GT, Cross M, et al. Global, regional, and national burden of other musculoskeletal disorders, 1990-2020, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. Lancet Rheumatol 2023;5(11):e670–e82.
[11] Steinmetz JD, Culbreth GT, Haile LM, Rafferty Q, Lo J, Fukutaki KG, et al. Global, regional, and national burden of osteoarthritis, 1990-2020 and projections to 2050: a systematic analysis for the Global Burden of Disease Study 2021. Lancet Rheumatol 2023;5(9):e508–e22.
[12] Wu A-M, Cross M, Elliott JM, Culbreth GT, Haile LM, Steinmetz JD, et al. Global, regional, and national burden of neck pain, 1990-2020, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. Lancet Rheumatol 2024;6(3):e142–e55.
[13] Guan S-Y, Zheng J-X, Feng X-Y, Zhang S-X, Xu S-Z, Wang P, et al. The impact of population ageing on musculoskeletal disorders in 204 countries and territories, 1990-2021: global burden and healthcare costs. Ann Rheum Dis 2025.
[14] Cheng X, Tan L, Gao Y, Yang Y, Schwebel DC, Hu G. A new method to attribute differences in total deaths between groups to population size, age structure and age-specific mortality rate. PLoS One 2019;14(5):e0216613.
[15] Chen N, Fong DYT, Wong JYH. Health and economic outcomes associated with musculoskeletal disorders attributable to high body mass index in 192 countries and territories in 2019. JAMA Netw Open 2023;6(1):e2250674.
[16] World Health Organisation. World Report on Ageing and Health. Geneva: WHO; 2015.
[17] Briggs AM, Sumi Y, Banerjee A. The World Health Organization guideline for non-surgical management of chronic primary low back pain in adults: implications for equitable care and strengthening health systems globally. Glob Health Res Policy 2025;10(1):26.
[18] World Health Assembly. Framework on integrated, people-centred health services: report by the Secretariat. Geneva: World Health Organization; 2016.
[19] World Health Organization. Integrated care for older people (ICOPE) implementation framework: guidance for systems and services. Geneva: WHO; 2019.
[20] World Health Organization. Package of interventions for rehabilitation: module 2: musculoskeletal conditions. Geneva: WHO; 2023.
[21] World Health Organization. WHO guideline for non-surgical management of chronic primary low back pain in adults in primary and community care settings. Geneva: WHO; 2023.
[22] World Health Organization. Integrated care for older people (ICOPE): guidance for person-centred assessment and pathways in primary care. 2nd ed. Geneva: World Health Organization; 2024 CC BY-NC-SA 3.0 IGO.
[23] World Health Organization, Regional Office for South-East Asia. South-East Asia Regional Strategy on Healthy Ageing: 2024–2030. Delhi: World Health Organization, Regional Office for South-East Asia; 2025.
[24] NCD Countdown 2030 collaborators. NCD Countdown 2030: efficient pathways and strategic investments to accelerate progress towards the Sustainable Development Goal target 3.4 in low-income and middle-income countries. Lancet. 2022;399(10331):1266–78.
[25] World Health Organization and International Bank for Reconstruction and Development/The World Bank. Tracking universal health coverage: 2023 global monitoring report. Geneva: World Health Organization and the International Bank for Reconstruction and Development/The World Bank; 2023.
[26] United Nations General Assemby. Political declaration of the fourth high-level meeting of the General Assembly on the prevention and control of noncommunicable diseases and the promotion of mental health and well-being. Equity and integration: transforming lives and livelihoods through leadership and action on noncommunicable diseases and the promotion of mental health and well-being. New York: General Assembly of the United Nations; 2025.
[27] Briggs AM, Persaud JG, Deverell ML, Bunzli S, Tampin B, Sumi Y, et al. Integrated prevention and management of non-communicable diseases, including musculoskeletal health: a systematic policy analysis among OECD countries. BMJ Glob Health 2019;4(5):e001806.
[28] Huckel Schneider C, Parambath S, Young JJ, Jain S, Slater H, Sharma S, et al. From local action to global policy: a comparative policy content analysis of national policies to address musculoskeletal health to inform global policy development. Int J Health Policy Manag 2022;12(1):1–20.
[29] Briggs AM, Jordan JE, Sharma S, Young JJ, Chua J, Foster HE, et al. Context and priorities for health systems strengthening for pain and disability in low- and middle-income countries: a secondary qualitative study and content analysis of health policies. Health Policy Plan 2023;38(2):129–49.
[30] Briggs AM, Huckel Schneider C, Slater H, Jordan JE, Parambath S, Young JJ, et al. Health systems strengthening to arrest the global disability burden: empirical development of prioritised components for a global strategy for improving musculoskeletal health. BMJ Glob Health 2021;6(6):e006045.

Ann Rheum Dis 85 (2026) 227–234

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Review

How good enough are the new therapies for lupus nephritis? A narrative review and a position paper

Frédéric A. Houssiau [1,2,*]

[1] *Service de Rhumatologie, Cliniques universitaires Saint-Luc, Brussels, Belgium*
[2] *Institut de Recherche Expérimentale et Clinique, UCLouvain, Belgium*

ARTICLE INFO

Article history:
Received 27 July 2025
Received in revised form 23 September 2025
Accepted 5 October 2025

ABSTRACT

In this narrative review, the author, a privileged witness to exciting developments in the field of lupus nephritis over almost 4 decades, describes and comments on the new therapies that have recently become available, after years of trial failures. He stresses the use of combination therapy, now widely supported by expert recommendations to mitigate the risk of chronic kidney disease. He reviews the early days of cellular therapy and proposes some further lines of research.

LUPUS NEPHRITIS IN A NUTSHELL

According to a US population study using nationwide death certificates performed between 2000 and 2015, systemic lupus erythematosus (SLE) is the seventh cause of mortality in women, after exclusion of unintentional injury, homicide, and suicide. These figures are even worse in US Black or Hispanic women, with SLE being the fifth cause of mortality in this age range [1]. Lupus nephritis (LN) is the most common severe manifestation of SLE and thereby largely contributes to this excess mortality. It affects 40% of adults with SLE and up to 80% of children. Despite major progress over the last 3 decades, 20% of patients with LN, even those followed in expert centres, still develop chronic kidney disease (CKD) stage G3 (CKD defined by an estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m^2). Between 5% and 10% will develop end-stage kidney disease (ESKD) in the (very) long term. These figures are likely much worse in countries where access to care is not universal.

It should be stressed that all patients with LN, even those presenting with a 'normal' eGFR, suffer from chronic disease of the kidneys because any episode of LN impacts the nephron cargo. This might stay unnoticed because compensatory mechanisms operate in the remaining nephrons (including hyperfiltration) and thereby maintain a 'normal' eGFR. Yet, this undetected loss of nephrons will contribute to the physiological loss related to aging and thereby compromise renal function in the very long term. This basic concept should drive our efforts to identify kidney involvement in patients with SLE as soon as possible, to spot patients at greater risk for CKD progression, and to promptly start treatment combining (1) optimal nephroprotection; (2) standard immunosuppression with glucocorticoids (GCs) and other immunosuppressants (ISs); and (3) targeted therapies.

SHORT HISTORICAL PERSPECTIVE

In 1950, Philip Hench, Edward Kendall, and Tadeusz Reichstein were granted the Nobel Prize in Physiology or Medicine for their serendipitious discovery of the benefits of GC in patients with rheumatic disease. I urge you to read their 'Preliminary report' [2] and Hench's Nobel Lecture (search 'Nobel Lecture of Philip Hench' on the web)! Steroids still save more lupus lives than any other drug in the acute phase. Yet, steroid therapy, when used alone, turned out to be insufficient in the long term to treat kidney involvement.

Pivotal LN trials performed by investigators from the National Institutes of Health (NIH) demonstrated the superiority of high-dose intravenous (IV) pulse cyclophosphamide (CY) given monthly for 6 months, then quarterly for 2 to 3 additional years [3–6]. Although this regimen, used for 2 decades, clearly contributed to saving kidneys, it has been associated with an

DOI of original article: http://dx.doi.org/10.1016/j.ero.2025.07.006.

*Correspondence to Prof. Frédéric A. Houssiau, Service de Rhumatologie, Cliniques universitaires Saint-Luc, Brussels, Belgium.
E-mail address: frederic.houssiau@uclouvain.be

Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.10.004

unacceptable rate of premature gonadal failure (38%-52%), compromising further parenthood in young ladies [7].

Since the NIH studies, we have experienced 3 major breakthroughs. First, a switch from the high-dose NIH CY regimen to the low-dose Euro-Lupus regimen (6 fixed doses of 500 mg IV CY given every 2 weeks followed by azathioprine), which was shown, in the *Euro-Lupus Nephritis Trial*, to be equally efficacious, including in the long term [8,9], without impacting the ovarian reserve [10]. Second, several controlled trials demonstrated the benefits of mycophenolate mofetil (MMF) given in combination with GC, both to induce a response [11,12] and to avoid further flares [13]. The excellent efficacy/toxicity profile of MMF explains why this drug is nowadays the most widely prescribed IS for LN, although some physicians still prefer low-dose IV CY in the first month of therapy to ensure full adherence. Third, long avoided because of their potential nephrotoxicity, calcineurin inhibitors (CNIs), especially tacrolimus (TAC) combined to MMF, came into play [14], taking advantage of their major antiproteinuric properties.

Although IV CY, MMF, and TAC were never labelled by medical agencies, these drugs have been recommended by several scientific societies as treatment of LN [15,16]. Thus, in 2019, the European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) advised either low-dose Euro-Lupus IV CY or MMF, combined to GC (IV methylprednisolone [MP] pulses followed by oral prednisolone or equivalent), hydroxychloroquine, and optimal nephroprotection [15]. By no doubts, implementing these recommendations has improved the prognosis of LN. Yet, results are far from optimal, even in expert centres, with still 20% of patients never achieving complete renal remission (CRR), 35% experiencing renal relapses, and 20% reaching CKD G3 (our own unpublished data on 283 patients with LN).

To improve these figures, many targeted therapies have been tested over the last 2 decades, based on a better knowledge of the pathophysiology of LN, tackling anti-DNA antibodies, lymphoid cells, cytokines or costimulatory signals. At risk of forgetting some, one can mention tetrameric oligonucleotide abetimus sodium, anti-CD20 rituximab (RTX), anti-CD20 ocrelizumab, anti-CD80/86 abatacept, dual BLyS/APRIL inhibitor atacicept, anti-CD40L BG9588, anti-CD40 BI 655064, and anti-IL17 secukinumab. None of the corresponding trials met its primary endpoint, including the *LUNAR* RTX trial, the most disappointing ever [17]. The many reasons underlying these failures were discussed elsewhere [18].

NEW THERAPIES, AT LAST!

These failures did not discourage investigators and the pharma industry to test other new drugs in LN, always used as add-on therapy, ie, on top of standard of care (SOC), namely GC and either MMF or low-dose IV CY. In brief, phase 3 LN trials with anti-BLyS belimumab (BEL), CNI voclosporin (VOC), and anti-CD20 obinutuzumab (OBI) met their primary endpoint, and the corresponding molecules were recently (or will be soon) labelled as treatment for LN.

Short cogent summary of recent LN trials

In the *BLISS-LN* trial, class III ± V, IV ± V, or V patients with LN were treated with BEL or placebo (PBO) on top of SOC (either MMF or low-dose IV CY) [19,20]. After 2 years, addition of BEL increased the CRR rate by 10.3%, reduced renal flare rate by 55%, allowed GC spare, and positively impacted the eGFR trajectory, with very low toxicity, as expected from the experience gained over >10 years in nonrenal patients.

In the *AURORA* trial, class III ± V, IV ± V, and V patients with LN were treated with VOC or PBO on top of SOC (MMF) [21]. VOC displays 3 advantages compared with first-in-class CNI ciclosporin: more potent inhibition of calcineurin, less deleterious effects on glucose, and lipid metabolism and more homogeneous pharmacokinetics across patients, making blood level monitoring unnecessary. VOC, tested with a very low and promptly tapered GC regimen, increased CRR rate by 18% after 1 year, without deleterious effects on eGFR or on the chronicity index on posttreatment repeat kidney biopsies performed in a small subset of patients [22]. Renal flares were not prevented by the addition of VOC. The jury is still out as to whether the main effect of the drug stems from its potent antiproteinuric properties, keeping in mind that reduction of proteinuria is the major driver of CRR.

In the *REGENCY* trial, class III ± V or IV ± V patients with LN were treated with OBI or PBO on top of SOC (MMF) [23]. OBI is a glycoengineered type II monoclonal antibody (mAb). The presence of nonfucosylated sugars in its Fc region increases its affinity for the FcγRIIIa expressed on effector killer cells (eg, macrophages), which are responsible for B-cell depletion through antibody-dependent cellular cytotoxicity. The glycosylation pattern of OBI minimizes the differences in binding capacities linked to FcγRIIIa polymorphisms, which explains different levels of B-cell depletion between individuals treated with RTX, a nonglycoengineered anti-CD20 mAb [24]. After 76 weeks, OBI increased the CRR rate by 13.3%, but we do not have data yet on its effect on the eGFR trajectory. Of note, more severe infectious adverse events, mostly COVID-19 pneumonias, were observed because patients were recruited during the pandemic, reminding that B-cell depletion severely jeopardizes antiviral immune responses, as well as responses to vaccines.

Good enough?

In brief, the new therapies for LN induce more CRR in the short term and thereby likely less CKD progression in the long term, allow GC spare, have not been associated with major toxicity, and might even be cost-effective in the very long term.

At the first glance, a gain of only 10% to 18% in CRR rate with BEL, VOC, or OBI might seem marginal. In this respect, it should be stressed that patients who never reach CRR during prolonged follow-up run a 3-fold higher risk of CKD progression long term [25]. Therefore, any increase in CRR rate after 1 to 2 years of treatment is most welcome. Similarly, a positive impact on the eGFR trajectory and, even more importantly, a reduction in the number of patients with a sustained 30% to 40% eGFR reduction over time (a true surrogate for ESKD met with BEL) augur less ESKD in the long term.

Another milestone achievement of the new targeted therapies is that they allow GC spare. Thus, more patients treated with BEL (compared with SOC alone) achieved CRR together with a reduction of daily prednisolone dose <7.5 mg or <5 mg, with figures diverging from week 40 onwards. Similarly, more patients treated with OBI (compared with SOC alone) achieved CRR together with reduction of daily prednisolone dose <7.5 mg (42.7% vs 30.9%). GC spare was also achieved with VOC based on the use of a very low prednisolone starting daily dose (20-25 mg), very promptly tapered to 2.5 mg/day already after 16 weeks of treatment.

Severe adverse events (SAEs) were not more common with BEL + SOC (26%) vs SOC alone (30%) nor for VOC + SOC (21%)

vs SOC alone (21%). The same holds true for infectious SAE (15% with BEL + SOC vs 18% with SOC alone; 11% with VOC + SOC vs 10% with SOC alone). By contrast, as already mentioned *supra*, addition of OBI to SOC increases the risk of SAE (32% vs 18%) and of infectious SAE (17% vs 8%).

A very rough cost-benefit estimate, based on the Belgian health system (not a proper pharmacoeconomic analysis), suggests that combination therapy might be breakeven in the long term. Let us suppose that of 100 patients with LN, 10 will develop ESKD despite SOC, but only 7 (30% reduction) if add-on therapy is prescribed for 2 years. In my country, a 2-year treatment with subcutaneous BEL costs 23K€/patient, which would mean 2300K€ for a cohort of 100 patients. Renal replacement therapy by dialysis costs 50K€/year, which means, for 10 patients in ESKD for 15 years, a cost of 7500K€. A reduction from 10 to 7 patients requiring dialysis for 15 years would reduce the global costs of dialysis for the initial cohort of 100 patients by 2250 K€ (3 patients × 15 years × 50K€). These savings on dialysis costs exactly compensate the amount paid to treat 100 patients for 2 years with BEL. This purely financial discussion, which is illustrative and not applicable to other countries with different private-public health insurance systems, ignores even more important benefits for patients escaping renal replacement therapy, in terms of comorbidities and quality of life.

Despite this evidence, it should be remembered that—at best—only 40% of patients with LN experience CRR after initial therapy, thereby indicating that there is still room for further improvement, although a ceiling effect could dampen our hopes. On another note, we miss histological data obtained through the protocol repeat kidney biopsy performed after treatment. The primary outcome measure used in most LN trials, namely CRR, is driven by proteinuria. And yet we know that proteinuria results not only from active inflammation but also from damage, which means that proteinuria is not a sufficiently reliable witness of what happens in the kidneys [26]. Hereby, I strongly advocate that per-protocol posttreatment repeat biopsies be performed in LN trials to convince us that the new drugs modulate the cellular and molecular pathways at work.

NEW RECOMMENDATIONS, WITH A PINCH OF SALT

The results of the new trials have been recently implemented in the American College of Rheumatology (ACR) 2024 guideline for the screening, treatment, and management of LN [27] and in the EULAR 2025 recommendations for the treatment of SLE with kidney involvement [28]. Suffice is to say that they concur to propose combination therapy in all patients! Rather than comparing the 2 sets of recommendations head-to-head (which will be made anyway by others), we will outline some pivotal points.

Combination rather than triple therapy

Although this might seem rather semantic, the term “triple therapy” (GC, IS, and targeted drug) used in the ACR guideline is somewhat misleading because treatment of LN also includes hydroxychloroquine, antiproteinuric drugs, drugs used to prevent GC-associated side effects, etc. We favour the wording ‘combination therapy’, used in EULAR recommendations.

Combination for all

Combination therapy is now proposed to all patients with LN whenever feasible. This raises 2 issues. On the one hand, in most low-income and lower-middle-income countries, patients will have no access to these new expensive therapies, while LN is more severe in many of these areas of the world due to genetic predisposition (such as pathogenic *APO-L1* alleles) and delayed diagnosis. This ethical question could be solved, eg, by obtaining large discounts on these medications for low-income countries. On the other hand, in upper-middle-income and high-income countries, where most patients with LN can be offered combined therapy following current recommendations, another question arises: do all patients with LN really need combination therapy? My answer is straightforward: not all need it, but the problem is that we are not smart enough (with current biomarkers *lato sensu*) to identify those very patients who would benefit from combination therapy because baseline clinical and pathological parameters do not discriminate patients with a good or poor long-term outcome [29,30]. It has been proposed to treat 3 months with SOC before deciding who should benefit from an add-on therapy, ie, those patients who do not reduce their proteinuria by at least 25% at month 3 [31]. Alas, according to our own Louvain cohort, this criterion does not discriminate between patients who or will not suffer from renal impairment in the long-term (unpublished data). Conversely, data from our trials and real-world series indicate that one must wait until month 12 of treatment to identify patients with good or poor outcome 7-10 years later [32,33], with the risk of missing a window of opportunity, especially in those not reaching the 12-month proteinuric target. In other words, it is correct to state that some patients will be overtreated, but this should be weighed against the risk of CKD progression/ESKD and their dire consequences in terms of morbidity and mortality.

Please pulse

The recommendations stress the importance of IV pulse MP therapy. In this respect, a systematic review and meta-analysis recently performed on 3231 patients randomised in the control arms of LN trials recently demonstrated that a similar rate of CRR could be achieved by 25 mg/d prednisolone administered together with IV MP pulses compared with 40 mg/d prednisolone without IV MP, thereby proving the steroid-sparing effect of IV MP. Quite importantly, addition of IV MP pulses did not impact the serious infection rate. This analysis also confirms that the use of a starting daily prednisolone dose ≥40 mg greatly increases the risk of infections [34].

Withdraw steroids

On the same note, the recommendations stress the need to promptly reduce GC and possibly withdraw them, in any case before stopping the other IS. The reasons for GC withdrawal are manyfold. In a recent real-world nationwide population-based study using the French medico-administrative claim database, the use of as low a daily dose as 5 mg prednisolone was associated with an increased risk of cardiovascular diseases, infections, and osteoporosis [35]. This, again, illustrates, that GCs are the elephant in the room! That the so-called *Rituxilup* protocol (RTX, IV MP and no oral GC) [36] could not be validated in a controlled trial was a shame but it paved the way to the currently recruiting *Obilup* protocol, which will fill in the gap (NCT04702256). Patients with LN are randomised to receive

either a combination of IV MP, MMF, and oral GC or a combination of IV MP, MMF, and OBI (no oral GC), with the hope that OBI will be a welcome replacement for oral GC therapy. This would be a milestone achievement.

Tacrolimus propelled to the top

Somewhat surreptitiously, TAC moved from an outsider status to a front-runner, taking advantage of the positive trial obtained with his classmate VOC. In the EULAR/ERA-EDTA 2019 recommendations [15] and in the KDIGO 2021 guidelines [16], CNIswere considered as a second choice, despite some positive TAC trials mainly in Asian patients with LN [14]. TAC is now considered on the same line as VOC by both ACR [27] and EULAR [28]. In other words, VOC took TAC to the top! This side benefit of the AURORA trial is good news not only for low-income countries but also for the wealthier countries in which VOC was refused reimbursement by the health authorities. For those, TAC will be the VOC of the poor!

Common sense will guide choices

The choice between different (current and future) combination therapies can hardly be pigeonhole in recommendations. Unless we can rely on biomarkers accurately predicting the response to a given combination, we will largely proceed by a trial and error approach, using our clinical common sense, considering some of the principles mentioned in the ACR 2024 guidelines, but mainly considering patient's history, previous use of drugs, and comorbidities. At the bedside, choices will be made case per case, using a patient-physician shared decision process.

Long is better than short

The decision to stop IS is always difficult because the topic has been understudied. It has been known for 20 years that IS therapy can be withdrawn in patients in remission and, not unexpectedly, that a long period of treatment and remission before withdrawal is associated with a lower risk of relapse [37]. In the more recent randomised *WIN-Lupus* trial, performed in patients with LN in stable renal remission for 2-3 years, IS treatment was stopped in half of the patients and further continued in the others. Renal relapse rate was twice higher in patients who discontinued (27.3% vs 12.5%) [38]. Another recent retrospective analysis performed on 303 patients with LN demonstrated that sustained clinical remission protects against CKD progression. The longer the sustained remission, the higher the chance of its persistence and the lower risk of flares [39]. This explains why, altogether, the EULAR 2025 recommendations propose at least 3 years of treatment and the ACR 2024 guideline 3 to 5 years. The picture becomes more complicated with the new therapies because we do not have enough hindsight with the recent new drugs to evaluate their long-term toxicity. This is especially true for OBI as long-term extension studies are still in the pipeline.

More attention to pure membranous LN

Pure class V membranous LN (between 10% and 20% of all LN cases) were included in the *BLISS-LN* and the *AURORA* trials (but not in the *REGENCY* trial). The small numbers do not allow to draw conclusions but, despite few data are available, today's trend is to treat these patients very much like 'proliferative' (I prefer 'hypercellular') class III/IV cases. The proteinuria threshold justifying intervention beyond nephroprotection and hydroxychloroquine has been considerably lowered in the ACR 2024 compared with the KDIGO 2021 guidelines. Gone are the days when we started thinking about prescribing IS in pure membranous LN when the patient was nephrotic. Combination therapy should be offered in patients when proteinuria becomes >1 g/d. In such patients, CNIs could be the first choice.

Beyond immunosuppression

Rather than lengthy discussions on the respective benefits of a given combination therapy over another, more time should be spend to (try to) optimise adherence to therapy, to prevent side effects of IS therapy and to implement all measures of nephroprotection, such as optimal blood pressure control, antiproteinuric treatment, SGLT-2 inhibitors, salt intake restriction, avoidance of nephrotoxic drugs, exercise, lipid control, smoking cessation, and weight control. This takes much time but makes such a difference.

AN ELECTRIFYING CAR-T CELL PAPER IN NATURE MEDICINE

Everything has been written about this CD19-CAR-T cell paper from the Erlangen group showing dramatic efficacy in 5 refractory patients with LN [40] and paving the way for cellular therapy in SLE, LN, and other autoimmune diseases (AIDs). It was followed by several other series from the same team [41] and other groups [42]. More than a dozen pharma trials have been launched, testing various CAR-T cell products and competing for refractory patients. We seldom experienced such an enthusiastic lupus research drive. Yet and not surprisingly, additional data [43–45] indicate that not all patients experience full remission as initially described and that rare SAEs, such as macrophage activation syndrome and severe infections, must be deplored. Many other issues need to be addressed, such as feasibility, costs, patient selection, long-term efficacy, long-term adverse events, type of CAR construct transfected in T cells, need for lymphodepletion, possibility of retreatment, off-the-shelf allogenic CAR-T cells, CAR-NK cells; all work in progress but not the topic of this narrative review. Nevertheless, the profound B-cell depletion observed in lymph nodes, together with major architecture disruption (loss of follicular dendritic cell and T follicular helper cells) [46], suggests that CAR-T cell therapy suppresses and/or restores key dysregulated paths.

OTHER LN DRUGS IN THE PIPELINE

The potential of CAR-T cell therapy should not overshadow other promising lines of research. Thus, the efficacy of anti-interferon type I receptor mAb anifrolumab (ANI), registered so far for nonrenal lupus [47,48], breached ground for testing the drug in LN, the more so as we know that an IFN signature is detected in most lupus kidneys [49]. After a promising phase 2 *TULIP-LN* study [50], ANI is currently tested in a phase 3 *IRIS* LN trial. Anti-CD19 inebilizumab and anti-BAFF-R ianalumab are also currently evaluated in LN. Other LN trials may start soon with SP1-receptor modulator cenerimod, anti-CD38 daratumumab, or anti-CD40-L dapirolizumab pegol.

WILL BITES BEAT CAR-TS?

Bispecific antibodies are engineered mAb with dual binding sites designed to bring different cell types together, one arm binding to tumour cells (or other cells one wishes to eliminate), whereas the other targets immune cells such as T cells or NK cells, resulting in killing of the undesirable cell through T cell or NK-cell machinery in an antigen/HLA-class II independent way. The terms BiTE (bispecific T-cell engager) and bispecific killer engager (BiKE) are used when T cells and NK cells are the killer effector cells, respectively. BiTEs are currently evaluated as potential treatment for AIDs where B-cells are purported to play a central role. Thus, blinatumomab, a CD3/CD19 BiTE, labelled for the treatment of refractory acute B-lymphoblastic leukaemia, was shown clinically efficacious in 6 patients suffering from rheumatoid arthritis, with strong remodelling of peripheral blood B-cells, ie, depletion of activated memory B-cells replaced by nonclass-switched IgD-positive naive B-cells [51]. Teclistamab, a CD3/BCMA BiTE, was reported to induce remission in a refractory case of LN [52] and to improve disease activity in 4 other cases of AID [53]. A phase 1 trial testing the toxicity/efficacy of a CD20/TCR/CD8 BiTE is recruiting patients with AID, with the hope that killing of B-cells through CD8 T-cell recruitment will lead to less cytokine-release syndromes.

Altogether, although CAR-T cell therapy led to a revival of B-cell depletion in LN, the possibility that BiTEs could beat CAR-T cells is not too far-fetched, given the intrinsic difficulties of CAR-T cell therapy, such as an expensive and time-consuming process, the need for a conditioning regimen, and the difficulties for retreatment in case of relapse. Although such difficulties should be avoided with BiTEs, we critically need data to support the hypothesis that BiTEs will be part of the therapeutic arsenal against LN.

CLUES FOR BETTER CARE

Earlier detection of kidney involvement in patients with SLE, individualised treatment based on molecular and cellular signatures, early identification of poor responders through a per-protocol posttreatment repeat kidney biopsy or through repeated measures of urinary biomarkers (liquid biopsy), and worldwide availability of new therapies are the major challenges for the next years.

Early detection of kidney involvement

The ACR 2024 and EULAR 2025 recommendations advise performing a kidney biopsy when proteinuria is ≥0.5 g/d. However, in the real world, biopsies are mostly performed in patients with higher proteinuria levels for various reasons, including the time needed to control a first abnormal result, to convince the patient that a biopsy is needed and to obtain an appointment for the procedure. Moreover, most clinical studies impose a higher proteinuria cut-off as inclusion criteria, mostly ≥1 g, if not ≥1.5 g, with a biopsy performed within a given time frame before randomisation, thereby possibly postponing the diagnosis (and the treatment) of LN. These delays might explain why chronic changes, such as glomerular and interstitial fibrosis, are detected in the so-called 'new' cases of LN, suggesting that the process is at work for several months. One should at least strictly comply with the 0.5 g/d proteinuria rule. A bolder proposal is to biopsy all patients with SLE with a supraphysiologic proteinuria (ie, >0.15 g/d), especially those with high serological activity, such as low serum complement levels and high titres of anti-DNA antibodies. In the future, newly identified urinary biomarkers (*vide infra*) might help select patients with low-grade proteinuria who should benefit from a kidney biopsy.

Precise medicine

Although targeted therapies for LN have been eagerly awaited, patients and physicians now face another difficult choice, moving from a 'no drug nightmare' to a 'which drug dilemma'. Should BLyS or type I IFNs be blocked? Should B-cells be depleted? Should T-cells be targeted? *Mutatis mutandis*, the jury is still out as to whether a rheumatoid arthritis patient should be given a tumour necrosis factor or an IL6(R) blocker, a JAK inhibitor or a B-cell depleting drug, despite very smart work performed on synovial biopsies, applying the best-omics technologies aimed at identifying cellular and molecular pathways at work to select the most appropriate treatment [54–56], rather than using a standard trial and error approach. We need a similar theranostic approach in LN, possibly supported by artificial intelligence–driven analyses of kidney biopsies. This said, the picture is much more sophisticated in autoimmunity than in cancer, where germline or somatic mutations have been identified which can be specifically targeted. In other words, we might miss the Holy Grail!

Early identification of poor responders

Since we cannot currently assign at first attempt *the* most appropriate combination therapy based on a validated algorithm, an early treatment switch should be decided in case of nonattainment of a validated target known to predict good long-term renal outcome. As already alluded to, a proteinuric target is not ideal as proteinuria reflects both inflammation and damage and does not perfectly match histopathology findings; some patients remain proteinuric without active disease, whereas others display activity without overt proteinuria [26]. Posttreatment per-protocol repeat kidney biopsies, performed independently of the clinical evolution, might help identify poor responder patients in which a treatment switch would be welcome. This is the rationale of the international *ReBioLup* study (www.rebiolup.com) performed under the leadership of the UCLouvain and the Karolinska Institutet. In this study, patients are treated according to SOC. By randomisation, half of them undergo a per-protocol repeat kidney biopsy after 12 months of treatment. If the activity index at the repeat biopsy is >3, treatment is intensified, with the hope to achieve more CRR at month 24 and less CKD G3 at year 5, because knowledge of residual disease activity will have implemented appropriate treatment changes at month 12. Repeated measurements of urinary biomarkers over time recently gained a lot of credit thanks to the possibility of measuring thousands of urine proteins by high-throughput proteomics [57]. The advantages of a liquid biopsy over a tissue biopsy are manifold, beyond obvious logistic and security issues. A liquid biopsy can be repeated and theoretically tests the full nephron cargo. A panel of 12 biomarkers, including CD163 [58] and IL16 [59], is currently being validated as a potential predictor of long-term renal outcome [60].

Worldwide availability

Many patients with LN live in low-income countries, where lupus is more severe than in other parts of the world. Sadly, access to new lupus drugs (and to care in general) is very limited in these countries, and their price is paradoxically similar, if not

higher, than in high-income countries [61]. As a result of this paradox, more GCs are used leading to more infections and damage accrual, exactly the opposite of what is observed in wealthy countries. Although there is no universal clue to solve this access to care, the role of scientific societies should not be overlooked, not only in the field of education but also in management. Thus, in a completely different domain, the World Federation of Haemophilia has developed a unique expanded humanitarian aid program with coagulation products donated by the pharma industry, allowing it to treat >20,000 severe haemophiliacs in low/middle-income countries [62]. Everything is different between LN and severe haemophilia, but the concept of supporting the care of patients living in low/moderate-income countries should become a daily concern.

CONCLUSION

Being involved in LN research for the last 35 years, I have been a privileged witness to all therapies developed over almost 4 decades. Yet, I never experienced such exciting times as in the past 5 years, as illustrated by the fact that more than half of the references quoted in this narrative review are dated 2020 onwards! Three drugs have been (will be) specifically labelled for LN, with many others in the pipeline. Even cellular therapy might be on board. Gone is the time when lupus remained 'le parent pauvre' of the rheumatic diseases, because all trials with targeted therapies failed. This remarkable achievement is attributable to outstanding science, persistence of patients, researchers, and clinicians, and exemplary cooperation with patients and between different medical specialties.

Fugit irreparabile tempus, augebitur scientia.

Competing interests

FAH reports a relationship with GSK that includes: consulting or advisory, funding grants, and paid expert testimony. FAH reports a relationship with Roche that includes: funding grants. FAH reports a relationship with AstraZeneca Pharmaceuticals LP that includes: consulting or advisory. FAH reports a relationship with Otsuka Pharmaceutical Co Ltd that includes: consulting or advisory. FAH reports a relationship with Biogen that includes: consulting or advisory. FAH reports a relationship with Idorsia Pharmaceuticals Ltd that includes: consulting or advisory. FAH is a member of the Editorial Board of *Annals of the Rheumatic Diseases.*

CRediT authorship contribution statement

Frédéric A. Houssiau: Writing – review & editing, Writing – original draft, Conceptualization.

Acknowledgements

We are very grateful to Dr Antoine Enfrein for the critical reading of this manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Patient consent for publication

Not applicable.

Ethics approval

Not applicable.

Provenance and peer review

Commissioned and peer reviewed.

Orcid

Frédéric A. Houssiau: http://orcid.org/0000-0003-1451-083X

REFERENCES

[1] Yen EY, Singh RR. Brief report: Lupus – an unrecognized leading cause of death in young females: a population-based study using nationwide death certificates, 2000-2015. Arthritis Rheumatol 2018;70:1251–5.
[2] Hench PS, Kendall EC, Slocumb CH, Polley HF. The effect of a hormone of the adrenal cortex (17-hydroxy-11-dehydrocorticosterone: compound E) and of pituitary adrenocortical hormone in arthritis: preliminary report. Ann Rheum Dis 1949;8:97–104.
[3] Austin 3rd HA, Klippel JH, Balow JE, le Riche NG, Steinberg AD, Plotz PH, et al. Therapy of lupus nephritis: controlled trial of prednisone and cytotoxic drugs. N Engl J Med 1986;314:614–9.
[4] Boumpas DT, Austin 3rd HA, Vaughn EM, Klippel JH, Steinberg AD, Yarboro CH, et al. Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. Lancet 1992;340:741–5.
[5] Gourley MF, Austin 3rd HA, Scott D, Yarboro CH, Vaughan EM, Muir J, et al. Methylprednisolone and cyclophosphamide alone or in combination, in patients with lupus nephritis. A randomized, controlled trial. Ann Intern Med 1996;125:549–57.
[6] Illei GG, Austin HA, Crane M, Collins L, Gourley MF, Yarboro CH, et al. Combination therapy with pulse cyclophosphamide plus pulse methylprednisolone improves long-term renal outcome without adding toxicity in patients with lupus nephritis. Ann Intern Med 2001;135:248–57.
[7] Boumpas DT, Austin 3rd HA, Vaughan EM, Yarboro CH, Klippel JH, Balow JE. Risk for sustained amenorrhea in patients with systemic lupus erythematosus receiving intermittent pulse cyclophosphamide therapy. Ann Intern Med 1993;119:366–9.
[8] Houssiau FA, Vasconcelos C, D'Cruz D, Sebastiani GD, de Ramon Garrido Ed E, Danieli MG, et al. Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. Arthritis Rheum 2002;46:2121–31.
[9] Houssiau FA, Vasconcelos C, D'Cruz D, Sebastiani GD, de Ramon Garrido E, Danieli MG, et al. The 10-year follow-up of the Euro-Lupus Nephritis Trial comparing low-dose and high-dose intravenous cyclophosphamide. Ann Rheum Dis 2010;69:61–4.
[10] Tamirou F, Husson SN, Gruson D, Debiève F, Lauwerys BR, Houssiau FA. Brief report: the Euro-Lupus low-dose intravenous cyclophosphamide regimen does not impact the ovarian reserve, as measured by serum levels of anti-Müllerian hormone. Arthritis Rheumatol 2017;69:1267–71.
[11] Chan TM, Li FK, Tang CS, Wong RW, Fang GX, Ji YL, et al. Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. Hong Kong-Guangzhou Nephrology Study Group. N Engl J Med 2000;343:1156–62.
[12] Ginzler EM, Dooley MA, Aranow C, Kim MY, Buyon J, Merrill JT, et al. Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis. N Engl J Med 2005;353:2219–28.
[13] Dooley MA, Jayne D, Ginzler EM, Isenberg D, Olsen NJ, Wofsy D, et al. Mycophenolate versus azathioprine as maintenance therapy for lupus nephritis. N Engl J Med 2011;365:1886–95.
[14] Liu Z, Zhang H, Liu Z, Xing C, Fu P, Ni Z, et al. Multitarget therapy for induction treatment of lupus nephritis: a randomized trial. Ann Intern Med 2015;162:18–26.
[15] Fanouriakis A, Kostopoulou M, Cheema K, Anders H-J, Aringer M, Bajema I, et al. 2019 update of the joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of lupus nephritis. Ann Rheum Dis 2020;79:713–23.
[16] KDIGO glomerular diseases work group. KDIGO 2021 clinical practice guideline for the management of glomerular diseases. Kidney Int 2021;100:S1–276.

[17] Rovin BH, Furie R, Latinis K, Looney RJ, Fervenza FC, Sanchez-Guerrero J, et al. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the lupus nephritis assessment with rituximab study. Arthritis Rheum 2012;64:1215–26.
[18] Houssiau FA. Why will lupus nephritis trials not fail anymore? Rheumatology (Oxford) 2017;56:677–8.
[19] Furie R, Rovin BH, Houssiau F, Malvar A, Onno Teng YK, Contreras G, et al. Two-year, randomised, controlled trial of belimumab in lupus nephritis. N Engl J Med 2020;383:1117–28.
[20] Rovin BH, Furie R, Onno Teng YK, Contreras G, Malvar A, Yu X, et al. A secondary analysis of the belimumab international study in lupus nephritis trial examined effects of belimumab on kidney outcomes and preservation of kidney function in patients with lupus nephritis. Kidney Int 2022;101:403–13.
[21] Rovin BH, Onno Teng YK, Ginzler EM, Arriens C, Caster DJ, Romero-Diaz J, et al. Efficacy and safety of voclosporin versus placebo for lupus nephritis (AURORA-1): a double-blind, randomized, multicenter, placebo-controlled, phase 3 trial. Lancet 2021;397:2070–80.
[22] Rovin BH, Cassol C, Parikh SV, Saxena A, Solomons N, Birardi V, et al. Effect of long-term voclosporin treatment on renal histology in patients with active lupus nephritis with repeat renal biopsies. Arthritis Rheumatol 2025;77:1387–93.
[23] Furie RA, Rovin BH, Garg JP, Santiago MB, Aroca-Martínez G, Zuta Santillán AE, et al. Efficacy and safety of obinutuzumab in active lupus nephritis. N Engl J Med 2025;392:1471–83.
[24] Freeman CL, Sehn LH. A tale of two antibodies: obinutuzumab versus rituximab. Br J Haematol 2018;182:29–45.
[25] Pirson V, Enfrein A, Houssiau FA, Tamirou F. Absence of renal remission portends poor long-term kidney outcomes in lupus nephritis. Lupus Sci Med 2021;8:e000533.
[26] Parodis I, Adamichou C, Aydin S, Gomez A, Demoulin N, Weinmann-Menke J, et al. Per-protocol repeat kidney biopsy portends relapse and long-term outcome in incident cases of proliferative lupus nephritis. Rheumatology 2020;59:3424–34.
[27] Sammaritano LR, Askanase A, Bermas BL, Dall'Era M, Duarte-García A, Hiraki LT, et al. 2024 American College of Rheumatology (ACR) guideline for the screening, treatment, and management of lupus nephritis. Arthritis Rheumatol 2025;77:1115–35.
[28] Fanouriakis A, Kostopoulou M, Andersen J, Aringer M, Arnaud L, Bae S-C, et al. 2025 EULAR recommendations for the management of SLE with kidney involvement. Ann Rheum Dis 2025;83:15–29.
[29] Houssiau FA, Vasconcelos C, D'Cruz D, Sebastiani GD, de Ramon Garrido E, Danieli MG, et al. Early response to immunosuppressive therapy predicts good renal outcome in lupus nephritis: lessons from long-term followup of patients in the Euro-Lupus Nephritis Trial. Arthritis Rheum 2004;50:3934–40.
[30] Gatto M, Radice F, Saccon F, Calatroni M, Frontini G, Trezzi B, et al. Clinical and histological findings at second but not at first kidney biopsy predict end-stage kidney disease in a large multicentric cohort of patients with active lupus nephritis. Lupus Sci Med 2022;9:e000689.
[31] Mejia-Vilet JM, Malvar A, Arazi A, Rovin BH. The lupus nephritis management renaissance. Kidney Int 2022;101:242–55.
[32] Dall-Era M, Cisternas MG, Smilek DE, Straub L, Houssiau FA, Cervera R, et al. Predictors of long-term renal outcome in lupus nephritis trials: lessons learned from the Euro-Lupus nephritis cohort. Arthritis Rheumatol 2015;67:1305–13.
[33] Tamirou F, Lauwerys BR, Dall'Era M, Mackay M, Rovin B, Cervera R, et al. A proteinuria cut-off of 0.7 g/day after 12 months of treatment best predicts long-term renal outcome in lupus nephritis: data from the MAINTAIN Nephritis Trial. Lupus Sci Med 2015;2:e000123.
[34] Figueroa-Parra G, Cuéllar-Gutiérrez MC, González-Treviño M, Sanchez-Rodriguez A, Flores-Gouyonnet J, Meade-Aguilar JA, et al. Impact of glucocorticoid dose on complete response, serious infections, and mortality during initial therapy of lupus nephritis: a systematic review and meta-analysis of the control arms of randomized controlled trials. Arthritis Rheumatol 2024;76:1408–18.
[35] Arnaud L, Fabry-Vendrand C, Todea R, Vidal B, Cottin J, Bureau I, et al. Real-world oral glucocorticoid use in SLE: a nation-wide population-based study using the French medico-administrative (SNDS) claim database. Lupus Sci Med 2025;12:e001428.
[36] Condon MB, Ashby D, Pepper RJ, Cook HT, Levy JB, Griffith M, et al. Prospective observational single-centre cohort study to evaluate the effectiveness of treating lupus nephritis with rituximab and mycophenolate mofetil but no oral steroids. Ann Rheum Dis 2013;72:1280–6.
[37] Moroni G, Gallelli B, Quaglini S, Banfi G, Rivolta E, Messa P, et al. Withdrawal of therapy in patients with proliferative lupus nephritis: long-term follow-up. Nephrol Dial Transplant 2006;21:1541–8.
[38] Jourde-Chiche N, Costedoat-Chalumeau N, Baumstarck K, Loundou A, Bouillet L, Burtey S, et al. Weaning of maintenance immunosuppressive therapy in lupus nephritis (WIN-Lupus): results of a multicentric randomized controlled trial. Ann Rheum Dis 2022;81:1420–7.
[39] Gatto M, Frontini G, Calatroni M, Reggiani F, Depascale R, Cruciani C, et al. Effect of sustained clinical remission on the risk of lupus flares and impaired kidney function in patients with lupus nephritis. Kidney Int Rep 2024;9:1047–56.
[40] Mackensen A, Müller F, Mougiakakos D, Böltz S, Wilhelm A, Aigner M, et al. Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus. Nat Med 2022;28:2124–32.
[41] Müller F, Taubmann J, Bucci L, Wilhelm A, Bergmann C, Völkl S, et al. CD19 CAR T-cell therapy in autoimmune disease—a case series with follow-up. N Engl J Med 2024;390:687–700.
[42] Wang W, He S, Zhang W, Zhang H, DeStefano VM, Wada M, et al. BCMA-CD19 compound CAR T cells for systemic lupus erythematosus: a phase 1 open-label clinical trial. Ann Rheum Dis 2024;83:1304–14.
[43] Morand E, Kandane-Rathnayake R, Cortés-Hernández J, Amoura Z, Weinmann-Menke J, Maurer B, et al. OP0079 Clinical, cellular kinetics, pharmacodynamics and biomarker data up to 12 months after YTB323 (rapcabtagene autoleucel); a rapidly manufactured CD19 CAR-T therapy, from an open-label, phase 1/2 study in severe refractory SLE. Ann Rheum Dis 2025;84:68–70.
[44] Wu C, Fu Y, Liu J, Ye Y, Wang R, Li G, et al. OP0074 Preliminary results of CD19/BCMA dual-targeting FasTCAR-T cells GC012F (AZD0120) in patients with refractory systemic lupus erythematosus-an open-label, single-arm study. Ann Rheum Dis 2025;884:65.
[45] Sheikh S, Derebail V, Grover N, Gulati G, Abedi M, Sise M, et al. OP0202 RESET-SLE: clinical trial evaluating rese-cel (resecabtagene autoleucel), a fully human, autologous 4-1BB anti-CD19 CAR T cell therapy in non-renal SLE and lupus nephritis. Ann Rheum Dis 2025;84:168.
[46] Tur C, Eckstein M, Bucci L, Auth J, Bergmann C, Rauber S, et al. Effects of different B-cell-depleting strategies on the lymphatic tissue. Ann Rheum Dis 2025. doi: 10.1016/j.ard.2025.06.2120.
[47] Furie RA, Morand EF, Bruce IN, Manzi S, Kalunian KC, Vital EM, et al. Type I interferon inhibitor anifrolumab in active systemic lupus erythematosus (TULIP-1): a randomized, controlled, phase 3 trial. Lancet Rheumatol 2019;1:e208–19.
[48] Morand EF, Furie R, Tanaka Y, Bruce IN, Askanase AD, Richez C, et al. Trial of anifrolumab in active systemic lupus erythematosus. N Engl J Med 2020;382:211–21.
[49] Pamfil C, Makowska Z, De Groof A, Tilman G, Babaei S, Galant C, et al. Intrarenal activation of adaptive immune effectors is associated with tubular damage and impaired renal function in lupus nephritis. Ann Rheum Dis 2018;77:1782–9.
[50] Jayne D, Rovin B, Mysler EF, Furie RA, Houssiau FA, Trasieva T, et al. Phase II randomised trial of type I interferon inhibitor anifrolumab in patients with active lupus nephritis. Ann Rheum Dis 2022;81:496–506.
[51] Bucci L, Hagen M, Rothe T, Raimondo MG, Fagni F, Tur C, et al. Bispecific T cell engager therapy for refractory rheumatoid arthritis. Nat Med 2024;30:1593–601.
[52] Tobias A, Krönke J, Cheng Q, Keller U, Krönke G. Teclistamab-induced remission in refractory systemic lupus erythematosus. N Engl J Med 2024;391:864–6.
[53] Hagen M, Bucci L, Böltz S, Nöthling DM, Rothe T, Anoshkin K, et al. BCMA-Targeted T-cell-engager therapy fir autoimmune disease. N Engl J Med 2024;391:867–9.
[54] Humby F, Durez P, Buch MH, Lewis MJ, Rizvi H, Rivellese F, et al. Rituximab versus tocilizumab in anti-TNF inadequate responder patients with rheumatoid arthritis (R4RA): 16-week outcomes of a stratifies, biopsy-driven, multicenter, open-label, phase 4 randomised controlled trial. Lancet 2021;397:305–17.
[55] Rivellese F, Surace AE, Goldmann K, Sciacca E, Çubuk C, Giorli G, et al. Rituximab versus tocilizumab in rheumatoid arthritis: synovial biopsy-based biomarker analysis of the phase 4 R4RA randomized trial. Nat Med 2022;28:1256–68.
[56] Lewis MJ, Çubuk C, Surace AE, Sciacca E, Lau R, Goldmann K, et al. Deep molecular profiling of synovial biopsies in the STRAP trial identifies signatures predictive of treatment response to biologic therapies in rheumatoid arthritis. Nat Commun 2025;16:5374.
[57] Fava A, Buyon J, Magder L, Hodgin J, Rosenberg A, Demeke DS, et al. Urine proteomic signatures of histological class, activity, chronicity, and treatment response in lupus nephritis. JCI Insight 2024;9:e172569.
[58] Mejia-Vilet JM, Zhang XL, Cruz C, Cano-Verduzco ML, Shapiro JP, Nagaraja HN, et al. Urinary soluble CD163: a novel noninvasive biomarker of activity for lupus nephritis. J Am Soc Nephrol 2020;31:1335–47.

[59] Fava A, Rao DA, Mohan C, Zhang T, Rosenberg A, Fenaroli P, et al. Urine proteomics and renal single-cell transcriptomics implicate interleukin-16 in lupus nephritis. Arthritis Rheumatol 2022;74:829–39.
[60] Fava A, Concoff A, O'Malley T, Taghavi S, Warsi T, Kumar S, et al. Urinary biomarker panel to predict the probability of histologically active lupus nephritis (abstract). Arthritis Rheumatol 2024;76:1611–22.
[61] Mendoza-Pinto C, Etchegaray-Morales I, Ugarte-Gil MF. Improving access to SLE therapies in low and middle-income countries. Rheumatology (Oxford) 2023;62(Suppl 1):i30–5.
[62] Pierce GF, Adediran M, Diop S, Dunn AL, El Ekiaby M, Kaczmarek R, et al. Achieving access to haemophilia care in low-income and lower-middle-income countries: expanded humanitarian aid program of the World Federation of Hemophilia after 5 years. Lancet Haematol 2022;9:e689–97.

Ann Rheum Dis 85 (2026) 235–237

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Viewpoint

Precision medicine and the chaos theory in rheumatoid arthritis

Clément Triaille[1,2,*], Patrick Durez[1,3], Josef S. Smolen[4], Iain B. McInnes[5], Bernard Lauwerys[1], Nisha Limaye[6]

[1] *Pôle de pathologies rhumatismales systémiques et inflammatoires (RUMA), Institut de Recherche Expérimentale et Clinique (IREC), Université catholique de Louvain, Brussels, Belgium*
[2] *Service d'Hématologie, Oncologie et Rhumatologie pédiatrique, Cliniques Universitaires Saint-Luc, Brussels, Belgium*
[3] *Service de Rhumatologie, Cliniques Universitaires Saint-Luc, Brussels, Belgium*
[4] *Division of Rheumatology, Department of Medicine 3, Medical University of Vienna, Vienna, Austria*
[5] *College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK*
[6] *de Duve Institute, Université catholique de Louvain, Brussels, Belgium*

Predicting what the future holds is a quintessentially human aspiration. Beyond the purview of mystics, pragmatic prognostication has long preoccupied physicians. In the fifth century BC, Hippocrates stated: 'The best physician is one who can prevent and *predict*'. Some 2500 years later, the concept of 'precision medicine', ie, the ability to tailor treatment decisions based on accurate biomarkers, remains the holy grail in many medical specialties including rheumatology [1]. Disappointingly, despite intensive efforts, this concept has proven challenging to translate into practice in the field of inflammatory rheumatic diseases (iRMDs). As therapeutic options have increased over the 3 last decades across a variety of iRMDs and modes of action [2], the notion of '*the right drug for the right patient at the right time*' has attracted increasing interest—with enhanced expectations—from physicians and patients alike. Enticed by breakthrough advances in oncology, the rheumatology community has expended tremendous work and resources into gathering clinical, serologic, radiologic, pharmacologic, histologic, genetic, epigenetic, transcriptomic, metabolomic, proteomic, and other data to build predictive models. As these highly effective new therapies interfere with general inflammatory principles (rather than truly separate mechanisms operating in different patient subsets), precision medicine continues to be a challenge [3].

Rheumatoid arthritis (RA) provides a salutary exemplar. Blood and synovial tissues from patients with RA have been analysed with increasing granularity using technologies ranging from advanced imaging to spatial and single-cell transcriptomic profiling. Progressively, well-designed studies on large patient cohorts have replaced smaller-scale, exploratory work, with machine learning methods and comparative multiomics approaches now routinely employed [4–9]. Multiple studies have provided statistically significant data, leading to legitimate initial enthusiasm. However, absent validation has been a growing concern [10–12]. Major limitations have gradually become apparent in the quest for 'precision decisions' in iRMDs; some are summarised in Box 1. Few findings have proven either reliable and/or sufficiently applicable, and of sufficient predictive value, to be translated into daily clinical practice. Indeed, predictors of outcomes routinely assessed in clinical practice have hitherto not been surpassed by other molecular predictors.

These results are reminiscent of challenges encountered in systems research fields from meteorology to economics: characteristically, variables are so numerous, and their interactions and hierarchy so complex, that accurate prediction at the level of the individual becomes extremely elusive. Small, sometimes nonobvious—perhaps unidentifiable—differences in initial conditions can have seemingly disproportionate effects on ultimate measured outcomes. This has been referred to as 'chaos theory', or mechanistic chaos: the system under study is so intricate and dynamic that long-term prediction, or modelling, of its behaviour proves extremely difficult or even impossible, although it is governed by mechanistic laws (as opposed to stochastic events). Illustrative examples of this phenomenon are the movements of stock markets, (medium term) weather forecasts, and (by and large, with the exception of those with certain high-penetrance genetic variants [13]) prediction of lifetime cancer risk in individuals. Another common illustration of the chaos theory is the widely known metaphor of the 'butterfly effect': in a

*Correspondence to Dr Clément Triaille. (C. Triaille).
E-mail address: clement.triaille@uclouvain.be (C. Triaille).
Handling editor Kimme L. Hyrich.

https://doi.org/10.1016/j.ard.2025.07.005
Received 14 April 2025; Revised 30 May 2025; Accepted 8 July 2025

Box 1 Some major limitations learned in the quest of predictive biomarkers

- The immune system (with its myriad components) is incredibly dynamic, with considerable plasticity in both the short (a few hours) and long term (several months to years).
- Established determinants of response to therapies in a given individual include genetic susceptibility, unpredictable and variable environmental factors, and patient- and drug-specific factors.
- Each of these determinants seems to be associated with low effect size, and they are likely interdependent.
- Remission is not a stable state, and metrics of clinical response are composite scores with intrinsic limitations, eg, dependence on baseline disease activity, reliance on subjective factors with interobserver variability, and patient expectations.
- The right therapeutic choice for a given patient is not just a drug that induces remission, but also a drug that the patient agrees to take, is associated with an *a priori* tolerable risk/benefit balance, does not induce unacceptable side effects, and takes patient comorbidities into account.
- At present, the biotechnologies and resources necessary to combine and integrate the complexity of treatment response mechanisms are unlikely to (1) return results in an acceptable time frame, (2) be cost-effective, and (3) become widely accessible, *in the short-to-medium term*.

mechanistic, yet nonlinear system, a butterfly flapping its wings in South America could be the cause a tornado in Australia. Existence of such chaotic dynamics is increasingly recognised in biology, eg, in NF-κB (Nuclear Factor kB) transcription factor activity and the transcriptomic response of *Escherichia coli* to oxidative stress [14–16]. It has been postulated that chaotic response dynamics provide a selection advantage to a bacterial or a cell population (such as immune cells) in a complex environment by increasing the spectrum of different responses and ultimately, the adaptability of the organism to this environment.

We would do well to recognise, and respect, the potential for 'chaos' to impact biomarker development in RA. Numerous 'pathologic influencers' could impact tissue evaluation. Epigenetic, comorbid (eg, obesity) and exposome (eg, infection, vaccines, pollution, smoking, alcohol, and diet) dependent factors will likely be critical and yet are very difficult to include in primary blood or synovial biomarker studies. Even if one focuses on the primary articular 'RA lesion', one should consider the pronounced plasticity of immune cell phenotypes (and likely resident stromal cells in their niche) responding dynamically to internal (eg, intercellular interactions) and external (eg, therapeutic agents) stimuli. Immunologic migration to lymphoid tissues (mimicked by ectopic germinal centres in synovium) further complicates interpretation: do circulating, lymph node, or synovial immune cells reflect RA pathogenesis, the disruption caused by immune-targeted intervention, or an active immune response to an incidental, possibly irrelevant, recent infectious insult? Therefore, unsurprisingly, it has become increasingly clear that the determinants of individual clinical response to a given drug in RA are numerous, dynamic over time, and likely interdependent [17–19]. Whereas a technically accessible, cost-effective biomarker test would no doubt be rapidly and widely implemented, the complexity, time, cost, and variable accessibility of the (bio)technologies currently tested in RA represent a significant obstacle to reliable interpretation, validation, and ultimately, transition into daily practice.

The most robust predictive factors in RA have been known for a long time and are routinely assessed in clinical practice: high disease activity, seropositivity for anticitrullinated protein antibodies and rheumatoid factor, obesity, baseline bone erosions, smoking, and failure of a previous drug are all associated with lower probability of favourable outcomes under therapy [7,20–23]. Note the continuing utility of weighted scores such as the Disease Activity Score, the Simplified Disease Activity Index, or the Clinical Disease Activity Index, conceived based on the mining of clinical data; indeed, baseline disease activity, and even more so, change in clinical disease activity over the first 3 months, has been shown to be one of the best correlates of future response [21,24]. These are not *predictive* biomarkers per se but rather qualify as markers of biologic processes or disease 'state/activity' that in turn influence therapeutic outcomes [7,8,24].

Let's not throw away the baby with the bath water. Many precision medicine studies are based on biologically plausible hypotheses and designed to answer highly clinically relevant questions. Although a given omics-based readout (or combination thereof) may allow for a broad stratification of patients for likelihood of response, falling well short of the highly stringent predictive demands of precision medicine [9,10,25], its promise remains beguiling. The substantial efforts of our community are necessary and must be acknowledged for the important and fundamental new knowledge—and hypotheses—they have generated on the pathologic (molecular and cellular) mechanisms operating in RA. We contend that novel tools (particularly those that incorporate the spatial evaluation of affected tissues with exquisite granularity), combined with a fresh perspective (human and/or artificial intelligence generated!) may provide more accurate predictive risk scores [26–28]. Whether these can be quickly and cheaply implemented (and surpass current clinical predictors!) will in large part determine their real-world utility.

Both European Alliance of Associations for Rheumatology recommendations and American College of Rheumatology guidelines acknowledge the unpredictability of treatment response in RA: once the most accessible, cheap, and safe treatment (namely methotrexate) has failed, any targeted synthetic or biologic disease-modifying antirheumatic drug can legitimately be chosen for high-risk patients (based on clinical, radiological, and biological criteria). This should not imply that treatments be selected at random. We suspect that chaos in the pathologic RA lesion and its antecedents may render precision an unrealistic medium-term goal. On the other hand, a pragmatic application of decision making based on patient profile, preferences, compliance, extra-articular manifestations, and serologic measures assessed in standard clinical practice, comorbidities, and comedications could form a baseline upon which to then build estimations of 'molecular state'. The latter could be defined by currently available biopsy and poly-omic approaches. Drug safety profiles, reimbursement policies, and costs should also frame therapeutic strategy. A strict treat-to-target therapeutic approach should be applied in early RA with poor prognostic factors, with particular attention paid to the follow-up of patients at risk of unfavourable outcomes (such as structural damage or difficult-to-treat RA [29]). This is how rational, pragmatic therapeutic choices should be made in RA, unless unexpected future discoveries emerge from the chaos!

CRediT authorship contribution statement

Clément Triaille: Conceptualization, Writing – original draft. **Patrick Durez:** Writing – review & editing. **Josef S. Smolen:** Writing – review & editing. **Iain B. McInnes:** Writing – review & editing. **Bernard Lauwerys:** Writing – review & editing. **Nisha Limaye:** Writing – review & editing.

Acknowledgements

We thank Louis Triaille, PhD, UCLouvain-Saint Louis for idea-generating exchanges on the concept of chaos theory.

Funding

CT is partly funded by Wallonie-Bruxelles International World (Bourses d'excellence).

Competing interests

BLA is presently employed at UCB Biopharma, Brussels, Belgium. The other authors have no conflict of interest to disclose in relation to the present manuscript.

Patient consent for publication

Not applicable.

Ethics approval

Not applicable.

Provenance and peer review

Not commisionned; externally peer-reviewed.

Data availability statement

No new data were generated for this manuscript.

Orcid

Clément Triaille: http://orcid.org/0000-0001-9227-3820

REFERENCES

[1] Aletaha D. Precision medicine and management of rheumatoid arthritis. J Autoimmun 2020;110:102405.
[2] Smolen JS, Landewé RBM, Bergstra SA, Kerschbaumer A, Sepriano A, Aletaha D, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2022 update. Ann Rheum Dis 2023;82(1):3–18.
[3] Smolen JS, Aletaha D. Forget personalised medicine and focus on abating disease activity. Ann Rheum Dis 2013;72(1):3–6.
[4] Tao W, Concepcion AN, Vianen M, Marijnissen ACA, Lafeber FPGJ, Radstake TRDJ, et al. Multiomics and machine learning accurately predict clinical response to adalimumab and etanercept therapy in patients with rheumatoid arthritis. Arthritis Rheumatol 2021;73(2):212–22.
[5] Yoosuf N, Maciejewski M, Ziemek D, Jelinsky SA, Folkersen L, Müller M, et al. Early prediction of clinical response to anti-TNF treatment using multi-omics and machine learning in rheumatoid arthritis. Rheumatology (Oxford) 2022;61(4):1680–9.
[6] Koo BS, Eun S, Shin K, Yoon H, Hong C, Kim DH, et al. Machine learning model for identifying important clinical features for predicting remission in patients with rheumatoid arthritis treated with biologics. Arthritis Res Ther 2021;23(1):178.
[7] Salehi F, Lopera Gonzalez LI, Bayat S, Kleyer A, Zanca D, Brost A, et al. Machine learning prediction of treatment response to biological disease-modifying antirheumatic drugs in rheumatoid arthritis. J Clin Med 2024;13(13):3890.
[8] Humby F, Lewis M, Ramamoorthi N, Hackney JA, Barnes MR, Bombardieri M, et al. Synovial cellular and molecular signatures stratify clinical response to csDMARD therapy and predict radiographic progression in early rheumatoid arthritis patients. Ann Rheum Dis 2019;78(6):761–72.
[9] Cherlin S, Plant D, Taylor JC, Colombo M, Spiliopoulou A, Tzanis E, et al. Prediction of treatment response in rheumatoid arthritis patients using genome-wide SNP data. Genet Epidemiol 2018;42(8):754–71.
[10] Humby F, Durez P, Buch MH, Lewis MJ, Rizvi H, Rivellese F, et al. Rituximab versus tocilizumab in anti-TNF inadequate responder patients with rheumatoid arthritis (R4RA): 16-week outcomes of a stratified, biopsy-driven, multicentre, open-label, phase 4 randomised controlled trial. Lancet 2021;397(10271):305–17.
[11] Rivellese F, Nerviani A, Giorli G, Warren L, Jaworska E, Bombardieri M, et al. Stratification of biological therapies by pathobiology in biologic-naive patients with rheumatoid arthritis (STRAP and STRAP-EU): two parallel, open-label, biopsy-driven, randomised trials. Lancet Rheumatol 2023;5(11):e648–e59.
[12] Wientjes MHM, den Broeder AA, Welsing PMJ, Verhoef LM, van den Bemt BJF. Prediction of response to anti-TNF treatment using laboratory biomarkers in patients with rheumatoid arthritis: a systematic review. RMD Open 2022;8(2):e002570.
[13] Carbone M, ST Arron, Beutler B, Bononi A, Cavenee W, Cleaver JE, et al. Tumour predisposition and cancer syndromes as models to study gene-environment interactions. Nat Rev Cancer 2020;20(9):533–49.
[14] Heltberg ML, Krishna S, Jensen MH. On chaotic dynamics in transcription factors and the associated effects in differential gene regulation. Nat Commun 2019;10(1):71.
[15] Choudhary D, Foster KR, Uphoff S. Chaos in a bacterial stress response. Curr Biol 2023;33(24) 5404-14.e9.
[16] Mandelbrot BB. The fractal geometry of nature. San Francisco: W.H. Freeman; 1982. p. 460.
[17] Triaille C, Quartier P, De Somer L, Durez P, Lauwerys BR, Verschueren P, et al. Patterns and determinants of response to novel therapies in juvenile and adult-onset polyarthritis. Rheumatology (Oxford) 2024;63(3):594–607.
[18] Stark J, Chan C, George AJT. Oscillations in the immune system. Immunol Rev 2007;216:213–31.
[19] Gibbs JE, Ray DW. The role of the circadian clock in rheumatoid arthritis. Arthritis Res Ther 2013;15(1):205.
[20] Khader Y, Beran A, Ghazaleh S, Lee-Smith W, Altorok N. Predictors of remission in rheumatoid arthritis patients treated with biologics: a systematic review and meta-analysis. Clin Rheumatol 2022;41(12):3615–27.
[21] Capelusnik D, Aletaha D. Baseline predictors of different types of treatment success in rheumatoid arthritis. Ann Rheum Dis 2022;81(2):153–8.
[22] Vastesaeger N, Kutzbach AG, Amital H, Pavelka K, Lazaro MA, Moots RJ, et al. Prediction of remission and low disease activity in disease-modifying anti-rheumatic drug-refractory patients with rheumatoid arthritis treated with golimumab. Rheumatology (Oxford) 2016;55(8):1466–76.
[23] Smolen JS, Szumski A, Koenig AS, Jones TV, Marshall L. Predictors of remission with etanercept-methotrexate induction therapy and loss of remission with etanercept maintenance, reduction, or withdrawal in moderately active rheumatoid arthritis: results of the PRESERVE trial. Arthritis Res Ther 2018;20(1):8.
[24] Triaille C, Tilman G, Sokolova T, Loriot A, Marchandise J, De Montjoye S, et al. Disease activity drives transcriptomic heterogeneity in early untreated rheumatoid synovitis. Ann Rheum Dis 2023;82(12):1538–46.
[25] Lequerré T, Rottenberg P, Derambure C, Cosette P, Vittecoq O. Predictors of treatment response in rheumatoid arthritis. Joint Bone Spine 2019;86(2):151–8.
[26] MacDonald L, Elmesmari A, Somma D, Frew J, Di Mario C, Madhu R, et al. Synovial tissue myeloid dendritic cell subsets exhibit distinct tissue-niche localization and function in health and rheumatoid arthritis. Immunity 2024;57(12) 2843-62.e12.
[27] Frezza D, DiCarlo E, Hale C, Ramirez D, Mehta B, Slater D, et al. Computer vision analysis of rheumatoid arthritis synovium reveals lymphocytic inflammation is associated with immunoglobulin skewing in blood. Arthritis Rheumatol 2023;75(12):2137–47.
[28] Zhang F, Jonsson AH, Nathan A, Millard N, Curtis M, Xiao Q, et al. Deconstruction of rheumatoid arthritis synovium defines inflammatory subtypes. Nature 2023;623(7987):616–24.
[29] Watanabe R, Ebina K, Gon T, Okano T, Murata K, Murakami K, et al. Predictive factors and treatment outcomes associated with difficult-to-treat rheumatoid arthritis conditions: the ANSWER cohort study. Rheumatology (Oxford) 2024;63(9):2418–26.

Ann Rheum Dis 85 (2026) 238–245

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Recommendation

EULAR 2025 classification criteria for haemochromatosis arthropathy

Patrick DW Kiely[1,2,*], Stephanie Finzel[3], Bayram Farisogullari[4], Graeme J. Carroll[5], Geraldine McCarthy[6,7], John Stack[6], Simone Parisi[8], Graça Porto[9], Pascal Richette[10], Gyorgy Nagy[11,12,13], Marton Weidl[11], Ann Rosenthal[14], Pascal Guggenbuhl[15,16], Katarzyna J. Banaszkiewicz[17], Svenja Engelhardt[3,18], Jeremy D. Shearman[19], David Mitchell[20], Jane Barker[21], Valerie Brueton[21], Barbara Butzeck[21,22], Philip Coathup[21], Howard Don[21,23], Jacqueline Dowsett[21], Marie Duncan[21], Tracey Dunleavy[21], Ian Fish[21], Allin Hoggarth[21], Mark McKinnon[21], James Minter[21], Tim Osborne[21], Marguerite Smith[21], Christine Wright[21], Pedro M. Machado[4,24,25]

[1] *Department of Rheumatology, St George's University Hospitals NHS Foundation Trust, London, UK*
[2] *Institute of Medical and Biomedical Education, City St George's, University of London, London, UK*
[3] *Department of Rheumatology and Clinical Immunology, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg im Breisgau, Germany*
[4] *Department of Neuromuscular Diseases, University College London, London, UK*
[5] *Department of Rheumatology, Fremantle and Fiona Stanley Hospitals, Perth, Australia*
[6] *Division of Rheumatology, Mater Misericordiae University Hospital, Dublin, Ireland*
[7] *School of Medicine, University College Dublin, Dublin, Ireland*
[8] *Rheumatology Unit, Department of General and Specialist Medicine, Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino, Turin, Italy*
[9] *Centro Hospitalar Universitário de Santo António, Unidade Local de Saúde de Santo António, Porto, Portugal*
[10] *Université de Paris, AP-HP Lariboisière, Service de Rhumatologie, Paris, France*
[11] *Department of Rheumatology and Immunology, Semmelweis University, Budapest, Hungary*
[12] *Heart and Vascular Center, Semmelweis University, Budapest, Hungary*
[13] *Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary*
[14] *Department of Medicine, Division of Rheumatology, Medical College of Wisconsin, Milwaukee, WI, USA*
[15] *Univ Rennes, INSERM, Inrae, CHU Rennes, Institut NuMeCan (Nutrition, Metabolisms and Cancer), Rennes, France*
[16] *Rheumatology Department, Rennes University Hospital, Rennes, France*
[17] *Outpatients Endocrinology Clinic, University Clinical Center in Gdańsk, Gdańsk, Poland*
[18] *Department of Internal Medicine, Cardiology, Klinikum Freising, Germany*
[19] *Department of Gastroenterology, South Warwickshire University Foundation Trust and Warwick Medical School, Warwick, UK*
[20] *Clongriffin Medical Centre, Dublin, Ireland*
[21] *Patient Research Partners*
[22] *European Federation of Associations of Patients with Haemochromatosis, Croissy-sur-Seine, France*

*Correspondence to Prof Patrick Kiely, Department of Rheumatology, St George's University Hospitals NHS Foundation Trust, London, UK.
E-mail address: Patrick.kiely@nhs.net (P.D. Kiely).
Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.10.003

[23] *Haemochromatosis International, Barnstaple, Devon, UK*
[24] *National Institute for Health Research, University College London Hospitals Biomedical Research Centre, University College London Hospitals NHS Foundation Trust, London, UK*
[25] *Department of Rheumatology, Northwick Park Hospital, London North West University Healthcare NHS Trust, London, UK*

ARTICLE INFO

Article history:
Received 19 May 2025
Received in revised form 26 July 2025
Accepted 5 October 2025

ABSTRACT

Objectives: This study aims to develop classification criteria for haemochromatosis arthropathy (HA).
Methods: A task force was convened per European Alliance of Associations for Rheumatology standardised operating procedures, including physicians experienced in genetic haemochromatosis and its arthropathy and patient research partners. Candidate classification criteria items were selected via systematic literature review, consensus, and Delphi process. Derivation cohorts of patients with C282Y homozygous *HFE* gene mutation, joint pain, and iron loading, along with disease mimics (primary generalised osteoarthritis or calcium pyrophosphate deposition disease), were recruited from routine care and assessed against candidate items. Group comparison and diagnostic performance analyses identified variables with the greatest discriminatory capacity. A subset of these variables, agreed upon by consensus (considering both statistical results and the HA disease construct), was modelled using multivariable logistic regression and receiver operating characteristic curve analysis to develop classification criteria models. The final model was agreed upon through discussion, voting, and consensus, taking both statistical performance and disease *Gestalt* (face validity) into account.
Results: A derivation cohort of 154 patients with HA and 120 patients with disease mimics was recruited. A point-based classification model was developed using 8 variables covering age of symptom onset, clinical, and radiographic features at the metacarpophalangeal, distal interphalangeal, and ankle joints, and surgery of hips or ankles. A score ≥5 out of 11 provides 93.3% specificity and 71.4% sensitivity for classifying HA.
Conclusions: An international multicentre task force has developed the first classification criteria for HA from a unique derivation cohort using rigorous methodology. Criteria should be used to include patients in research studies and await external validation in separate cohorts.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- The pathogenic link between mutations of the *HFE* gene, hepcidin deficiency, iron loading, and haemochromatosis arthropathy (HA) is poorly understood.
- Understanding of HA is based on observational studies detailing case series and patient-reported outcomes, and there are no disease-modifying agents for HA.
- The lack of classification criteria for HA has impaired research development for this arthropathy.

WHAT THIS STUDY ADDS

- We present the first classification criteria for HA to be used for people with joint pain, the C282Y homozygous *HFE* mutation, and past evidence of iron loading.
- The criteria include 8 items covering core features of HA, each easily assessed in routine clinical practice, requiring clinical examination and radiographs of hands and ankle joints.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Classification criteria for HA will enable the inclusion of homogenous patient populations in research studies and clinical trials.
- Classification criteria for HA will stimulate interest and research into aetiopathogenesis and therapeutic interventions for this arthropathy.

INTRODUCTION

A distinctive arthropathy associated with genetic haemochromatosis (GH) was first described in 1964 [1]. Since then, patient surveys and clinical case series have confirmed a high prevalence of joint pain in up to 87% of people with GH [2–8]. Several characteristic features have been consistently identified, including large and small joint disease, especially involving the second and third metacarpophalangeal (MCP) and ankle joints, an osteoarthritic phenotype with florid subchondral cysts seen on X-ray and magnetic resonance imaging (MRI), exuberant osteophytes described as ‘hooks’, chondrocalcinosis, and rapid progression of joint damage sometimes resulting in arthroplasty at a young age [2,3,5–7,9–12].

Cases of haemochromatosis arthropathy (HA) are most commonly reported in people with the C282Y homozygous mutation in the *HFE* gene and high ferritin [2,6,7,13–15]. However, our understanding of pathogenesis of HA is incomplete with limited published research and some observations from case series, population studies, and patient surveys raising speculation that iron may not be the only pathogenic factor. For example, the presence and severity of arthropathy are not consistently reflective of the severity of iron loading [16] and clinically indistinguishable joint disease is seen in patients with major and minor *HFE* mutations in the absence of iron loading [17–20]. Furthermore, an iron-independent effect of *HFE* mutations is suggested by an increased risk of diabetes in people with the C282Y homozygous mutation and normal levels of ferritin and transferrin saturation [21]. Finally, while deironing with venesection reverses many manifestations of GH, it has a limited impact on the arthropathy, with most patients reporting no improvement in joint pain and new joints becoming involved after completion of venesection [2,3,5,6,15]. These inconsistencies illustrate the poor understanding of the link between mutations of the *HFE* gene, arthropathy, and the role of iron loading, emphasising the need for more research.

The aim of this work was to create the first classification criteria for HA to enable good-quality research studies and clinical trials into this unusual and poorly understood rheumatic disease.

METHODS

A task force (TF) was convened according to European Alliance of Associations for Rheumatology (EULAR) standardised operating procedures [22, https://www.eular.org/recommendations/points-to-consider-task-forces-project-applications"], comprising a convenor (PK), a EULAR-approved methodologist (PMM), 10 rheumatologists with an interest and experience of HA (including the convenor), 2 members of the Emerging EULAR Network (EMEUNET), 2 fellows, 2 physicians with experience of GH (haematology, hepatology), a primary care physician, a healthcare professional working with GH, and 15 patient research partners including representatives of Haemochromatosis International and the European Federation of Associations of Patients with Haemochromatosis.

Three sequential phases were followed to create the EULAR classification criteria:

Phase 1. Review the features of HA, taking into consideration findings from a fresh systematic literature review (SLR) and opinions of the TF. Create and clearly define candidate classification criteria items by the Delphi process. Selection and definition of inclusion criteria for derivation cohorts of patients with HA and disease mimics by consensus.

Phase 2. Recruitment of patients with HA and disease mimics fulfilling inclusion criteria, and assessment of the presence or absence of candidate classification criteria variables in each case. Demographic and clinical features of the cohorts were collected. Radiographs of hands and other affected joints were scored for chondrocalcinosis, hook osteophytes, joint space narrowing, and subchondral cysts according to atlas definitions [23–25].

Phase 3. Analysis of the prevalence of candidate classification criteria variables in patients with HA and disease mimics, including group comparison analyses (using independent samples t-test for quantitative variables and Chi-square test for categorical variables), was conducted. This was followed by assessment of diagnostic performance (sensitivity and specificity) and univariable logistic regression analysis (dependent variable: diagnosis of HA vs disease mimics) for significant variables in group comparisons, using an alpha of 0.05 as the significance cutoff, to identify variables with the greatest discriminatory capacity. A select number of these variables were agreed upon after discussion and consensus by TF members, taking both the statistical results and reflection of the HA disease construct into account. These variables were subsequently modelled using multivariable logistic regression and receiver operating characteristic curve analysis to create a selection of sum-of-points classification criteria models. Multivariable logistic regression was used to assess variable redundancy/collinearity and to determine the weight (ie, number of points) assigned to each variable, as informed by the regression coefficients. The minimum sample size was determined to ensure statistical robustness and minimise overfitting in these multivariable logistic regression models. We followed established recommendations for a minimum of 10 events per variable, as proposed by Peduzzi et al [26] and further supported by Vittinghoff and McCulloch [27]. Given our estimate that the final classification models might include up to 10 to 12 variables, this corresponds to a requirement of 100 to 120 cases per group, assuming a balanced case-control design. Accordingly, we aimed to enrol at least 120 cases and 120 controls, satisfying the lower bound of the events per variable criterion and supporting stable estimation of model parameters. Receiver operating characteristic curve analysis was used to determine the cutoff point for classifying patients as HA. Several candidate models were created, and the final model was agreed upon by discussion, voting and consensus, taking both diagnostic performance (sensitivity and specificity) and reflection of the disease *Gestalt* (face validity) into account.

RESULTS

Phase 1

An in-person meeting of the TF in early 2020 reviewed findings from the SLR [28] and created an initial list of 42 candidate classification criteria items. Following a Delphi process, 16 items achieved at least 75% agreement by the TF and were accepted. Five items achieved 65% to 74% agreement and were discussed and modified, followed by a second Delphi vote in which 1 item achieved at least 75% agreement and was accepted. In the second Delphi round, 2 items achieved 65% to 74% agreement; these were then discussed and modified but neither achieved 75% agreement in a third Delphi round (Supplementary Table S1). Of the 17 accepted items, 2 concerned the use of a homunculus for joint scoring, 12 were deemed mandatory, and 3 concerning ultrasound features were deemed optional/exploratory as not all recruiting centres had access to this imaging modality. A case report form (CRF) itemising and defining all 15 candidate classification criteria items was compiled (Supplementary Fig S1).

Three derivation cohorts of patients were defined for the study:

Group 1. C282Y homozygous HFE mutation, joint pain, and evidence of iron overload:

Iron overload was defined by at least one of 4 criteria: (1) transferrin saturation >45% on 2 occasions, (2) liver histopathology (biopsy) shows excess iron stain, (3) MRI shows excess iron in liver but not in the spleen, and (4) patients required at least 16 phlebotomies induction before maintenance.

Group 2. Any HFE mutation not fulfilling group 1 requirements, and joint pain:

It includes C282Y homozygous without iron overload according to group 1 criteria and patients with any other single or combination *HFE* mutation (C282Y heterozygous, C282Y/H63D compound heterozygous, H63D homozygous, and H63D heterozygous) with or without iron overload.

Group 3. Disease mimics:

(1) Primary generalised osteoarthritis: either nodal distribution in the hands (first carpo-metacarpal, proximal interphalangeal, distal interphalangeal (DIP) joints) or hip or knee or foot (any site).
(2) Calcium pyrophosphate deposition (CPPD) disease diagnosed either by (i) an expert physician or (ii) radiographic chondrocalcinosis or (iii) calcium pyrophosphate crystals seen in joint fluid.

Phase 2

Participants aged 18 years or older, who were able to provide informed consent, were recruited for the derivation cohorts of patients fulfilling criteria for groups 1, 2, and 3. Recruitment took place between early 2022 and mid-2023, with

commencement delayed by the global COVID-19 pandemic between 2020 and 2022. Patients were recruited from the clinical practices of 11 rheumatologists and 1 haematologist across 12 centres and 10 countries.

Patients were assessed for each item on the CRF by the recruiting rheumatologist, and for patients recruited from the practice of the haematologist by 2 visiting rheumatologists from the TF (PK, SF). The total number recruited was group 1: 154, group 2: 81, group 3: 120 (Supplementary Table S2).

In group 1, the criteria for iron loading were fulfilled in 72/154 (46.8%) for transferrin saturation >45% on 2 occasions, 77/154 (50%) for at least 16 phlebotomies induction before maintenance, and 5/154 (3.2%) for excess iron stain on liver biopsy. No patients had been assessed for iron loading by MRI criteria. Those who fulfilled the criteria for transferrin saturation >45% on 2 occasions had also undergone induction phlebotomy.

In group 3, 81/120 (67.5%) had primary generalised osteoarthritis and 39/120 (32.5%) CPPD disease (for subtypes, see Supplementary Table S3). All cases were screened for iron loading and confirmed not to have an elevation of serum ferritin or transferrin saturation at enrolment.

Phase 3

Data from the 12 mandatory candidate classification criteria items (and resulting extensive list of candidate variables) comparing group 1 and group 3 were analysed for the purpose of creation of classification criteria for HA. Data from the 3 optional items concerning US findings and all data from group 2 will be subject to separate analysis and are not a focus of this work.

Group comparison analyses revealed multiple statistically significant and clinically relevant differences between groups 1 and 3 for individual candidate classification criteria variables, including demographic and clinical variables, patient-reported and physician-recorded joint pain and swelling and radiographic features, and groups of these variables (Supplementary Table S4). The findings of the initial analyses were presented to the TF at an in-person meeting (23/33 members present) in mid-2024. Following discussion, 25 variables or combinations of variables were selected with the best combination of specificity and sensitivity and face validity (ie, reflection of the HA disease construct) comparing groups 1 and 3 (Supplementary Table S5).

These 25 variables were tested in many combinations to produce sum-of-points models for classification criteria. From over 150 models generated, 4 were selected favouring the highest specificity over sensitivity and minor variations in a representative spread of 8 variables, including age of symptom onset, clinical MCP and DIP joint involvement, hip or ankle surgery and radiographic MCP, and ankle joint space narrowing, cysts, and hook osteophytes. A fifth model was selected with additional variables to capture additional features of HA, and a sixth model was selected, also with additional variables and more balanced specificity and sensitivity (Supplementary Table S6A-F).

These 6 models were circulated remotely to the TF to rank in preference. Opinions of 31/33 TF members were returned (Supplementary Fig S2), in which model 1, with specificity 93.3% and sensitivity 71.4%, was the preferred model of 61% and the first or second choice model of 90% of the TF. The preference poll results were presented to the TF at a virtual meeting (24/33 members present), and following discussion of the merits of individual variables, model 1 was voted to be the best performing model by 100% of TF members. This model includes core characteristics of HA, each easy to assess in routine practice, in which a score of 5 or more out of 11 fulfils the classification criteria for HA (Table 1). The precise definition of the 8 variables within the classification criteria is shown in Table 2, an image and description of the iron fist in Figure 1, and examples of joint space narrowing grades 2 and 3, subchondral cysts, and hook osteophytes at the MCP and ankle joints are shown in Figures 2 and 3.

Table 1
Classification criteria for haemochromatosis arthropathy

Variable	Score
Joint symptom onset before 50 y of age	2
Iron fist	1
Absence of any DIP joint soft-tissue swelling and bony swelling and deformity	2
Hip or ankle surgery	Ankle only: 2 Hip only: 1 Ankle and hip: 2
MCP joint 2 or 3 or 4 or 5 tenderness, clinical examination	1
X-ray: any MCP joint—joint space narrowing grade 3 or surgery or subchondral cyst	1
X-ray: ankle joint—joint space narrowing grade 3 or subchondral cyst[a]	1
X-ray: hook osteophytes MCP joint 2 or 3 or ankle joint[a]	MCP 2/3 only: 1 Ankle only: 2 MCP 2/3 and ankle: 2
Classification criteria met	Total score ≥5

DIP, distal interphalangeal, MCP, metacarpophalangeal.
To be used in people with joint pain, the C282Y homozygous *HFE* mutation and evidence of iron overload. Specificity 93.3%; sensitivity 71.4%.
[a] Item not scored if ankle surgery either side.

DISCUSSION

The EULAR classification criteria for HA are to be used in people with joint pain, the C282Y homozygous *HFE* mutation, and past evidence of confirmed iron loading, here defined as either serum transferrin saturation >45% on 2 occasions, a requirement for at least 16 phlebotomies during induction deironing or excess iron stain on liver biopsy. They are intended for use to recruit patients to research studies (clinical trials and observational studies) and are not intended for diagnostic purposes.

The strengths of these criteria are that they are derived from a large derivation cohort of 154 HA cases recruited from 10 countries, providing global representation of this disease. The criteria include 8 variables, each easily assessed in routine clinical practice, covering core features of HA, including age of onset before 50 years of age, involvement of MCP, hip and ankle joints utilising clinical signs and radiographic features, the absence of DIP joint disease, and the frequent need for hip or ankle surgery. These are the features that best distinguish HA from primary generalised osteoarthritis and CPPD disease, the closest mimics. The selected model favoured specificity (93.3%) over sensitivity (71.4%) to minimise the chance of false positive cases being included in clinical trials. To achieve the threshold of at least 5 points out of a maximum 11, a minimum of 3 criteria must be met, and this threshold cannot be reached by involvement of only 1 joint site alone (out of MCP, hip, ankle) without an additional item also being present, such as age of onset, absence of DIP disease, or involvement of other joint sites.

The iron fist is a characteristic clinical sign occurring when there is a reduced range of flexion of the MCP joints and should be scored from its appearance, as illustrated in Figure 1. It should not be scored if the MCP joints exhibit impaired flexion

Table 2
Definition of variables within the classification criteria for haemochromatosis arthropathy

Variable	Definition
Joint symptom onset before 50 y of age	Age of onset of persisting pain, stiffness or swelling in any joint. Exclude transient joint symptoms, eg, due to trauma
Iron fist	Score 1 whether unilateral or bilateral See Figure 3
Absence of any DIP joint soft-tissue swelling and bony swelling and deformity	All DIP joints clinically normal with no palpable synovial or bony swelling or deformity. Intention to exclude nodal osteoarthritis or psoriatic arthritis. DIP joint deformity following trauma is allowed
Hip or ankle surgery	Joint fusion and arthroplasty For ankle score 2 whether unilateral or bilateral surgery. For hip score 1 whether unilateral or bilateral surgery For ankle and hip surgery score 2 whether unilateral or bilateral surgery at each joint site Surgery should have been performed for primary arthropathy, do not score if surgery was performed as a consequence of trauma
MCP joint 2 or 3 or 4 or 5 tenderness, clinical examination	Score 1 if tenderness on clinical examination of any of the second to fifth MCP joints of either hand
X-ray: any MCP joint – joint space narrowing grade 3 or surgery or subchondral cyst	Score 1 if any MCP joint has grade 3 joint space narrowing, or surgical fusion or arthroplasty or subchondral cyst/s (see reference [25])
X-ray: ankle joint – joint space narrowing grade 3 or subchondral cyst*	Ankle joint space narrowing grade 3 or subchondral cyst/s in any of 5 regions: lateral view, anterior, and posterior tibiotalar space, Mortise view, medial and superior tibiotalar and talofibular joint space (see reference [24]) Score 1 whether unilateral or bilateral, and whether either joint space narrowing grade 3 or subchondral cyst or both. *This item is not scored if either ankle has had surgery. Only score for primary ankle arthritis; do not score if ankle arthritis is a consequence of trauma.
X-ray: hook osteophytes* MCP joint 2 or 3 or ankle joint	MCP joint: osteophyte attached to the radial aspect of the second or third metacarpal head, characteristically large and hook-like in appearance. Score 1 if present in one or more second or third MCP joints (see reference [25]) Ankle joint: osteophyte attached to the distal tibia seen on lateral view, either anterior or posterior. Score 2 if present in either or both ankles (see reference [24]) *This item is not scored at the ankle if either ankle has had surgery. A score of 1 is still given for MCP hook osteophytes in a person with ankle surgery

DIP, distal interphalangeal, MCP, metacarpophalangeal.
* Item not scored if ankle surgery either side.

Figure 1. The iron fist. The iron fist is a physical sign due to incomplete full flexion of the second and/or third MCP joints. When making a fist, the PIP joints of the second and/or third fingers are raised above the level of the PIP joints of the fourth and fifth fingers, and the fingertips of the second and third fingers may not touch the palm. PIP, proximal interphalangeal, MCP, metacarpophalangeal.

for reasons other than primary arthropathy, such as previous trauma, fracture, or finger contractures.

Although DIP joint disease is seen in some cases of HA, its absence was a highly discriminatory criterion compared to its prevalence in nodal OA; this subgroup making up 61/81 (75%) of the primary generalised osteoarthritis disease mimic cohort. Inclusion of this item in the classification criteria should not be taken to imply that DIP joint disease is never a feature of HA.

For the ankle joint, variables should only be scored where there is primary arthropathy and any radiographic changes or surgery primarily as a consequence of trauma, fracture, malalignment, ligamentous injuries, or congenital abnormalities should not be scored. The same proviso applies to hip surgery, scored for primary arthropathy and not for surgery following trauma or congenital abnormalities.

The term ‘hook osteophytes’ is specifically used to describe the shape of often large osteophytes growing from the radial aspect of the head of the second or third metacarpal bone in people with HA. The TF recognised that similar large osteophytes are frequently seen at other joints in HA, and inclusion of these at any site was voted as one of the candidate classification criteria items (Supplementary Table S1), and specifically at the ankle for inclusion in the variables for modelling (Supplementary Table S5). In this derivation cohort, they were found to be present at any site in 55.8%, at the MCP 2/3 joints in 45.5% and the ankle in 20.1%, compared with 10%, 8.3%, and 0% of disease mimics, with specificity 90%, 91.7% and 100% respectively (Supplementary Table S4). We do not specify how large hook

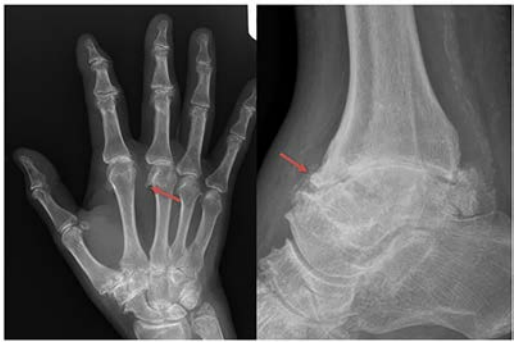


Figure 2. Hook osteophytes and joint space narrowing. Hook osteophytes at MCP 3 (A), attached to the radial aspect of the metacarpal head, and at the ankle joint (B), attached to the anterior distal tibia (see red arrows). In these images, MCP 2 and 3 and the ankle joints all have grade 2 joint space narrowing. MCP, metacarpophalangeal.

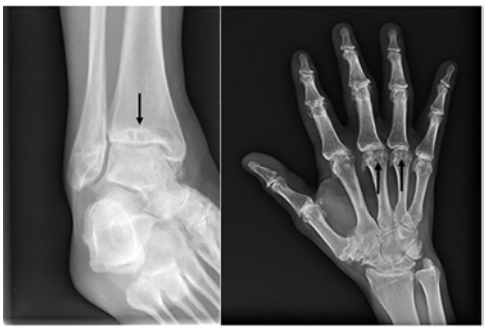


Figure 3. Subchondral cysts and joint space narrowing. Subchondral cysts of ankle (A) and MCP joints (B) (see black arrows). In this image, MCP joints 2 and 3 have a joint space narrowing grade 3, and there is a hook osteophyte at MCP 3. MCP, metacarpophalangeal.

osteophytes at the distal tibia need to be, to be scored as present in the criteria, leaving this to the subjective opinion of the rheumatologist or radiologist, in keeping with the nature of large osteophytes seen in HA.

Fatigue is a highly prevalent feature of GH and HA, reported at diagnosis in over 60% of patients [3]. In this derivation cohort, fatigue severity, assessed by visual analogue scale, was scored statistically higher in HA cases than disease mimics; however, the difference was not sufficiently discriminatory to be included in the final model. For example, a fatigue score greater than 5 had a low specificity of 59.7% and sensitivity of 55.8% (Supplementary Table S4).

While GH is a related metabolic disease for CPPD, several characteristics distinguish HA from CPPD. First is the age of onset of joint symptoms, with classification criteria for HA indicating a threshold of less than 50 years of age whereas classification criteria for CPPD indicate a threshold of greater than 60 years [29]. Second, radiographic chondrocalcinosis is not as prevalent in HA as CPPD, with reports from small case series in HA varying from 5% to 41% [7,30–32], and 31% of HA cases in this larger derivation cohort compared to 92% of the CPPD disease mimics. Furthermore, hook osteophytes, as discussed, are a more prevalent feature of HA (Supplementary Table S4).

The *HFE* genotype of disease mimics was unknown, and while some cases might have had the C282Y homozygous *HFE* mutation with osteoarthritis or CPPD, none were iron overloaded at enrolment to the cohort. If such cases were unwittingly included with the mimics, their features of arthropathy would have detracted from the differences seen between the disease mimics and the HA cohort, and so we are confident that this does not detract from the validity of the criteria.

HA is a rare arthropathy, and the collection of large numbers is difficult, taking nearly 2 years to recruit this large derivation cohort of 154 cases in 12 centres globally. Nonetheless, the classification criteria require validation in a separate independent cohort and should be considered provisional classification criteria until then.

In conclusion, we present the first classification criteria for HA, based on EULAR standardised operating procedures and rigorous methodology. They should be used to facilitate recruitment to clinical trials, ensuring high-quality studies into aetiopathogenesis and therapeutic interventions for people with HA. This somewhat neglected arthropathy deserves attention, with no disease-modifying agents identified, and a lack of clarity concerning the role of iron loading and development of joint disease. We hope these classification criteria will raise awareness of this unusual arthropathy and prompt renewed interest and clinical trials. Collection of a second cohort will be necessary for validation of these criteria.

Phase 1. We thank Abhishek Abhishek, Axel Braner, Pierre Brissot, Peter Korsten, David Lovell, and Annick van Clooster for contributions to phase 1 of this project.

Phase 2. We thank Dr Barbara Butzeck and the Grönemeyer Institute in Bochum for providing facility and ultrasound machine to enable SF to enrol patients from the region who were unable to travel to the centre in Freiburg. We thank Graça Melo for the enrolment of patients in Porto, and all the patients, study nurses, and trial coordinators involved in recruitment to this project.

PDWK convened and led the TF, prepared the CRF, assessed patients and scored radiographs in London and Porto, and drafted the first version of the manuscript. SF was the deputy TF convenor, trainee methodologist, and collected ultrasound data on all cases from Germany (Freiburg and Bochum) and Portugal. PMM was the methodologist, who led the Delphi exercise in phase 1, and the analyses in phase 3. BF is an EMEUNET member and performed all analyses under supervision of PMM. PDWK, SF, GJC, PR, PG, GN, GM, JDS, SP, KJB, and AR were recruiting rheumatologists. MW recruited patients in Hungary, and SE performed the SLR; both are fellows. SP is an EMEUNET member. DM is a primary care doctor. JDS is a gastroenterologist and Chair of the British Society for Gastroenterology/British Association for the Study of the Liver Haemochromatosis Special Interest Group. GP is a haematologist and recruited patients in Porto for assessment by PDWK and SF. HD is President of Haemochromatosis International. BB is Vice President of the European Federation of Associations of Patients with Haemochromatosis. All coauthors gave critical comments on the manuscript and approved the final version.

Six members left the TF between phase 1 and phase 2 and are acknowledged. MW joined the TF in phase 2. BF joined the TF in phase 3.

EULAR funded this project (CLI117) to create Classification Criteria for Haemochromatosis Arthropathy. The EULAR Council was not involved in design, analyses, or interpretation of results.

FUNDING

EULAR funded this project (CLI117) to create Classification Criteria for Haemochromatosis Arthropathy. The EULAR Council was not involved in design, analyses, or interpretation of results.

Competing interests

PK has received consulting/speaker's fees from Alphasigma/Galapagos, Eli Lilly and Novartis, all unrelated to this work. SF has received consulting/speaker's fees from Abbvie, Alphasigma/Galapagos, AstraZeneca, Biotest, Celltrion, Janssen/J&J, Novartis, NovoNordisk and UCB, all unrelated to this work.

PM has received consulting/speaker's fees from Abbvie, Bristol Myers Squibb, Celgene, Eli Lilly, Alphasigma/Galapagos, Greywolf, Janssen, MSD, Novartis, Orphazyme, Pfizer, Roche and UCB, all unrelated to this work.

Contributors

PDWK convened and led the TF, prepared the CRF, assessed patients and scored radiographs in London and Porto, and drafted the first version of the manuscript. SF was the deputy TF convenor, trainee methodologist, and collected ultrasound data on all cases from Germany (Freiburg and Bochum) and Portugal. PMM was the methodologist, who led the Delphi exercise in phase 1, and the analyses in phase 3. BF is an EMEUNET member and performed all analyses under supervision of PMM. PDWK, SF, GJC, PR, PG, GN, GM, JDS, SP, KJB, and AR were recruiting rheumatologists. MW recruited patients in Hungary, and SE performed the SLR; both are fellows. SP is an EMEUNET member. DM is a primary care doctor. JDS is a gastroenterologist and Chair of the British Society for Gastroenterology/British Association for the Study of the Liver Haemochromatosis Special Interest Group. GP is a haematologist and recruited patients in Porto for assessment by PDWK and SF. HD is President of Haemochromatosis International. BB is Vice President of the European Federation of Associations of Patients with Haemochromatosis. All coauthors gave critical comments on the manuscript and approved the final version. Six members left the TF between phase 1 and phase 2 and are acknowledged. MW joined the TF in phase 2. BF joined the TF in phase 3.

Patient consent for publication

The task force included 15 patient research partners, of whom 2 were representatives of Haemochromatosis International and the European Federation of Associations of Patients with Haemochromatosis. Patients gave informed consent to participate in the project, for their pseudoanonymised data to be shared and pooled for analyses and for publication of the classification criteria.

Ethics approval

Patient involvement, data collection, and sharing were granted ethical approval by the institutional review boards of the recruiting centres according to local country and centre requirements (Australia, France, Hungary, Ireland, Italy, Germany, Poland, Portugal, UK, USA).

Provenance and peer review

Not commissioned, externally peer reviewed.

Data availability statement

Data sharing requests can be submitted after 1 year following publication of the study results, to the corresponding author, who will provide a data access request form. Data sharing requests will be considered by the Task Force on a case-by-case basis, and data will be shared if the request is considered reasonable, of scientific interest, and legally and ethically possible.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ard.2025.10.003.

Orcid

Patrick DW Kiely: http://orcid.org/0000-0002-8091-2717
Stephanie Finzel: http://orcid.org/0000-0002-7625-9612
Graeme J. Carroll: http://orcid.org/0000-0002-2324-2227
Pascal Richette: http://orcid.org/0000-0003-2132-4074
Jeremy D. Shearman: http://orcid.org/0000-0002-7314-6233
Pedro M. Machado: http://orcid.org/0000-0002-8411-7972

REFERENCES

[1] Schumacher Jr HR. Hemochromatosis and arthritis. Arthritis Rheum 1964;7:41–50.
[2] Richette P, Ottaviani S, Vicaut E, Bardin T. Musculoskeletal complications of hereditary hemochromatosis: a case control study. J Rheumatol 2010;37(10):2145–50.
[3] Richardson A, Prideaux A, Kiely P. Haemochromatosis: unexplained metacarpophalangal or ankle arthropathy should prompt diagnostic tests: findings from two UK observational cohort studies. Scand J Rheumatol 2017;46(1):69–74.
[4] Living with the impact of iron overload [Internet]. Available from: https://www.haemochromatosis.org.uk/Handlers/Download.ashx?IDMF=c519f05d-d656-4ec3-8edf-7cbf39bb57d4. Accessed October 30, 2025.
[5] Mc Donnell SM, Preston BL, Jewell SA, Barton JC, Edwards CQ, Adams PC, et al. A survey of 2851 patients with hemochromatosis. Symptoms and response to treatment. Am J Med 1999;106(6):619–24.
[6] Sahinbegovic E, Dallos T, Aigner E, Axmann R, Manger B, Englbrecht M, et al. Musculoskeletal disease burden of hereditary hemochromatosis. Arthritis Rheum 2010;62(12):3792–8.
[7] Carroll GJ, Breidahl WH, Bulsara MK, Olynyk JK. Hereditary haemochromatosis is characterised by a clinically definable arthropathy that correlates with iron load. Arthritis Rheum 2011;63(1):286–94.
[8] Brissot P, Ball S, Rofail D, Cannon H, Jin VW. Hereditary haemochromatosis: patient experiences of the disease and phlebotomy treatment. Transfusion 2011;51(6):1331–8.
[9] Elmberg M, Hultcrantz R, Simard JF, Carlsson Å, Askling J. Increased risk of arthropathies and joint replacement surgery in patients with genetic hemochromatosis: a study of 3,531 patients and their 11,794 first degree relatives. Arthritis Care Res 2013;65(5):678–85.
[10] Husar-Memmer E, Stadlmayr A, Datz C, Zwerina J. HFE-related haemochromatosis: an update for the rheumatologist. Curr Rheumatol Rep 2014;16(1):393.
[11] Guggenbuhl P, Brissot P, Loréal O. Miscellaneous non-inflammatory musculoskeletal conditions. Haemochromatosis: the bone and the joint. Best Pract Res Clin Rheumatol 2011;25(5):649–64.
[12] Kiely PDW. Haemochromatosis arthropathy-a conundrum of the Celtic curse. J R Coll Physicians Edinb 2018;48(3):233–8.
[13] Allen KJA, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, Nicoll AJ, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. N Engl J Med 2008;358(3):221–30.
[14] Valenti L, Fracanzani AL, Rossi V, Rampini C, Pulixi E, Varenna M, et al. The hand arthropathy of hereditary haemochromatosis is strongly associated with iron overload. J Rheumatol 2008;35(1):153–8.
[15] Harty LC, Lai D, Connor S, Dunne A, Ali M, Ryan J, et al. Prevalence and progress of joint symptoms in hereditary hemochromatosis and symptomatic response to venesection. J Clin Rheumatol 2011;17(4):220–2.
[16] Nguyen CD, Morel V, Pierache A, Lion G, Cortet B, Flipo R-M, et al. Bone and joint complications in patients with hereditary hemochromatosis: a cross-sectional study of 93 patients. Ther Adv Musculoskel Dis 2020;12:1–14.
[17] Carroll GJ. Primary osteoarthritis in the ankle joint is associated with finger metacarpophalangeal osteoarthritis and the H63D mutation in the HFE gene: evidence for a haemochromatosis-like polyarticular osteoarthritis phenotype. J Clin Rheumatol 2006;12(3):109–13.

[18] Becerra-Fernandez E, Panopalis P, Menard HA. Idiopathic hindfoot problems as an early rheumatologic manifestation of hereditary haemochromatosis type I. Arthritis Rheum 2011;63(Suppl):S762.
[19] Alizadeh BZ, Njajou OT, Hazes JMW, Hofman A, Slagboom PE, Pols HAP, et al. The H63D variant in the HFE gene predisposes to arthralgia, chondrocalcinosis and osteoarthritis. Ann Rheum Dis 2007;66(11):1436–42.
[20] Ross JM, Kowalchuk RM, Shaulinsky J, Ross L, Ryan D, Phatak PD, et al. Association of heterozygous hemochromatosis C282Y gene mutation with hand osteoarthritis. J Rheumatol 2003;30(1):121–5.
[21] Mottelson M, Helby J, Nordestgaard BG, Ellervik C, Mandrup-Poulsen T, Petersen J, et al. Mortality and risk of diabetes, liver disease, and heart disease in individuals with haemochromatosis HFE C282Y homozygosity and normal concentrations of iron, transferrin saturation, or ferritin: prospective cohort study. BMJ 2024;387:e079147.
[22] Van der Heijde D, Aletaha D, Carmona L, Edwards CJ, Kvien TK, Kouloumas M, et al. 2014 update of the EULAR standardised operating procedures for EULAR -endorsed recommendations. Ann Rheum Dis 2015;74(1):8–13.
[23] Altman RD, Gold GE. Atlas of individual radiographic features in osteoarthritis, revised. Osteoarthritis Cartilage 2007;15:A1–A56.
[24] Kraus VB, Kilfoil TM, Hash II TW, McDaniel G, Renner J, Carrino JA, et al. Atlas of radiographic features of osteoarthritis of the ankle and hindfoot. Osteoarthritis Cartilage 2015;23(12):2059–85.
[25] Adamson TC, Resnik CS, Guerra Jr J, Vint VC, Weisman MH, Resnick D. Hand and wrist arthropathies of hemochromatosis and calcium pyrophosphate deposition disease: distinct radiographic features. Radiology 1983;147 (2):377–81. doi: 10.1148/radiology.147.2.6300958.
[26] Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol 1996;49(12):1373–9.
[27] Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. Am J Epidemiol 2007;165(6):710–8.
[28] Engelhardt S, Buroh S, Schwarzer G, Kerschbaumer A, Braner A, Herber M, et al. •Clinical and imaging features of haemochromatosis arthropathy: a systematic literature review and meta-analysis to inform the development of EULAR classification criteria. EULAR Rheumatology Open, Volume 1, Issue 3, 260 - 268
[29] Abhishek A, Tedeschi SK, Pascart T, Latourte A, Dalbeth N, Neogi T, et al. The 2023 ACR/EULAR classification criteria for calcium pyrophosphate deposition disease. Arhtritis Rheum 2023;75(10):1703–13.
[30] Huaux JP, Geubel A, Koch MC, Malghem J, Maldague B, Devogelaer JP, et al. The arthritis of hemochromatosis. A review of 25 cases with special reference to chondrocalcinosis, and a comparison with patients with primary hyperparathyroidism and controls. Clin Rheumatol 1986;5 (3):317–24.
[31] Faraawi R, Harth M, Kertesz A, Bell D. Arthritis in haemochromatosis. J. Rheumatol. 1993;20(3):448–52.
[32] Dymock IW, Hamilton EBD, Laws JW, Williams R. Arthropathy of haemochromatosis. Clinical and radiological analysis of 63 patients with iron overload. Ann Rheum Dis 1970;29(5):469–76.

Ann Rheum Dis 85 (2026) 246–253

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Rheumatoid arthritis

Association between socioeconomic status and patient delay in rheumatoid arthritis: linking self-reported and national registry data

Peter Alsing[1], Ellen-Margrethe Hauge[1,2], Stine Daugaard Pedersen[3], Annette de Thurah[1,2,*]

[1] *Department of Rheumatology, Aarhus University Hospital, Aarhus, Aarhus N, Denmark*
[2] *Department of Clinical Medicine, Aarhus University, Aarhus, Denmark*
[3] *Department of Rheumatology, Diagnostic Centre, Silkeborg Regional Hospital, Silkeborg, Denmark*

ARTICLE INFO

Article history:
Received 29 July 2025
Received in revised form 13 September 2025
Accepted 2 October 2025

ABSTRACT

Objectives: Early treatment is critical for improving outcomes in rheumatoid arthritis (RA), with patient-initiated delays being a key barrier. Socioeconomic status (SES) influences health behaviour, but its role in the timing of first general practitioner (GP) contact after RA symptom onset remains unclear. This study examined the association between SES and time to help-seeking in people newly diagnosed with RA.

Methods: Data on incident RA were collected from the Danish Rheumatology Database. Patients' self-reported time from symptom onset to first GP contact was collected via questionnaire data. Responses were linked to national registries for SES information, including education, wealth, cohabitation status, and occupation. Median delays and IQRs were calculated overall, and by SES strata. Multiple logistic regression provided adjusted odds ratios (aOR) and 95% CIs. Selection bias was investigated by comparing the SES between responders and nonresponders.

Results: Median patient delay was 59 days (IQR 15-182). Medium education level was associated with longer delay compared to high level (aOR 1.96 [CI: 1.01-3.79]); low occupational status showed a tendency towards longer delay (aOR 1.58 [CI: 0.47-5.33]). No consistent associations were found for wealth or cohabitation. Delays were longer among younger patients, those without comorbidities, and those with lower disease activity. Significantly lower SES was seen among nonresponders.

Conclusions: Educational level showed the strongest socioeconomic association with patient delay. The nonresponse analysis highlighted a possible overrepresentation of socially vulnerable patients among nonresponders, reinforcing the need to address equity in early RA care pathways.

INTRODUCTION

The prognosis of rheumatoid arthritis (RA) improves if treatment is initiated within 12 weeks of symptom onset [1], often referred to as a 'window of opportunity' [2,3]. International studies have shown that only 22% to 33% of patients are assessed by a rheumatologist within the recommended timeframe [4,5]. Overall, the treatment delay in RA has declined over the last few years due to improvements from healthcare providers and system factors such as new approaches to diagnosis and clinical awareness [5,6]. However, patient factors are often overlooked, and in RA, the patient delay, defined as the

*Correspondence to Prof. Annette de Thurah, Department of Rheumatology, Aarhus University Hospital, Aarhus, Aarhus N, Denmark.
E-mail address: annethur@rm.dk (A. de Thurah).
Handling editor Josef S Smolen

https://doi.org/10.1016/j.ard.2025.10.001

WHAT IS ALREADY KNOWN ON THE TOPIC

- Early diagnosis and treatment of patients with rheumatoid arthritis (RA) is critical for improving outcomes related to joint health and functioning.
- The largest contributing factor in the delayed treatment of RA is patient-related delay, with scarce knowledge regarding the socioeconomic disparities affecting this part of the overall delay.

WHAT THIS STUDY ADDS

- Educational attainment showed the most consistent socioeconomic association with help-seeking delay, highlighting a potential target for early treatment strategies—even within a universal healthcare system.
- Nonresponse analysis revealed markedly lower SES among nonresponders, underpinning the need to address equity in early RA care. Furthermore, suggesting that socioeconomic disparities in help-seeking behaviour may be underestimated in survey-based RA research.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Highlighting the influence of different socioeconomic factors in relation to patient-related delay in RA care, these findings contribute valuable information for targeted treatment to reduce delays and promote health equity.

time from symptom onset to help-seeking at the general practitioner (GP) [7], also contributes to the overall treatment delay [4–6,8–10]. A review assessing treatment delay in RA found patient delay to be the largest contributing factor to the overall delay, with a median time of about 3.5 months [11]. Previously, we published a study evaluating the use of primary healthcare before the RA diagnosis among 7200 newly diagnosed Danish patients and found that individuals with RA had significantly more contacts with GPs during the year preceding the diagnosis compared to individuals without RA [12]. The study investigated how patients perceive their first RA symptom and how this affects their help-seeking behaviour with the GP and found a median delay of 63 days. The conclusion was that younger patients, especially those with low treatment expectations and high symptom variability, delayed seeking help [13]. In Denmark, healthcare is tax-funded and provided free of charge to all residents. General practice serves as the primary entry point and gatekeeper to the wider healthcare system, with the exception of emergency care and ear-nose-throat or eye specialists. More than 98% of Danish residents are registered with a specific general practice, ensuring continuity of care and coordinated access to specialised services [14].

In recent years, social inequality in health has become an increasing focus in public health research and politics [15], with socioeconomic status (SES) being recognised as an important factor in health-related outcomes and behaviour [16]. A recent cohort study from the UK among 22 million people with autoimmune diseases found that individuals with RA had 1.5 times the risk of having lower SES compared to the general population [17]. Studies suggest that patients with RA and low SES experience higher disease activity, increased disability, and poor quality of life as well as physical and mental health [18–20]. Furthermore, an observational study conducted in the Netherlands involving 878 patients with RA discovered that those with low SES utilised primary healthcare services significantly less than those with high SES [19]. No previous studies have investigated the extent to which SES affects patient delay in receiving early treatment. Understanding the factors influencing early treatment for patients with RA will help us effectively target treatment strategies towards patients who are at the highest risk of experiencing delays and assess the impact of social disparities in health on patient delay. Thus, the aim of this study is to examine the relationship between SES and the delay in first contact with a GP due to the onset of the first RA symptoms.

METHODS

Design and study population

The study was conducted as a nationwide cross-sectional study, based on questionnaire data [13], linked with data from Danish national registries. The study population was identified through the national Danish Rheumatology database (DANBIO), which is a nationwide database on inflammatory diseases, with information on RA since 2006 [21]. From here, we included patients ≥18 years of age and diagnosed with RA (International Classification of Diseases 10th revision [ICD-10] codes: M05.3, M05.9, M05.8, M06) in Denmark from 1 January 2021 to 10 December 2021. These patients were sent a questionnaire through the Danish national secure digital mailbox 'e-Boks' [22], excluding patients not having access to the digital mailbox. The questionnaire contained information about how and when they had experienced the first symptom of RA, and when they decided to contact their GP with these symptoms. The index date was the date of presenting RA symptoms to a GP. For cases where the self-reported date of the first RA symptom is missing, we replaced it with the date recorded in DANBIO when possible (n = 13). Patients were excluded if no valid interval between the first symptom and the GP consultation could be established (n = 27) [13]. Patients with missing information on any of the clinical disease activity measures were excluded from the primary analyses (n = 117). To compare responders and nonresponders regarding differences in sociodemographic characteristics and SES, we conducted nonresponse analyses on both all responders and the responders with information on disease activity. Further methodological description of the cohort, including information on responders and nonresponders, can be found elsewhere [13].

Data sources

The Danish Civil Registration System was used for individual-level linkage across registries and the questionnaire dataset, based on the unique civil personalised registration number given to all Danish residents upon birth or immigration [23]. The following registries were also linked with the dataset based on the questionnaire data: the DANBIO, Statistics Denmark, and the Danish National Patient Registry (DNPR).

Outcome

The outcome of interest was patient delay, defined as the time in days from onset of symptoms to first consultation with a GP concerning RA-related symptoms. Data were obtained from the questionnaire and introduced as follows: '*In the following sections, we will ask about several dates related to the onset of your rheumatoid arthritis symptoms. Please answer the questions as accurately as possible based on your memory and any calendar records you might have. We understand it can be challenging to recall these*

details, but we encourage you to provide the most accurate information possible' [13].

Exposure

The exposure was SES, including 4 distinct markers to adequately describe the concept. The 4 markers of SES were educational level, wealth (income or liquid assets), cohabiting status, and occupational level. Information on all markers was obtained from Statistics Denmark, the central authority on statistics in Denmark [24]. Educational level was defined as the highest attained educational level at the index date, and divided into 3 categories: 'low' defined as primary- and lower secondary education, 'medium' defined as upper secondary- or vocational education, and 'high' defined as short-cycle tertiary education, bachelor's degree, or higher, based on the International Standard Classification of Education [25]. This aspect of SES reflected cognitive resources and knowledge in relation to health risks and health-promoting behaviour [26]. The patient's income or liquid assets represented material resources, affecting living conditions, self-esteem, social standing, and access to quality healthcare and nutrition, among other mechanisms [26,27]. For patients aged under 65 years, data consisted of an equivalised disposable income based on the total household income, while for patients aged 65 years or older, the estimate was based on family liquid assets. To account for yearly variation, an average of the 5 years before index date was calculated, with all estimates adjusted for inflation. Both household income and family liquid assets were divided into tertiles, then categorised as 'low', 'medium', and 'high', and combined into 1 variable referred to as 'wealth'. Information on cohabiting status was obtained at the index date, and dichotomised into 'cohabiting', defined as married or cohabiting, or 'living alone', defined as living alone, divorced, or widowed. This aspect of SES reflected social support [28], with living alone being equivalent to low SES. Occupational level represented social status and risk in regard to the workplace environment [26]. This marker was categorised based on the International Standard Classification of Occupations [29]. Categories were defined as 'high occupational level', equal to skill levels 3 and 4, consisting of managers, professionals, technicians, and associate professionals, 'medium occupational level', equal to skill level 2, consisting of clerical support workers, service and sales workers, skilled agricultural, forestry and fishery workers, craft and related trades workers, plant and machine operators, and assemblers, 'low occupational level' equal to skill level 1, consisting of elementary occupations, and 'outside the workforce' consisted of retired patients or patients receiving public transfer payments, related to sickness or social benefits, as their primary source of income.

Other variables/covariates

To address potential confounding factors, we utilised sex, age, comorbidity, and clinical disease activity measures. Information on age and sex was retrieved from the questionnaire, with age being categorised into '≥18-50 years', '51-64 years', and '≥65 years' for descriptive purposes and used as a continuous variable for the regression analyses. Ethnicity was categorised into 'Danish' and 'Immigrants, and descendants of immigrants', based on information from Statistics Denmark. Comorbidity burden was defined according to Charlson's Comorbidity Index (CCI) [30], based on diagnostic codes from hospital contacts registered in the DNPR during the 5-year period preceding the index date. The weighted scores were based on updated estimates from Quan et al [31]. DNPR has complete nationwide coverage of diagnoses and discharges, based on the ICD [32]. CCI scores were categorised into 'no comorbidities' or '≥1 comorbidities. Information on clinical disease activity measures was obtained from DANBIO [33]. Clinical disease activity measures included the number of swollen and tender joints, as well as C-reactive protein (CRP) levels, analysed both as a continuous variable and dichotomised into low (<8 mg/L) and high (≥8 mg/L) levels. The Disease Activity Score-28 (DAS28-CRP) score was used descriptively as a composite measure of disease activity, which includes information on tender joint count, swollen joint count, CRP levels, and a global health assessment. Here, it was categorised into low disease activity (score <3.2), moderate disease activity (score 3.2-5.1), and high disease activity (>5.1) [12,33]. In Table 1, the low and moderate DAS28-CRP categories were merged for descriptive clarity and to ensure data confidentiality.

Responder/nonresponder analysis

Survey-based studies are often subject to selection bias, with responders typically having higher SES than nonresponders. This can lead to systematic underestimation, especially in a study like ours, where SES is our main exposure. Therefore, examining differences between responders and nonresponders is crucial to assess the extent of potential bias [34–36].

Statistical analysis

Descriptive data were presented as numbers (n) and corresponding percentages for categorical and dichotomous data. Continuous data were presented as mean and SD if normally distributed, otherwise as median and IQR, corresponding to the 25th and 75th percentile. For the nonresponse analyses, we utilised the Kruskal-Wallis test for continuous variables and the Pearson Chi-squared test for categorical variables. The outcome of patient delay was non-normally distributed and therefore presented as median time in days, with corresponding IQR. The association between markers of SES was analysed using multivariable logistic regression analysis, with the outcome of patient delay dichotomised into <3 and ≥3 months from onset of symptoms to first contact with the GP. For each SES marker, high SES was used as the reference category. Crude and adjusted odds ratios (aOR) were presented with corresponding 95% CIs. The aORs were adjusted for sex, age, comorbidity, and clinical disease activity measures, including a continuous measure of CRP levels and number of tender and swollen joints. For all applicable statistical tests, a significance level of <0.05 was applied.

All statistical analyses were conducted using Stata/MP 18.0 (StataCorp LLC).

Permission and ethics

The study was conducted adhering to the principles outlined in the Declaration of Helsinki and registered by the Danish law on data protection and the European Union's General Data Protection Regulation. All patients were provided with information about the study before answering the questionnaire, emphasising that their participation was voluntary with the right to withdraw their consent at any time. All data were handled on Statistics Denmark's secure server platforms, with only aggregated results being presented. Permission to obtain data from the Danish Rheumatologic Quality Database has been approved

Table 1
Characteristics of the population overall and by markers of socioeconomic status

	Overall	Educational level			Wealth			Cohabiting status		Occupational level			
Factor		Low	Medium	High	Low	Medium	High	Living alone	Cohabiting	Low	Medium	High	Outside the workforce
	n = 227	n = 46	n = 108	n = 73	n = 77	n = 75	n = 75	n = 59	n = 168	n = 15	n = 60	n = 56	n = 92
Sex													
Female	153 (67.4)	32 (69.6)	70 (64.8)	51 (69.9)	54 (70.1)	50 (66.7)	49 (65.3)	47 (79.7)	106 (63.1)	8 (53.3)	40 (66.7)	41 (73.2)	60 (65.2)
Male	74 (32.6)	14 (30.4)	38 (35.2)	22 (30.1)	23 (29.9)	25 (33.3)	26 (34.7)	12 (20.3)	62 (36.9)	7 (46.7)	20 (33.3)	15 (26.8)	32 (34.8)
Age													
Age in years, Mean (SD)	58 (15)	63 (13)	57 (15)	56 (16)	55 (18)	57 (15)	62 (12)	57 (18)	58 (14)	49 (14)	51 (14)	51 (12)	69 (11)
18-50 years	61 (26.9)	9 (19.6)	26 (24.1)	26 (35.6)	27 (35.1)	22 (29.3)	12 (16)	17 (28.8)	44 (26.2)	6 (40)	23 (38.3)	25 (44.6)	5 (5.4)
51-64 years	81 (35.7)	12 (26.1)	44 (40.7)	25 (34.3)	21 (27.3)	25 (33.3)	35 (46.7)	19 (32.2)	62 (36.9)	9 (60)[a]	37 (61.8)[a]	61 (55.4)[a]	87 (94.6)[a]
>64 years	85 (37.4)	25 (54.4)	38 (35.2)	22 (30.1)	29 (37.6)	28 (37.4)	28 (37.3)	23 (39)	62 (36.9)				
Ethnicity													
Danish	213 (93.8)												
Immigrants and descendants	14 (6.2)												
Comorbidity													
No comorbidities	194 (85.5)				64 (83.1)	64 (85.3)	66 (88)	50 (84.8)	144 (85.7)				
One or more comorbidities	33 (14.5)				13 (16.9)	11 (14.7)	9 (12)	9 (15.2)	24 (14.3)				
C-reactive protein (CRP)													
CRP (mg/L), Median (IQR)	10 (4-25)	10.5 (4.6-25)	12 (4-29)	7.7 (3-20)	13 (5-31)	10 (4-22)	8 (3-26)	11 (3-27)	10 (4-25)	25 (7-43)	8.5 (4-21)	4.6 (2.9-16)	12.5 (6.3-37)
Low disease activity	103 (45.4)	18 (39.1)	46 (42.6)	39 (53.4)	32 (41.6)	33 (44)	38 (50.7)	28 (47.5)	75 (44.6)	6 (40)	30 (50)	34 (60.7)	32 (34.8)
High disease activity	124 (54.6)	28 (60.9)	62 (57.4)	34 (46.6)	45 (58.4)	42 (56)	37 (49.3)	31 (52.5)	93 (55.4)	9 (60)	30 (50)	22 (39.3)	60 (65.2)
DAS28-CRP													
DAS28-CRP score, Mean (SD)	4.7 (1.15)	4.7 (1.2)	4.8 (1.2)	4.7 (1.1)	4.8 (1.2)	4.7 (1.2)	4.7 (1.0)	4.4 (1.0)	4.8 (1.2)	4.5 (1.2)	4.7 (1.1)	4.5 (1.0)	5 (1.3)
Low to moderate disease activity	104 (45.8)	23 (50)	49 (45.4)	32 (43.8)	32 (41.6)	38 (50.7)	34 (45.3)	26 (44.1)	78 (46.4)		32 (53.3)	29 (51.8)	37 (40.2)
High disease activity	54 (23.8)	8 (17.4)	28 (25.9)	18 (24.7)	19 (24.7)	18 (24)	17 (22.7)	12 (20.3)	42 (25)		13 (21.7)	12 (21.4)	25 (27.2)
Missing	69 (30.4)	15 (32.6)	31 (28.7)	23 (31.5)	26 (33.8)	19 (25.3)	24 (32)	21 (35.6)	48 (28.6)		15 (25)	15 (26.8)	30 (32.6)
Swollen joints													
Number of swollen joints, Median (IQR)	4 (2-8)	5 (3-9)	4 (2-8)	5 (2-9)	4 (2-8)	4 (2-8)	5 (2-9)	4 (2-7)	5 (2-9)	3 (2-5)	5 (3-8)	4 (2-8)	5 (3-9)
Tender joints													
Number of tender joints, Median (IQR)	6 (3-10)	6 (3-8)	6 (4-10)	6 (3-10)	6 (3-10)	6 (3-10)	6 (3-10)	5 (2-8)	6 (3-10)	4 (3-10)	6 (4-9)	5 (3-9)	6 (3-11)
Family liquid assets x 10^3, Median (IQR)					32 (14-119.2)	240.9 (219.5-305.4)	577.5 (440.1-1720.4)						
Income in euros x 10^3, Median (IQR)					30.5 (23.8-34.9)	44.6 (40.2-48.1)	63 (55.3-69)						

[a]Agegroups '51-64 years' and '>64 years' merged into one group due to small numbers and data protection

= Cells include ≥3 observations; results are aggregated to prevent identification or back-calculation. Where this was not possible, data are not reported.

by the Danish Data Protection Agency (ref: 1-16-02-279-21) and the DANBIO steering group. According to Danish law, approval by the regional committee on health research ethics in the Central Denmark Region was not required, as no biomedical intervention was performed.

RESULTS

A total of 1163 individuals were sent a questionnaire, of which 404 patients responded. Those with missing information on any of the 3 clinical measures of disease activity, CRP, tender- or swollen joints, were excluded from the primary analyses (n = 117). This resulted in a final study population of n = 227 for the SES markers educational level, wealth and cohabiting status, and 223 patients for occupational level (Fig 1). Characteristics of the study population are shown in Table 1. The average age of participants was 58 years, with a majority being female (67%). The majority were of Danish origin (94%) and had no comorbidities (86%). The median CRP levels were 10 mg/L and the mean DAS28-CRP score was 4.7.

The overall median patient delay was 59 (IQR 15-182) days (Table 2). Younger patients experienced a median delay of 79 days (IQR 30-317) compared to 47 days (IQR 9-123) for those over 64 years of age. Patients without comorbidities had a longer delay of 61 days (IQR 16-182) compared to 38 days (IQR 11-236) for those with comorbidities. Disease activity also

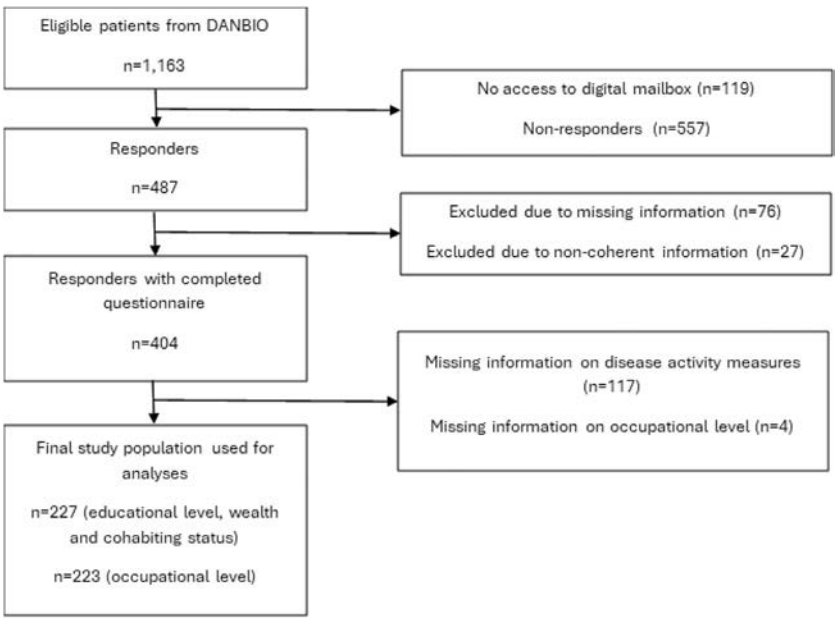


Figure 1. Flowchart describing the inclusion and exclusion process of the study population. DANBIO, Danish Rheumatology Database; N, number.

Table 2
Median (IQR) patient delay in days, overall, by markers of socioeconomic status and sociodemographic factors

Factor	N (%)	Median patient delay (d)	IQR
Overall	227 (100)	59	15-182
Sex			
Female	153 (67.4)	61	15-162
Male	74 (32.6)	49	16-220
Age (y)			
18-50	61 (26.9)	79	30-317
51-64	81 (35.7)	61	14-219
>64	85 (37.4)	47	9-123
Comorbidity			
No comorbidities	194 (85.5)	61	16-175
One or more comorbidities	33 (14.5)	38	11-236
CRP			
Low disease activity	103 (45.4)	87	22-317
High disease activity	124 (54.6)	41	9-126
DAS28 -CRP			
Low	14 (6.2)	125	11-443
Medium	90 (39.6)	69	19-193
High	54 (23.8)	46	10-133
Missing	69 (30.4)	59	15-219
Markers of SES			
Education			
Low	46 (20.3)	51	11-133
Medium	108 (47.6)	77	15-232
High	73 (32.1)	59	17-151
Wealth			
Low	77 (34)	49	9-212
Medium	75 (33)	61	19-174
High	75 (33)	73	14-175
Cohabiting status			
Living alone	59 (26)	49	9-150
Cohabiting	168 (74)	63	16-214
Occupational status			
Low	15 (6.7)	76	9-365
Medium	60 (26.9)	49	19-255
High	56 (25.1)	86	20-225
Outside the workforce	92 (41.3)	50	11-130

P, C-reactive protein; N, number; SES, socioeconomic status.

influenced delay, with lower CRP levels leading to a delay of 87 days (IQR 22-317) versus 41 days (IQR 9-126) for high CRP levels.

Markers of SES

No clinically relevant differences in median patient delay were observed across the socioeconomic groups (Table 2). However, regression analyses indicated that compared to individuals with high educational level, individuals with a medium educational level had significantly longer delays (aOR 1.96 [CI: 1.01-3.79]), while a similar but nonsignificant association was observed for lower educational level (aOR 1.26 [CI: 0.55-2.91]) (Fig 2). Individuals with a low occupational level experienced a longer delay compared to those with a high occupational level, although this was not statistically significant with an aOR of 1.58 (CI: 0.47-5.33). Lower wealth and living alone were both associated with shorter patient delay, though not statistically significant (wealth: aOR 0.73 [CI: 0.36-1.46]; living alone: aOR 0.54 [CI: 0.28-1.07]).

Exploratory analyses, including interaction terms between age group and education, did not reveal any statistically significant interactions (Supplementary Fig S1).

Responder/nonresponder analysis

Comparing nonresponders and all responders (n = 404), significant differences were observed between age and all 4 markers of SES, with nonresponders having a lower degree of SES. For the comparison between the subpopulation with available clinical values (n = 227) and nonresponders, a significantly lower degree of SES was observed in educational level and cohabiting status for nonresponders (Table 3).

DISCUSSION

Main findings

Educational level showed the strongest socioeconomic association with patient delay. The aORs showed a tendency for patients with low educational level to have a longer patient delay than patients with higher educational level. Wealth and occupational level showed little to no difference between aORs in the different groups. Patients living alone tended to have a shorter patient delay compared with patients who were cohabiting. The nonresponse analysis did, however, support significant differences between the responders and nonresponders in regard to SES, with nonresponders having a lower degree of SES.

Comparison with other studies

The study population was comparable to similar studies investigating treatment delay in patients with RA, regarding age, sex, and disease activity [4–6,20,37]. Several studies have investigated treatment delays in relation to time from onset of symptoms to diagnosis or treatment, however with insufficient information regarding patient-related delays and/or information on SES. Recent studies investigating patient delay have reported delays of 2 months [38], and 5.4 weeks [9], which is in line with the results of the current study. However, these study populations differed from our study regarding sex, with a larger female proportion, and a lower mean age. A review and meta-analysis included 12 studies assessing patient delay, finding a median patient delay of about 3.5 months [11].

Previous studies have investigated the association between SES and diagnosis or treatment delay, finding significantly longer delays for patients with low SES compared with high SES [20,39]. These studies, however, lacked information regarding the isolated effect of SES on patient delay, and thus information on patient-related behaviour before first contact with the healthcare system.

To our knowledge, only a few studies have previously investigated the association of SES and patient delay in patients with RA. An Australian study found no significant association between educational level and patient delay, with a median patient delay of 8.7 (IQR 2.9-18.8) weeks [37]. However, only 75 of 135 eligible patients were assessed due to missing data regarding GP visits, creating a potential source of selection bias.

Our study focused specifically on the help-seeking phase, ie, the time from first symptom experience to initial GP consultation [7]. Previous studies have mainly examined later phases, showing that lower educational attainment is associated with adverse outcomes, reduced healthcare use, and longer treatment delays [18–20]. Our findings extend this evidence by demonstrating that such disparities already manifest in the very first phase of the pathway to treatment,

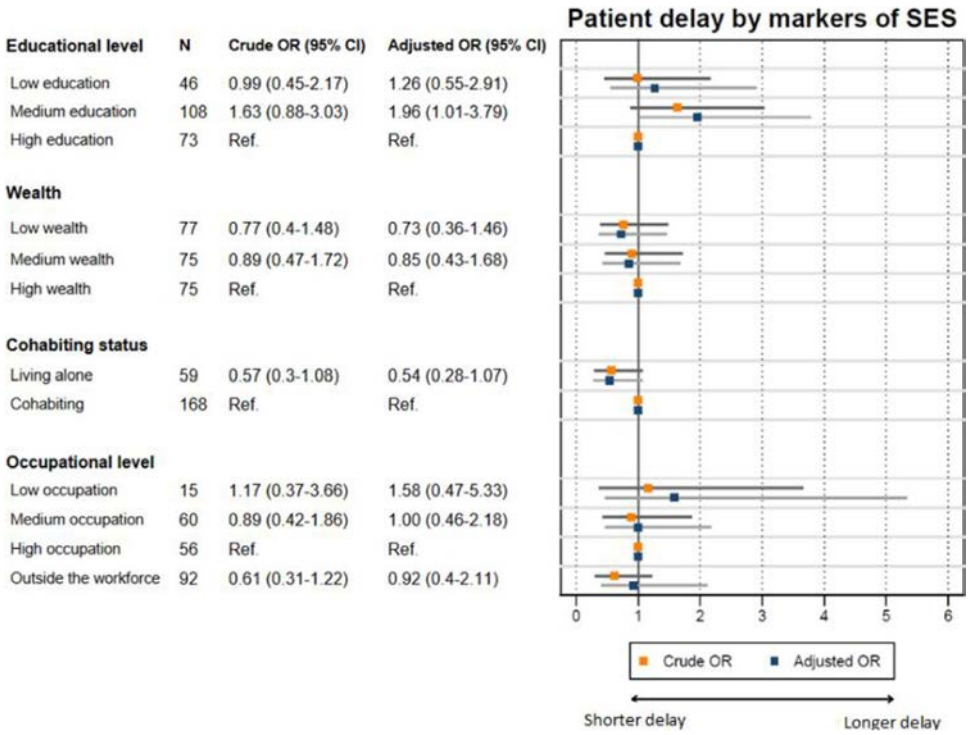


Figure 2. Logistic regression analyses of the association between markers of socioeconomic status and patient delay (<3 mo or ≥3 mo). Presented with crude and adjusted odds ratios, adjusted for sex, age, comorbidity, and disease activity measures, with corresponding 95% CIs. N, number; OR, odds ratio; Ref, reference category; SES, socioeconomic status.

when patients decide whether and when to seek medical help.

Strengths and limitations

To address the aim of the study with data of high validity, data were obtained from several data sources. Nationwide self-reported data were used to assess patients' perception of their own disease history before first contact with the healthcare system. Information on clinical disease activity measures contributed to a more comprehensive clinical picture of the study population. This information minimised the potential confounding effect of disease activity, as high disease activity has been associated with a shorter patient delay [4]. We were also able to obtain register-based individual-level data of high validity and completeness [40,41].

Our study has some limitations that merit further discussion. First, the outcome of interest was self-reported survey data, which often underrepresents socially disadvantaged individuals [42]. The nonresponder analysis showed that responders had higher SES than nonresponders (Table 3), suggesting that we may have underestimated the association between patient delay and lower educational level or living alone. Although we cannot rule out selection bias given the relatively large number of nonrespondents, findings from a related study using the same dataset suggest that health-seeking behaviour is shaped primarily by health-related behaviour and psychological factors [13]. Second, participation in DANBIO requires patients to consult a GP, be referred to a rheumatologist, and begin treatment. Individuals who do not seek care are therefore excluded, which, together with the lower SES among nonrespondents, indicates that the actual impact of SES on patient delay may be stronger than what we report. Third, a potential 'healthy worker effect' may have influenced our findings, as patients with severe disease or work disability could be underrepresented, leading to an underestimation of socioeconomic disparities in patient delay.

Further, using self-reported data to capture patients' recollections of their disease history may introduce recall bias. However, this bias is likely to be nondifferential as it does not systematically vary between comparison groups and thereby does not introduce a systematic distortion in the estimates.

The tendency for patients with higher educational levels to have a shorter patient delay is in alignment with the well-known correlation between health literacy and educational level [43]. This may be partly explained by higher health literacy in this group, enabling earlier recognition of symptoms and timely help-seeking. As we did not have direct measures of health literacy in this dataset, we could not explore its potential mediating role; future studies should therefore investigate health literacy directly to clarify how it may link educational level with patient delay.

The external validity of our findings should be interpreted in the context of the Danish healthcare system, where universal access to GP and specialist care may have reduced the influence of 'wealth' as an SES factor. In countries with fewer financial or structural supports, SES-related delays could therefore be stronger.

The observed tendency for cohabiting patients to experience longer patient delays compared to those living alone may be partly explained by differences in sex distribution. Eighty percent of patients living alone were female, compared to 63% in the cohabiting group. A recent report from the Danish Health Authority supports this interpretation, showing that women are more likely to consult their GP than men—by more than 10 percentage points [44].

Table 3
Baseline characteristics for responders and nonresponders

	Nonresponders n = 759	Responders n = 404	Test*	Responders included in regression analyses n = 227	Test*
Sex					
Female	478 (63)	276 (68.3)	0.069	153 (67.4)	0.223
Male	281 (37)	128 (31.7)		74 (32.6)	
Age	60.2 (15.5)	56.8 (14.8)	<0.001	57.9 (15.2)	0.052
Age group (y)					
18-50	189 (24.9)	126 (31.2)	<0.001	61 (26.9)	0.131
51-64	230 (30.3)	144 (35.6)		81 (35.7)	
>64	340 (44.8)	134 (33.2)		85 (37.4)	
Educational level					
Low education	228 (30.4)	74 (18.3)	<0.001	46 (20.3)	<0.001
Medium education	360 (47.9)	194 (48)		108 (47.6)	
High education	163 (21.7)	136 (33.7)		73 (32.1)	
Wealth					
Low wealth	248 (33.3)	135 (33.4)	0.987	77 (34)	0.987
Medium wealth	248 (33.4)	135 (33.4)		75 (33)	
High wealth	248 (33.3)	134 (33.2)		75 (33)	
Family liquid assets $\times 10^3$, median (IQR)	1512 (333-2873)	1868 (904-3318)	0.021	1746 (889-3198)	0.118
Equivalised disposable income $\times 10^3$, median (IQR)	318 (236-392)	346 (269-346)	0.002	334 (256-414)	0.073
Cohabiting status					
Living alone	252 (33.2)	105 (26)	0.011	59 (26)	0.04
Cohabiting	507 (66.8)	299 (74)		168 (74)	
Occupational level					
Low occupation	56 (7.5)	23 (5.8)	<0.001	15 (6.7)	0.117
Medium occupation	197 (26.3)	110 (27.6)		60 (26.9)	
High occupation	137 (18.2)	119 (29.9)		56 (25.1)	
Outside the workforce	360 (48)	146 (36.7)		92 (41.3)	

N, number.
* Pearson Chi-squared test was used for categorical variables, and Kruskal-Wallis for continuous variables.

CONCLUSION

Educational level showed the strongest socioeconomic association with patient delay, with a tendency for patients with higher educational levels to seek help earlier than patients with lower educational levels. This study highlights how different socioeconomic factors influence patient-related delays in RA care. These findings are key to developing targeted interventions that reduce delays and promote health equity.

Competing interests

AdT reports financial support was provided by Danish Rheumatism Association. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Contributors

PA was responsible for conceptualisation, methodology, formal analysis, investigation, data curation, writing – original draft, and visualisation. AdT was responsible for conceptualisation, methodology, investigation, writing – original draft, visualisation, supervision, and funding acquisition. E-MH and SDP were responsible for writing – review and editing.

Funding

The study was supported by The Danish Rheumatism Association and Praksisforskningsfonden, Central Denmark Region. The funders were not involved in any aspects of the study.

Patient consent for publication

Not applicable.

Ethics approval

According to Danish law, approval by the regional committee on health research ethics in the Central Denmark Region was not required as no biomedical intervention was performed.

Provenance and peer review

Not commisioned; externally peer reviewed.

Data availability statement

The data and analyses were handled on secure servers provided by Statistics Denmark. These data are not publicly available, adhering to the Danish data protection legislation, as the data contains personal data which cannot be shared. Only aggregated results are presented in the current study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ard.2025.10.001.

Orcid

Annette de Thurah: http://orcid.org/0000-0003-0103-4328

REFERENCES

[1] van der Linden MP, le Cessie S, Raza K, van der Woude D, Knevel R, Huizinga TW, et al. Long-term impact of delay in assessment of patients with early arthritis. Arthritis Rheum 2010;62:3537–46.
[2] Burmester GR, Pope JE. Novel treatment strategies in rheumatoid arthritis. Lancet 2017;389:2338–48.
[3] Combe B, Landewe R, Daien CI, Hua C, Aletaha D, Álvaro-Gracia JM, et al. 2016 update of the EULAR recommendations for the management of early arthritis. Ann Rheum Dis 2017;76:948–59.
[4] De Cock D, Meyfroidt S, Joly J, Van der Elst K, Westhovens R, Verschueren P. A detailed analysis of treatment delay from the onset of symptoms in early rheumatoid arthritis patients. Scand J Rheumatol 2014;43:1–8.
[5] Raza K, Stack R, Kumar K, Filer A, Detert J, Bastian H, et al. Delays in assessment of patients with rheumatoid arthritis: variations across Europe. Ann Rheum Dis 2011;70:1822–5.
[6] Kumar K, Daley E, Carruthers DM, Situnayake D, Gordon C, Grindulis K, et al. Delay in presentation to primary care physicians is the main reason why patients with rheumatoid arthritis are seen late by rheumatologists. Rheumatology (Oxford) 2007;46:1438–40.
[7] Scott SE, Walter FM, Webster A, Sutton S, Emery J. The model of pathways to treatment: conceptualization and integration with existing theory. Br J Health Psychol 2013;18:45–65.
[8] Van der Elst K, De Cock D, Vecoven E, Arat S, Meyfroidt S, Joly J, et al. Are illness perception and coping style associated with the delay between symptom onset and the first general practitioner consultation in early rheumatoid arthritis management? An exploratory study within the CareRA trial. Scand J Rheumatol 2016;45:171–8.
[9] Stack RJ, Nightingale P, Jinks C, Shaw K, Herron-Marx S, Horne R, et al. Delays between the onset of symptoms and first rheumatology consultation in patients with rheumatoid arthritis in the UK: an observational study. BMJ Open 2019;9:e024361.
[10] Ellingwood L, Kudaeva F, Schieir O, Bartlett S, Bessette L, Boire G, et al. A quarter of patients time their early rheumatoid arthritis onset differently than physicians. RMD Open 2019;5:e000931.
[11] Barhamain AS, Magliah RF, Shaheen MH, Munassar SF, Falemban AM, Alshareef MM, et al. The journey of rheumatoid arthritis patients: a review of reported lag times from the onset of symptoms. Open Access Rheumatol 2017;9:139–50.
[12] de Thurah A, Jensen H, Maribo T, Jensen MB, Sandbæk A, Hauge EM, et al. Use of primary health care and radiological imaging preceding a diagnosis of rheumatoid arthritis: a nationwide cohort study in Denmark. Rheumatology (Oxford) 2023;62:555–64.
[13] Pedersen SD, Nielsen BD, Assmann ML, Hauge EM, de Thurah A. Early diagnosis of rheumatoid arthritis: associations between patients' perceptions of initial symptoms and the timing of seeking help from the general practitioner. Scand J Rheumatol 2025;54 242-51-10.
[14] Pedersen KM, Andersen JS, Søndergaard J. General practice and primary health care in Denmark. J Am Board Fam Med 2012;25(Suppl 1):S34–8.
[15] United Nations. Transforming our world: the 2030 agenda for sustainable development [Internet]. [updated 2025 Aug 1; cited 2025 Aug 1]. Available from: https://sustainabledevelopment.un.org/content/documents/21252030%20Agenda%20for%20Sustainable%20Development%20web.pdf
[16] Braveman P, Gottlieb L. The social determinants of health: it's time to consider the causes of the causes. Public Health Rep 2014;129(Suppl 2):19–31.
[17] Conrad N, Misra S, Verbakel JY, Verbeke G, Molenberghs G, Taylor PN, et al. Incidence, prevalence, and co-occurrence of autoimmune disorders over time and by age, sex, and socioeconomic status: a population-based cohort study of 22 million individuals in the UK. Lancet 2023;401:1878–90.
[18] Zou Y, Cheung PP, Teoh LK, Chen C, Lahiri M. Sociodemographic factors as determinants of disease, disability and quality of life trajectories in early rheumatoid arthritis: a multi-ethnic inception cohort study. Int J Rheum Dis 2020;23:55–64.
[19] Jacobi CE, Mol GD, Boshuizen HC, Rupp I, Dinant HJ, Van Den Bos GA. Impact of socioeconomic status on the course of rheumatoid arthritis and on related use of health care services. Arthritis Rheum 2003;49:567–73.
[20] Molina E, Del Rincon I, Restrepo JF, Battafarano DF, Escalante A. Association of socioeconomic status with treatment delays, disease activity, joint damage, and disability in rheumatoid arthritis. Arthritis Care Res (Hoboken) 2015;67:940–6.
[21] Ibfelt EH, Jensen DV, Hetland ML. The Danish nationwide clinical register for patients with rheumatoid arthritis: DANBIO. Clin Epidemiol 2016;8:737–42.
[22] e-Boks. What is a digital postbox? [Internet]. [updated 2025 Aug 1; cited 2025 Aug 1]. Available from: https://global.e-boks.com/what-is-a-digital-postbox/
[23] Schmidt M, Pedersen L, Sørensen HT. The Danish Civil Registration System as a tool in epidemiology. Eur J Epidemiol 2014;29:541–9.
[24] Statistics Denmark. About us [Internet]. [updated 2025 Aug 1; cited 2025 Aug 1]. Available from: https://www.dst.dk/en/OmDS)
[25] Organisation for Economic Co-operation and Development (OECD) / Eurostat / UNESCO Institute for Statistics (UIS). ISCED 2011 Operational Manual: Guidelines for Classifying National Education Programmes and Related Qualifications. Paris: OECD Publishing; 2015.
[26] Hoffmann R, Kröger H, Geyer S. Social causation versus health selection in the life course: does their relative importance differ by dimension of SES? Soc Indic Res 2019;141:1341–67.
[27] Galobardes B, Shaw M, Lawlor DA, Lynch JW, Davey Smith G. Indicators of socioeconomic position (part 1). J Epidemiol Community Health 2006;60:7–12.
[28] Berkman LF, Glass T. Social integration, social networks, social support, and health. social epidemiology. New York, NY: Oxford University Press; 2000. p. 186–233.
[29] Office GIL. The International Standard Classification of Occupations (ISCO-08) companion guide [Internet]. [updated 2025 Aug 1; cited 2025 Aug 1]. Available from: https://ilostat.ilo.org/methods/concepts-and-definitions/classification-occupation/
[30] Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987;40:373–83.
[31] Quan H, Li B, Couris CM, Fushimi K, Graham P, Hider P, et al. Updating and validating the Charlson comorbidity index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. Am J Epidemiol 2011;173:676–82.
[32] Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol 2015;7:449–90.
[33] Regionernes Kliniske Kvalitetsudviklingsprogram. The Danish Rheumatology Database (DANBIO) - National Annual Report. 2023. [Internet]. [updated 2025 Aug 1; cited 2025 Aug 1]. Available from: https://www.sundk.dk/media/u3m1a3vu/034e79e423be4caa886171d508b770d2.pdf.
[34] Tolonen H, Dobson A, Kulathinal S. Assessing the quality of risk factor survey data: lessons from the WHO MONICA Project. Eur J Cardiovasc Prev Rehabil 2006;13:104–14.
[35] Tolonen H, Dobson A, Kulathinal S. Effect on trend estimates of the difference between survey respondents and non-respondents: results from 27 populations in the WHO MONICA Project. Eur J Epidemiol 2005;20:887–98.
[36] Galea S, Tracy M. Participation rates in epidemiologic studies. Ann Epidemiol 2007;17:643–53.
[37] Ison M, Duggan E, Mehdi A, Thomas R, Benham H. Treatment delays for patients with new-onset rheumatoid arthritis presenting to an Australian early arthritis clinic. Intern Med J 2018;48:1498–504.
[38] Naeem F, Khan SE, Saeed MA, Farman S. Diagnostic and therapeutic delay in rheumatoid arthritis patients: impact on disease outcome. Pak J Med Sci 2021;37:1001–7.
[39] Widdifield J, Paterson JM, Bernatsky S, Tu K, Thorne JC, Ivers N, et al. Access to rheumatologists among patients with newly diagnosed rheumatoid arthritis in a Canadian universal public healthcare system. BMJ Open 2014;4:e003888.
[40] Jensen VM, Rasmussen AW. Danish Education Registers. Scand J Public Health 2011;39:91–4.
[41] Baadsgaard M, Quitzau J. Danish registers on personal income and transfer payments. Scand J Public Health 2011;39:103–5.
[42] Reinikainen J, Tolonen H, Borodulin K, Härkänen T, Jousilahti P, Karvanen J, et al. Participation rates by educational levels have diverged during 25 years in Finnish health examination surveys. Eur J Public Health 2018;28:237–43.
[43] Beauchamp A, Buchbinder R, Dodson S, Batterham RW, Elsworth GR, McPhee C, et al. Distribution of health literacy strengths and weaknesses across socio-demographic groups: a cross-sectional survey using the Health Literacy Questionnaire (HLQ), 15. BMC Public Health; 2015. p. 678.
[44] Danish Health Authority. Social ulighed i sundhed og sygdom i Danmark—Udviklingen i Danmark i perioden 2013-2021. 2025. [Internet]. [updated 2025 Aug 1; cited 2025 Aug 1]. Available from: https://www.sst.dk/-/media/Udgivelser/2025/Ulighed/Social-ulighed-2013-2021.ashx.

Ann Rheum Dis 85 (2026) 254–264

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Rheumatoid arthritis

Short-chain fatty acids and their gut microbial pathways distinguish rheumatoid arthritis in discordant monozygotic twins

Rebecca B. Blank[1,†], Kevin Bu[2,†], Xinyuan Zhang[3,†], Weixi Chen[4], Ian Cunningham[1], Jeremy Sokolove[5], Lauren Lahey[5], Adriana Heguy[6], Rhina Medina[1], Carles Ubeda[7,8], Renuka R. Nayak[9,10], Jiyuan Hu[4], Adam Cantor[2], Jakleen Lee[2], Frances M.K. Williams[3,‡], Jose C. Clemente[2,‡], Jose U. Scher[1,11,‡,*]

[1] *Division of Rheumatology, Department of Medicine, NYU Grossman School of Medicine, New York, New York, USA*
[2] *Department of Genetics and Genomic Sciences, Precision Immunology Institute, Icahn School of Medicine at Mt. Sinai, New York, New York, USA*
[3] *Department of Twin Research and Genetic Epidemiology, School of Life Course Sciences, King's College London, London, UK*
[4] *Division of Biostatistics, Department of Population Health, NYU School of Medicine, New York, New York, USA*
[5] *Stanford University School of Medicine, Stanford, California, USA*
[6] *Department of Pathology, NYU School of Medicine, New York, New York, USA*
[7] *Fundación para el Fomento de la Investigación Sanitaria y Biomédica de la Comunitat Valenciana, FISABIO, Valencia, Spain*
[8] *CIBER en Epidemiología y Salud Pública, Madrid, Spain*
[9] *Rheumatology Division, Department of Medicine, University of California, San Francisco, California, USA*
[10] *Veterans Affairs Medical Center, San Francisco, California, USA*
[11] *NYU Colton Center for Autoimmunity, NYU Grossman School of Medicine, New York, New York, USA*

ARTICLE INFO

Article history:
Received 27 June 2025
Received in revised form 18 August 2025
Accepted 28 August 2025

ABSTRACT

Objectives: Although genetic risk factors, such as HLA-DRB1 alleles, contribute to the pathogenesis of rheumatoid arthritis (RA), the concordance rate in monozygotic (MZ) twins is low, suggesting that other factors are involved in disease development. Further, the relative contribution of nongenetic elements in identical twins has not been characterised. Here, we aimed to characterise host and microbial biomarkers of RA by studying MZ twins discordant for disease using a multiomics approach.

Methods: Eight pairs of MZ twins discordant for RA (N = 16) were enrolled in the United States (US). The gut microbiome was assessed using shotgun metagenomic sequencing. Autoantibodies, cytokines, and plasma proteins were measured in both plasma and faeces. Levels of short-chain fatty acids (SCFAs) from serum and faeces were quantified using gas chromatography mass spectrometry (GC-MS). Metagenomic data from a UK twin registry (TwinsUK) (N = 14) were used to validate findings in the US population.

Results: Although microbiome diversity and composition did not differ between twins, we observed a significant decrease in the SCFA-producing bacteria *Blautia faecis* and significantly

*Correspondence to Dr. Jose U. Scher, Division of Rheumatology, Department of Medicine, NYU Grossman School of Medicine, New York, New York, USA.
E-mail address: Jose.scher@nyulangone.org (J.U. Scher).

Handling editor Josef S. Smolen.

Rebecca B. Blank, Kevin Bu, Xinyuan Zhang share first authorship and Frances M.K. Williams, Jose C. Clemente, and Jose U. Scher share last authorship.
† Co-first authors.
‡ Co-last authors.

https://doi.org/10.1016/j.ard.2025.08.029

lower concentrations of faecal butyrate and propionate in affected RA twins in the US. TwinsUK showed a similar reduction in the SCFA-producers *Gemmiger formicilis* and *Faecalicatena fissicatena,* as well as bacterial SCFA metabolism pathways.

Conclusions: Multiomics biomarkers differentiate MZ twins discordant for RA. Faecal butyrate and propionate, as well as SCFA-producing bacteria, were decreased in affected twins. We found a similar decrease in SCFA-producing taxa in affected twins in a geographically distinct cohort in the UK. Our results suggest that, if further validated in larger cohorts, multiomics approaches may improve our understanding of RA pathogenesis and, potentially, contribute to more accurate diagnostics and coadjuvant therapies.

Key messages

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Genetic and environmental factors contribute to rheumatoid arthritis (RA) development and perpetuation.
- Genetics plays only a limited role in RA disease development, as suggested by the low disease concordance rate in monozygotic twins.
- Gut microbial taxa and their metabolites (eg, short-chain fatty acids [SCFAs]) are implicated in RA pathogenesis.
- Few RA studies have utilised a multiomics approach to understand disease pathogenesis, and none have controlled for the confounding effect of genetics.

WHAT THIS STUDY ADDS

- This study characterises, for the first time, the molecular landscape of RA using a multiomics approach while controlling for host genetics.
- Our results identified SCFA-producing gut microbes and intestinal SCFAs as potential biomarkers of nonprogression in unaffected monozygotic discordant RA twins.
- Analysis of a geographically distinct cohort of monozygotic twins found similarities in microbial taxa and pathways of SCFA producers in unaffected twins.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- The study of genetic and environmental factors in isolation limits our understanding of RA pathogenesis. The comprehensive multiomics characterisation of monozygotic twins discordant for RA, including the microbiome, metabolome, and proteome, provides an integrated molecular landscape of disease pathogenesis and may help with biomarker discovery for earlier diagnosis and potential SCFA-based therapeutics.

INTRODUCTION

Genetic factors, including susceptibility alleles, have been identified in the pathogenesis of rheumatoid arthritis (RA) [1,2] and can contribute to up to 60% of the risk of developing RA [3]. However, the concordance rate in monozygotic (MZ) twins is only 12% to 15% [4,5], suggesting that other factors must contribute to disease onset and perpetuation. Among those, autoantibodies, the microbiome, and the metabolome have all been associated with RA.

The gut microbiome has been shown to be associated with inflammatory arthritis in various murine models [6–8], and different studies have implicated microbial taxa in the pathogenesis of human RA, including periodontal microbes *Porphyromonas gingivalis* [9,10] or *Lactobacillus* salivarius [11] (present in both saliva and the gut) and gut microbes (eg, *Prevotella copri* [6,12,13] and *Subdoligranulum* [14]), among others [15]. In fact, some of these bacteria have been implicated in the induction of RA-associated pathogenic autoantibodies, which can be observed in both pre-RA [10] and symptomatic RA patients [9,11,13,14]. These microbes can subsequently activate innate, B- and T-cell responses that produce proinflammatory cytokines such as interleukin (IL)-17 [6,7,13], IL-1, IL-6, tumour necrosis factor (TNF)-α, and others [15]. Conversely, gut microbial fermentation byproducts, such as short-chain fatty acids (SCFAs), have been shown to induce a tolerogenic immune response [16], ameliorate inflammatory arthritis in murine models [17,18], and have been found to be decreased in serum concentrations in pre-RA individuals [19]. Although previous studies have investigated the contribution of each of these factors to RA in isolation, few have integrated different assays to characterise the molecular landscape of immune-mediated inflammatory disease [20–22], and to our knowledge, no study to date has used a multiomics approach while controlling for host genetics. Here, we combined microbiome, autoantibody, metabolome, and circulating plasma protein data in a cohort of MZ twins discordant for RA to identify disease signatures that discriminate between affected and unaffected twins. In an independent, geographically distinct cohort of MZ RA discordant twins, we validated the relevance of specific bacterial functions as potential biomarkers of nonprogression in RA.

METHODS

Participants

MZ twin pairs discordant for RA were recruited from the population-based Mid-Atlantic Twin Registry (MATR). Eight pairs of MZ twins were enrolled in the study. Diagnosis of RA was obtained through the patient's medical records. Upon enrolment, demographics and detailed medical history, including review of past and present medication, were collected. In addition, Multidimensional Health Assessment Questionnaire (MD-HAQ), swollen joint counts (SJCs), and tender joint counts (TJCs) were estimated. Stool, serum, and plasma samples were collected at the time of enrolment. An additional validation cohort of MZ twin pairs discordant for RA was recruited from a population-based UK twin registry (TwinsUK). Available data included faecal shotgun metagenomic data from the time of enrolment.

Patient and public involvement

Patients and/or the public were not involved in the design of our research.

Metagenomics

Faecal samples were collected, stored on freezer packs, and shipped to the Scher laboratory within 24 hours of collection, and immediately stored at −80°C as previously described [23]. Bacterial DNA extraction and shotgun metagenomic sequencing

to characterise the gut microbiome were performed as previously described [23]. Briefly, DNA was extracted from faecal samples using the MoBio Powersoil DNA Isolation Kit (QIAGEN). Metagenomic sequencing was performed using the NextSeq500 platform (Illumina) with 2 × 150 bp reads according to the manufacturer's instructions. Analysis of metagenomic sequencing data was performed using MetaPhlAn 4 [24] to estimate bacterial composition and Human Microbiome Project Unified Metabolic Analysis Network (HUMAnN) 3 [25] to determine bacterial functions.

Faecal sample collection, DNA extraction, and library preparation for the TwinsUK cohort were performed as previously described [26]. Metagenomics sequencing was performed by GenomeScan (https://genomescan.nl/) using an Illumina HiSeq 2500 with 2 × 125 bp reads. For TwinsUK, quality control of paired-end reads was performed using the YAMP pipeline (v.0.9.5.0, mode "qc") [26] with the "genomescan" parameter. Taxonomic profiling was performed with MetaPhlAn 4, and functional profiling was performed with HUMAnN 3.

Diversity metrics and visualisations were estimated as implemented in QIIME2 V2020.8.0 [27]. Shannon diversity was used to measure α-diversity, whereas Bray-Curtis was used for β-diversity. The Bray-Curtis distance matrix was used as input for principal coordinate analysis (PCoA). Comparison of microbiome diversity distances between 3 groups (intratwin, inter-RA, and interunaffected) was performed as follows: for α-diversity, the intratwin distances were obtained by computing the absolute value of the differences in Shannon entropy for each of the twin pairs ($|\alpha_{\text{Unaffected}} - \alpha_{\text{RA}}|$). For the inter-RA distances, the absolute value of the difference in Shannon entropy was computed for each pair of RA-affected twins, ie, for all (8 choose 2) = 28 pairs. Analogous distances were computed for the interunaffected group.

β-Diversity distances were computed in a similar fashion. The intratwin distances consisted of the 8 Bray-Curtis distances between unaffected and affected twins, ie, 1 per twin pair. The inter-RA differences consisted of aggregating all (8 choose 2) = 28 distances between each pair of individuals with RA, and an analogous calculation was performed for the interunaffected differences. The distances of these 3 groups (intratwin, inter-RA, and interunaffected) were then compared using the Kruskal-Wallis test. Correlations of bacterial diversity (Shannon entropy for α-diversity or Bray-Curtis intratwin for β-diversity) with SJC, TJC, C-reactive protein (CRP), and disease activity score-CRP (DAS28-CRP) were performed using Spearman correlation. Cohen's D effect size was computed using the method previously described [28].

Metabolomics

Faecal SCFA and medium-chain fatty acid (MCFA) measurements were performed at the University of Michigan Metabolomics Core (Michigan Medicine Biomedical Research Facilities). Briefly, SCFA analysis was performed by gas chromatography mass spectrometry (GC-MS) on an Agilent 69890N GC-5973 MS detector as previously described [29]. MCFA analysis was performed using Reversed-Phase Liquid Chromatography-Mass Spectrometry (RPLC-MS) on an Agilent 1290 Infinity II/6545 qTOF MS system with a JetStream Ionisation (ESI) source (Agilent Technologies) and the Acquity HSS T3 1.8 µm 50 mM column (Waters Corporation). Each sample was analysed twice, once in positive and once in negative ion mode. Mobile phase A was 100% water with 0.1% formic acid, and mobile phase B was 100% methanol with 0.1% formic acid. The gradient for both positive and negative ion modes was as follows: 2% B (0 minutes), 75% B (20 minutes), 98% B (22 minutes), 98% B (30 minutes), and 2% B (30.1 minutes). The column was then reconditioned for 7 minutes with 2% B before moving to the next injection. The flow rate was 0.46 mL/min, and the column temperature was 40°C. The injection volumes for the positive and negative modes were 5 µL and 8 µL, respectively. Source parameters were as follows: drying gas temperature 350°C, drying gas flow rate 10 L/min, nebuliser pressure 30 psig, sheath gas temperature 350°C, flow 11 L/min, and capillary voltage 3500 V, with internal reference mass correction. Data analysis of SCFAs and MCFAs was processed using MassHunter Quantitative Analysis version B.07.00 (Agilent Technologies). SCFAs and MCFAs were normalised to the nearest isotope-labelled internal standard and quantified using 2 replicated injections of 6 standards to create a linear calibration curve with an accuracy better than 80% for each standard. Other compounds in the analysis were normalised to the nearest internal standard, and the peak areas were used for differential analysis between groups. Samples were normalised to wet sample weight after quantification.

Serum samples were collected, centrifuged at 1700 × *g* for 10 minutes, and immediately stored at −80°C. Frozen serum sample aliquots were shipped on dry ice for SCFA metabolomics profiling by Metabolon Inc, as previously described [30], using the quantitative SCFA panel. Quantitative analysis was performed using Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) on an Agilent 1290 UHPLC/Sciex QTrap 5500 instrument.

Multiplex autoantibody assays

Autoantibody concentration was determined in stool and plasma using a multiplex flow cytometry assay as previously described [31,32]. Briefly, a panel of 103 citrullinated and noncitrullinated self-protein antigens were conjugated to fluorescent beads using the Bio-Plex Multiplex Immunoassay System (Bio-Rad Laboratories) and analysed using the Luminex 200 (Luminex). Pooled beads were mixed with either plasma or stool samples and incubated at room temperature. After washing, the sample dyed bead mixture was incubated with antihuman immunoglobulin (Ig) G antibody conjugated to phycoerythrin (PE) at room temperature. After the second wash, the bead mixture was passed through the laser detector Luminex 200, which identified the beads based on the fluorescence of the dyes. The quantity of autoantibody bound to each bead was determined by PE fluorescence and expressed as mean fluorescence intensity (MFI). Serum anticyclic citrullinated peptide (CCP) antibody was measured by QuantaLite CCP 3.1 IgG/IgA ELISA (INOVA Diagnostics).

Analysis of plasma cytokines and proteins

Plasma analytes were measured by proximity extension assay (PEA) (OLINK Technology, Bioxpedia) using a 92 inflammation-associated plasma protein biomarker panel. PEA was performed at the Human Immune Monitoring Center, Icahn School of Medicine, New York, NY, USA. PEA allows for the detection of very low concentrations of plasma analytes, and the final concentration is given in normalised protein expression (NPX) [33] units, which is an arbitrary unit on a log2 scale. Of the 92 analytes in

the panel, 6 analytes were removed from analysis because at least 33% of the sample measurements were below the limit of detection.

Statistical analyses

The association of clinical metrics (eg, age, sex assigned at birth, ethnicity, SJC, and TJC) with disease status was assessed using Fisher's exact test for dichotomous variables and paired t-tests for continuous variables. Analysis of inflammatory cytokines, fatty acids, and citrullinated peptides was performed using paired t-tests. Comparison of α-diversities, taxonomic abundances, and pathway abundances in unaffected vs affected twins was done using paired Wilcoxon signed-rank tests. Permutational multivariate analysis of variance (PERMANOVA) using 999 permutations was used to assess differences in composition between affected and unaffected twins. Comparison of α-diversity differences or β-diversity distances between subgroups (eg, comparing differences between individuals of the same diagnosis vs differences between individuals with the same genetics) was done using Kruskal-Wallis tests. Differential taxa enriched or depleted in affected twins were identified using Wilcoxon signed-rank tests. Correlations were computed using Spearman's correlation coefficient and further inspected for false positives using CUTIE [34].

Statistical analyses (paired and unpaired t-tests, Wilcoxon signed-rank tests, Mann-Whitney U test, and Cohen's D [35]) and plotting were performed using Python 3.7 and libraries (pandas [36] and seaborn [37]), and R 4.2.1 [38] and libraries (stats and ggplot2 [39]). Unless otherwise specified, functions, plotting, and testing were performed with default parameters.

RESULTS

Eight MZ twin pairs discordant for RA were enrolled in the United States, with a mean age of 56.3 years and a mean disease duration of 7.0 years (Table). The majority were female (87.5%), and all were non-Hispanic White. The RA-affected twins had moderate disease activity with a mean DAS28-CRP of 4.02; their mean TJC was 10.4, and their mean SJC was 6.25. Both affected and unaffected twins had a wide range of serum anti-CCP antibody levels, as quantified by CCP 3.1 IgG/IgA ELISA, with no significant difference between affected and unaffected twins. Most affected twins (75%) were currently or had previously taken methotrexate (MTX), and 62.5% were currently taking MTX at the time of the study. A total of 62.5% of affected twins were being treated with or had previous exposure to a biologic agent (Table); 4 affected twins had exposure to TNF inhibitors, and 1 had had exposure to multiple TNF inhibitors and abatacept and was on rituximab at the time of enrolment. None had taken or were currently taking a Janus kinase (JAK) inhibitor. Potential confounders (smoking, antibiotics, non-steroidal anti-inflammatory drugs [NSAIDs], and steroid usage) were not significantly associated with any of the categories tested (Table and Supplementary Table S1).

Gut microbiome of affected and unaffected twins

Alpha- and β-diversity were not significantly different between affected and unaffected twins (Mann-Whitney U test, $P = .95$ and PERMANOVA, $P = .80$, respectively; Fig 1A,B). We next compared the gut microbiome of individuals with a shared diagnosis vs individuals with a shared genetic background. There were no significant differences between intratwin and intradisease differences in the overall species diversity using Shannon entropy (Kruskal-Wallis test, $P = .67$; Supplementary Fig S1A). Namely, the distance within twin pairs was significantly lower than the distance within unrelated RA subjects or the distance within unrelated healthy subjects. These trends were recapitulated to a lesser extent in analyses of β-diversity, where twin pairs tended to have a gut microbiome more similar to each other than to unrelated individuals with a shared diagnosis, although not significantly (Kruskal-Wallis test, $P = .15$; Supplementary Fig S1B). Regarding specific features, we observed a depletion of *Blautia faecis* in affected twins compared with unaffected twins (Wilcoxon signed-rank test, $P = .04$; Fig 1C,D). Analysis of microbial functions also identified 7 pathways that were differentially abundant in affected and unaffected twins, including lysine metabolism, cyanoamino acid metabolism, and monobactam biosynthesis, which were more abundant in RA (Fig 1E, Supplementary Fig S2).

Current treatment with MTX was not associated with significant differences in α-diversity (Mann-Whitney U test, $P = .39$; Supplementary Fig S3A) or β-diversity (Mann-Whitney U test, $P = .57$; Supplementary Fig S3B). α-Diversity was also not significantly associated with disease activity, as measured by SJC

Table
Cohort demographics and clinical information

Characteristic	Unaffected (n = 8)	RA (n = 8)	*P* value
Age (y), mean (median)	56.3 (58.0)	56.3 (58.0)	1
Female, n (%)	7 (87.5)	7 (87.5)	1
White/Caucasian, n (%)	8 (100)	8 (100)	1
Disease duration (y), mean (median)	–	7.0 (4.0)	–
Disease activity parameters, mean (median)			
TJC-28	–	10.4 (9.5)	–
SJC-28	–	6.25 (4.0)	–
DAS 28-CRP	–	4.02 (4.0)	–
Serum CCP antibody (units), mean (SD)	13,314 (36,841)	27,203 (33,538)	.117
Ever smoked, n (%)	4 (50)	3 (37.5)	>.999
Current NSAID use, n (%)	4 (50)	5 (62.5)	>.999
Medication use ever, n (%)			
Prednisone	–	4 (50.0)	–
Methotrexate	–	6 (75.0)	–
Biologic agent	–	5 (62.5)	–

CCP, cyclic citrullinated peptide; DAS28-CRP, disease activity score-C-reactive protein; SJC, swollen joint count; TJC, tender joint count. NSAID, non-steroidal anti-inflammatory drug.

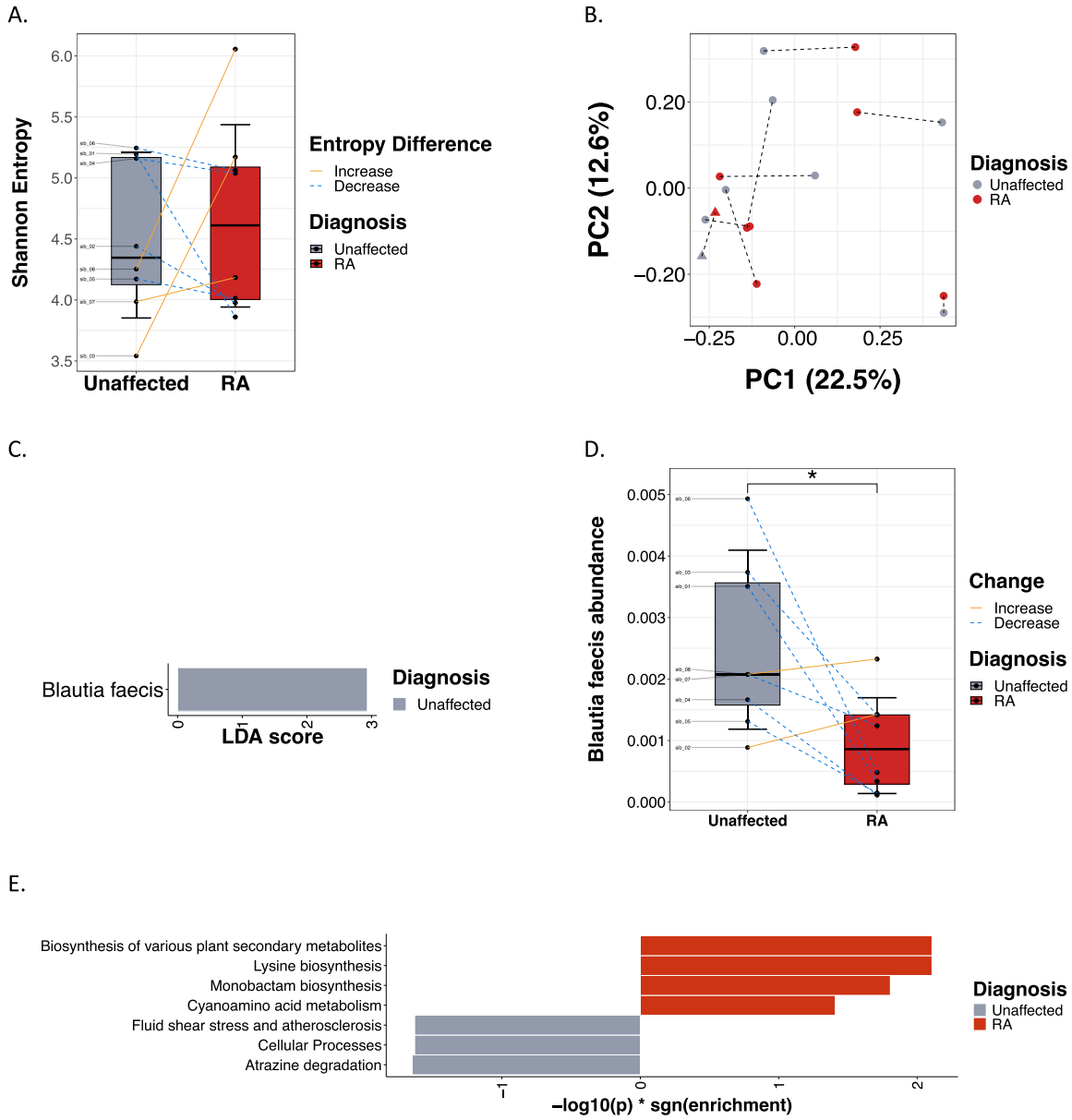


Figure 1. Gut bacterial composition and predicted functional pathways in affected and unaffected twins in the US cohort. A, Shannon α-diversity in participants, with box plots representing the median and IQR. Lines are drawn between monozygotic (MZ) pairs. B, Principal coordinate analysis (PCoA) of Bray-Curtis β-diversity between groups. C, Linear discriminant analysis effect size (LEfSe) showing differential species in affected twins compared with unaffected twins. D, Boxplot showing distribution of *Blautia faecis* abundance in unaffected and affected twins. E, Barplot of Wilcoxon signed-rank test *P* values for differential pathways. *$P < .05$, **$P < .01$. RA, rheumatoid arthritis. PC, principal component. LDA, linear discriminant analysis

(Spearman rho = 0.11; $P = .8$), TJC (Spearman rho = 0.36; $P = .39$), CRP (Spearman rho = −0.10; $P = .82$), and DAS28-CRP (Spearman rho = 0.12; $P = .78$) (Supplementary Fig S4A-D). Finally, we found no significant correlation between these 4 clinical measures and the intratwin Bray-Curtis distances of the affected twins (P = not significant), suggesting that disease severity is not associated with a larger difference in microbial composition between affected and unaffected twins.

Affected twins have a lower concentration of SCFAs

We next tested whether there were differences in fatty acid concentrations between affected and unaffected twins. Compared with their unaffected siblings, affected twins had significantly lower concentrations of faecal butyrate ($P = .03$), propionate ($P = .03$), and acetate ($P = .05$) and a significantly higher concentration of faecal valerate (paired t-test, $P = .04$; Fig 2A-D). There was no significant difference in serum SCFAs between the 2 groups, although unaffected twins had higher concentrations of SCFAs (paired t-test, $P = .41$ for 2-methylbutyric acid; $P = .31$ for isobutyric acid; $P = .67$ for acetic acid; $P = .15$ for hexanoic acid; Supplementary Fig S5).

Integrative analysis of faecal autoantibodies, SCFAs, and inflammatory phenotypes

We further explored whether autoantibodies considered to be pathogenic in RA were differentially expressed in twin pairs. Using a multiplex bead assay, we measured 103 faecal autoantibodies (Supplementary Table S2) against either citrullinated (ACPA) or noncitrullinated native proteins. Only 1 faecal autoantibody was significantly elevated in US-affected twins compared with their unaffected siblings (Supplementary Fig S6 and Supplementary Table S3). We then conducted Spearman

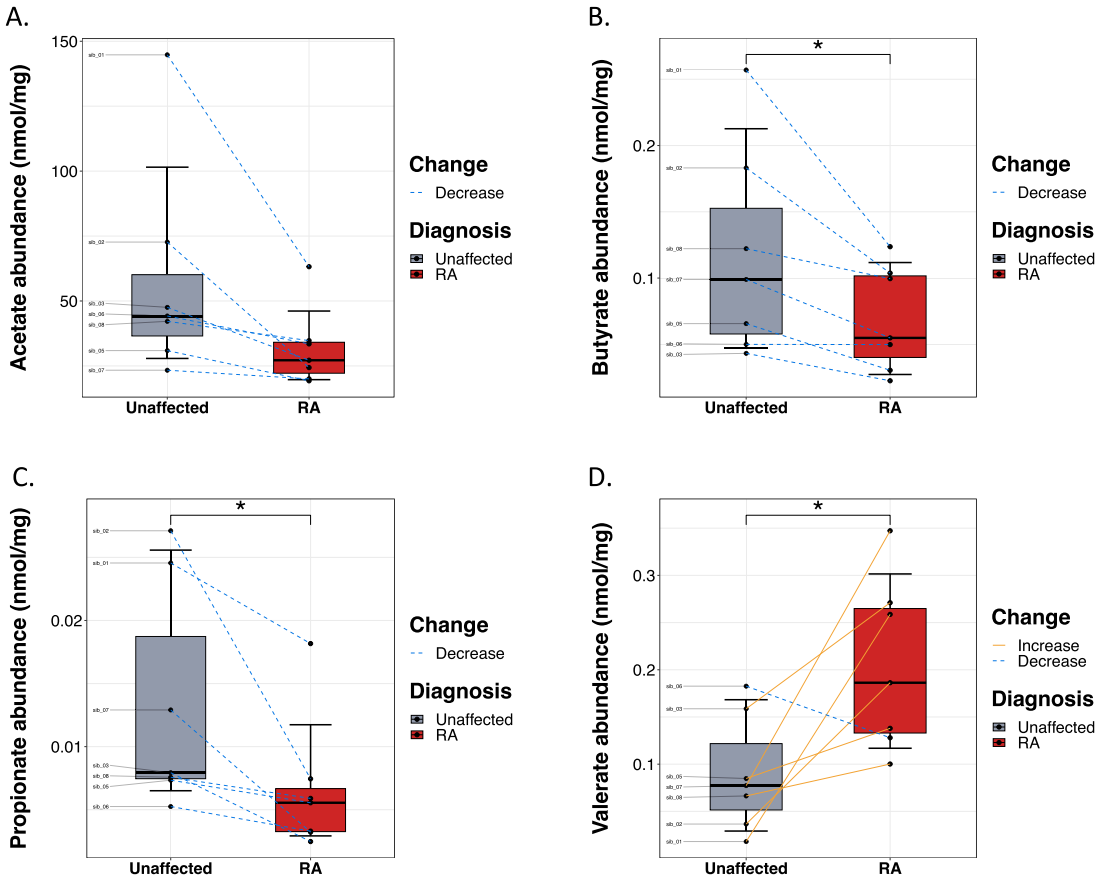


Figure 2. Comparison of faecal short-chain fatty acid (SCFA) concentrations between unaffected and affected twins. A, Affected twins have a lower faecal concentration of acetate. B, Butyrate faecal levels are depleted in affected twins. C, Propionate faecal concentrations are lower in affected twins. D, Valerate faecal levels are higher in affected twins. Lines are drawn between monozygotic (MZ) pairs. *P* values were calculated with a paired t-test; **P* < .05. RA, rheumatoid arthritis.

correlation analysis between taxa and functional pathways of cytokines, fatty acids, and autoantibody data in the US-affected and unaffected twins separately, focusing on the differential taxa (*B. faecis*) and 7 differential pathways. We found no significant correlations involving *B. faecis* and 34 differential pathways in the affected group (2 of which also involved differential inflammatory markers), and 30 significant correlations involving *B. faecis* and 48 differential pathways in the unaffected twins after filtering out false positive results. Out of the 30 significant correlations involving *B. faecis* in the unaffected twins, 2 were with cytokines and 28 were with faecal ACPAs. *B. faecis* was positively correlated with FMS-like tyrosine kinase 3 ligand (Flt3L) (rho = 0.90; *P* = .002) and negatively correlated with stem cell factor (SCF) (rho = −0.98; *P* = .00003; Supplementary Fig S7A,B) in unaffected twins. Additionally, all but 1 of the remaining 28 significant associations between *B. faecis* and faecal autoantibodies were negative. *B faecis* was not correlated with faecal butyrate, propionate, valerate, and acetate levels, although the correlation approached statistical significance for acetate (Spearman's rho = 0.45; *P* = .09). We next performed correlation analyses in unaffected and affected twins separately to assess the association between faecal SCFAs and faecal inflammatory cytokines. We found 70 correlations in the affected twins and 43 in the unaffected twins that were significant at *P* < .05, though none passed the false discovery rate correction. Notably, all of the correlations were negative in the affected twins, including correlations between faecal butyrate and IL-8 (rho = −0.86; *P* = .007), faecal butyrate and extracellular newly identified-receptor for advanced glycation end products (EN-RAGE) (rho = −0.76; *P* = .028), faecal propionate and IL-8 (rho = −0.93; *P* = .001), and faecal propionate and EN-RAGE (rho = −0.83; *P* = .01) (Supplementary Fig S8). Lastly, we compared the effect sizes of the differential features of each data type using the paired Cohen's D statistic (Fig 3 and Supplementary Table S5). Among the metagenomic pathways, lysine biosynthesis and atrazine degradation exhibited the largest effect sizes. The SCFAs butyrate, propionate, and valerate also had large effect sizes (D > 0.8), as did the faecal autoantibody Clusterin 231-250 sm-1 cyclic IgG and *B. faecis*.

Metagenomic analyses in a geographically distinct cohort of MZ twins discordant for RA

To validate findings observed in the US cohort, we obtained shotgun metagenomic data from a geographically independent cohort of 7 pairs of MZ twins discordant for RA from the TwinsUK Registry in the United Kingdom (UK). The cohort consisted of White women with a mean age of 63.4 years and a mean disease duration of 19.7 years (Supplementary Table S4). Five affected twins had previously or were currently taking MTX (71.4%), and 3 were being treated with or had previous exposure to a biologic agent (42.9%) (Supplementary Table S4). We found no significant difference in either α-diversity (Wilcoxon signed-rank test, *P* = .71) or β-diversity (PERMANOVA, *P* = .51) between affected and unaffected twins (Fig 4A,B). We identified different SCFA-producing bacterial taxa with decreased abundance in the affected twins, including *Gemmiger formicilis* and *Faecalicatena fissicatena* (Fig 4C). *Clostridia* were also significantly abundant in the affected twins, while

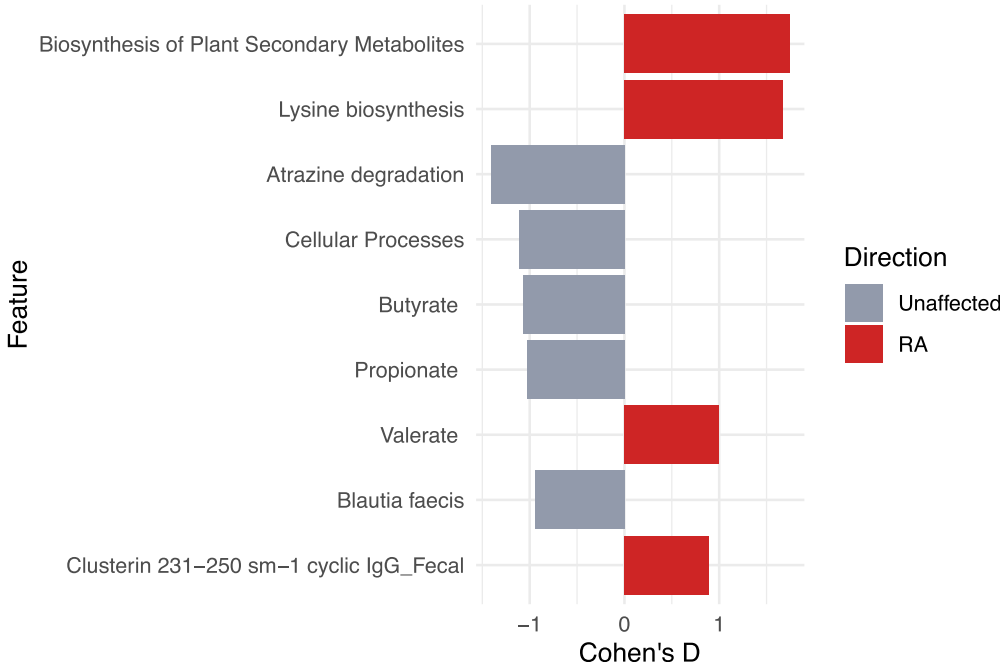


Figure 3. Cohen's D (paired) effect sizes for the top differential features of each data type, along with the directionality of enrichment. IgG, immunoglobulin G; RA, rheumatoid arthritis.

Escherichia coli was enriched in the unaffected twins (Fig 4C). In the US cohort, although not significantly, *Clostridia* was also more abundant in the affected twins (mean abundance ± SD, affected twins: 1.5e-4 ± 3.0e-4; unaffected twins: 0 ± 0; $P = .18$) and *E. coli* in the unaffected twins (affected: 1.2e-5 ± 2.5e-5; unaffected twins: 5.2e-3 ± 1.4e-2; $P = .14$).

Analysis of microbial functions identified 6 pathways that were differentially abundant in the affected and unaffected twins in the UK cohort, including propionate metabolism, ascorbate and aldarate metabolism, and biosynthesis of ansamycins, which were more abundant in the unaffected twins (Fig 4D). Propionate metabolism and biosynthesis of ansamycins, which were significantly more abundant in the unaffected twins in the UK cohort, exhibited the same trend as the unaffected twins in the US cohort, although the difference was not significant (Supplementary Table S6). We further investigated whether other SCFA metabolic pathways were differentially abundant in unaffected vs affected twins. Although not statistically significant in either the US or UK cohorts, there was also higher abundance of butyric acid metabolism and pyruvate metabolism (an intermediate in the acetic acid pathway) in unaffected twins (Supplementary Table S6).

DISCUSSION

Although studies on twin pairs have been previously conducted to determine concordance rates in RA, ours is the first to perform an integrated multiomics investigation of microbial, immune, and metabolic features that differentiate MZ twins discordant for RA. Two prior studies have used a multiomics approach to study RA, although they have been limited to studying gut microbiome and metabolomic differences between RA patients and unrelated (non-twins) healthy controls [21,22]. Another recent study assessed the differences in gut microbiome, colonic transcriptome, and peripheral blood immune profiles between patients with psoriasis and healthy controls [20]. Although we found no overall differences in microbial α- and β-diversity between affected and unaffected twins, we identified one species, *B faecis,* which was differentially abundant in the US cohort. *Blautia spp*. are butyrate and propionate producers [40], which may explain the increased levels of faecal SCFAs observed in the unaffected twins. Additionally, *B faecis*, which produces lactate and acetic acid and reduces expression of inflammatory cytokines such as TNF-α and IL-8 [40,41], was enriched in the unaffected twins. Flt3L, which has been implicated in both inflammatory [42] and regulatory [43,44] processes in RA, was positively correlated with *B faecis* abundance in the unaffected twins. We hypothesise that *B faecis* may modulate a regulatory process via its association with Flt3L, which could contribute to protection against RA. This hypothesis is supported by the negative correlation between faecal autoantibodies and *B faecis* in the unaffected twins. Furthermore, unlike serum autoantibodies, which are broadly elevated in affected RA twins compared with the unaffected twins [45], out of the 103 faecal autoantibodies tested, only 1 was elevated in affected twins. This suggests that faecal autoantibodies might not be a differentiating feature of MZ discordant twins per se, but might provide insights when paired with microbiome analyses.

The lack of overall differences in microbiome diversity between affected and unaffected twins was expected, given that affected twins had been treated or were undergoing treatment at time of sample collection. In fact, the gut microbiome of untreated patients with RA and healthy controls is significantly different [12], but these differences are partially reduced after treatment [8,11,46,22]. Effective RA treatment may also contribute to our observation that microbial composition was more similar between intrapair MZ twins than between unrelated individuals with the same disease state, either unaffected or RA. Although *P copri* has been previously reported to be enriched in early RA [6,12,13], we found that its abundance was not significantly different between unaffected and affected twins in either the US or UK cohorts. A recent study by Andréasson et al [47] showed that *P copri* abundance decreased after 3 months of treatment with either MTX or TNF inhibition, and *P copri* was

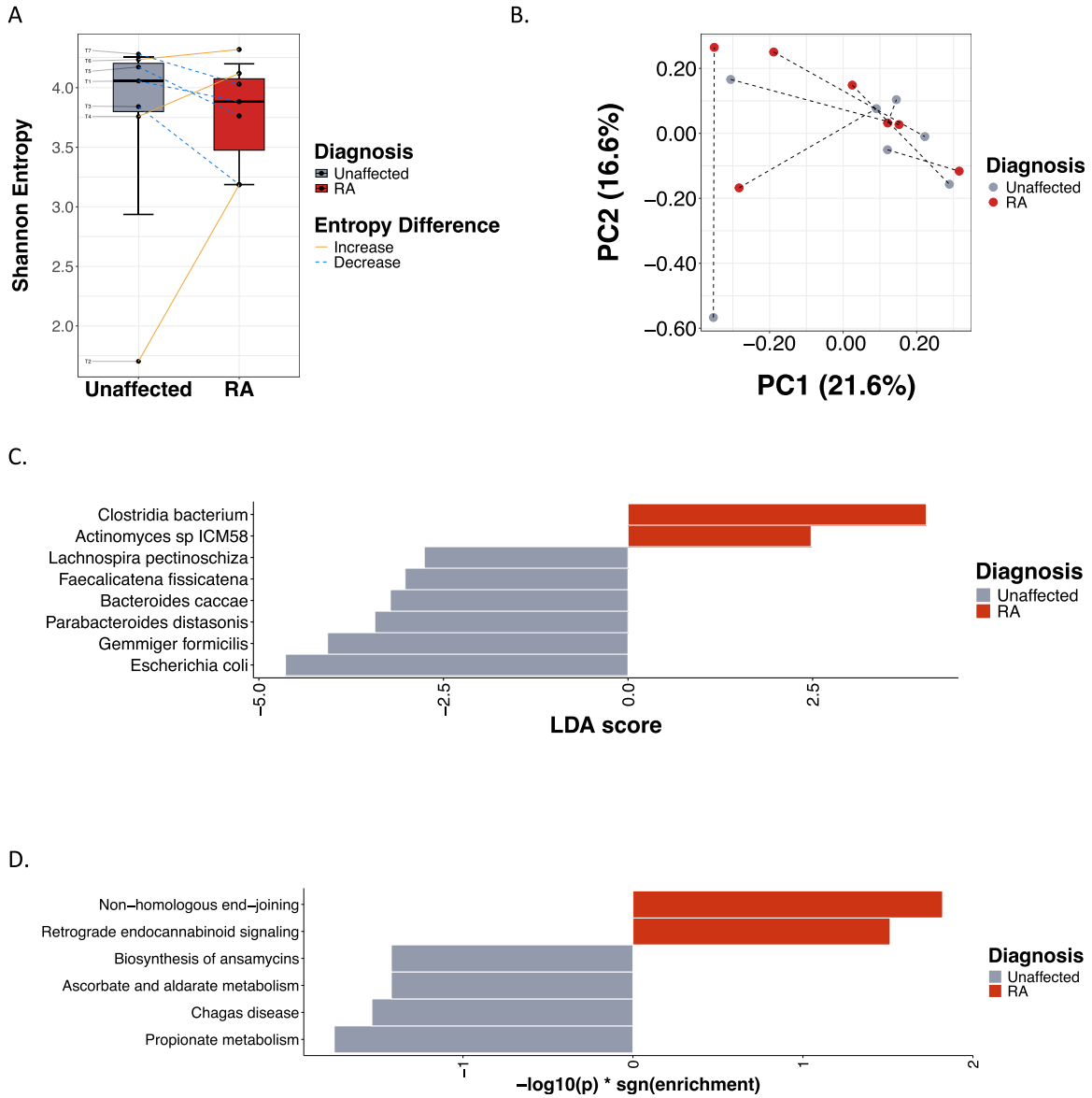


Figure 4. Gut bacterial composition and predicted functional pathways in affected and unaffected twins in the UK cohort (TwinsUK). A, Shannon α-diversity in participants, with box plots representing the median and IQR. Lines are drawn between monozygotic (MZ) pairs. B, Principal coordinate analysis (PCoA) of Bray-Curtis β-diversity between groups. C, Linear discriminant analysis effect size (LEfSe) showing differential species in affected twins compared with unaffected twins. D, Barplot of Wilcoxon signed-rank test *P* values for differential pathways. *$P < .05$; **$P < .01$. RA, rheumatoid arthritis.

negatively correlated with improved disease activity. Similarly, treatment with MTX has been previously reported to be associated with a reduction in *Bacteroides fragilis* [48]. Nayak et al [8] have also found that *P copri* decreased after MTX treatment in a gnotobiotic mouse model and in ex vivo cultures of gut microbial communities of RA patients. We thus hypothesise that the low *P copri* abundance in our twin cohorts might simply reflect the pathophysiology of established disease and the effect of treatment with MTX and other drugs.

Our study incorporates a validation cohort of a geographically distinct set of MZ twins from the UK, the TwinsUK Registry, who were also discordant for RA. There were several commonalities between the cohorts, including comparable age and medication use, and a lack of significant differences in microbial α- or β-diversity. Although the specific taxa found in the US and UK cohorts were distinct, we observed that those depleted in the affected twins, *Blautia spp.* in the US and *Gemmiger* and *Faecalicatena* in the UK, are all SCFA producers [40,49,50]. We also found that the enrichment of *Clostridia* and depletion of *E coli* in affected twins was consistent across both cohorts and in agreement with previous literature [51–53]. Importantly, we found that faecal butyrate and other SCFAs were elevated in unaffected US twins, which was paralleled by an enrichment in SCFA-producing bacteria in the unaffected UK twins. Functional pathway analysis of the UK cohort found that both propionate metabolism and biosynthesis of ansamycins were more abundant in the unaffected twins, a finding that was also observed in the unaffected US twins, although differences were not statistically significant. Increased propionate metabolism in the unaffected UK twins suggests that SCFAs, such as propionate, are an important discriminator between unaffected and affected twins and are in line with the observation that unaffected US twins have increased faecal SCFAs levels and that SCFAs are negatively associated with RA, as discussed above and elsewhere [17,18,22,54].

The anti-inflammatory properties and their protective role against disease progression of some gut microbial fermentation products, such as SCFAs, have been well-studied in both murine

models of RA and humans [17,18,54]. We found negative correlations between faecal butyrate and propionate and inflammatory cytokines in the affected US twins, suggesting that faecal SCFA concentration may regulate proinflammatory responses in affected twins. SCFAs, such as propionate, remain low in early RA patients, both before and after MTX treatment [22]. Although patients in our cohort have long-standing disease, we hypothesise that reduced SCFAs in affected twins are more likely due to the disease state and not an effect of the treatment regimen. Our finding that SCFAs are elevated in the faeces of unaffected twins suggests that they may play a protective role in individuals who are genetically predisposed to developing RA. Further work is required to characterise the long-term dynamics of intestinal SCFAs in response to various therapeutics.

Although our study is highly innovative in the multiomics interrogation of MZ twins discordant for RA from 2 geographically distinct cohorts, we acknowledge that our work has various limitations. Our sample size is limited due to the relatively low prevalence of RA in the general population, and particularly, in twins discordant for RA. One unaffected twin was eventually diagnosed with RA more than a year after the conclusion of the study; therefore, it is unlikely to have confounded our results. Most affected twins in our study were being treated with disease-modifying anti-rheumatic drugs (DMARDs) at the time of enrolment, and the mean duration of their disease was 7 years in the US cohort and 19.7 years in the UK cohort. Further, our design was cross-sectional in nature, which prevented us from formulating interpretations on the causality of disease pathogenesis and the effect of treatment. Other potential limitations include the use of specific DMARDs in each of the cohorts and the lack of detailed dietary information. Other medical and lifestyle factors that can influence the microbiome [55–57] may also be considered limitations, although we did not find significant differences between the cohorts, limiting their potential impact. Further work on these and similar cohorts is ongoing to expand our multiomics analyses, such as expanding our methodological capabilities with single-cell technologies.

Despite these limitations, our work identified several differentiating features between affected RA and unaffected identical twins in 2 geographically distinct cohorts. Unaffected twins in both cohorts harboured an abundance of SCFA producers, such as *Blautia, Gemmiger,* and *Faecalicatena*. US unaffected twins had higher concentrations of faecal SCFAs propionate and butyrate, and UK unaffected twins had increased abundance of propionate metabolism pathways. These findings were significant even when considering potential confounders. Taken together, our results suggest that SCFAs may be a biomarker of protection against the development of RA and highlight their potential as a therapeutic or preventive treatment approach. To our knowledge, our study is the first to use a multiomics approach to investigate microbial, metabolomic, and molecular features in twins discordant for RA. The unique setting of our cohorts thus provides a novel avenue to investigate RA pathogenesis while controlling for genetic predisposition and highlights the need to integrate metabolomics and host molecular features into microbiome studies.

Competing interests

RBB, KB, WC, IC, AH, RM, RRN, JH, AC, JL, JS, LL, CU, JCC, XZ, and FMKW have no conflicts to declare. JUS has served as a consultant for Janssen, Novartis, Pfizer, Sanofi, Amgen, UCB, Bristol Myers Squibb Co, and AbbVie, and has received funding for investigator-initiated studies from Janssen and Pfizer.

CRediT authorship contribution statement

Rebecca B. Blank: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Kevin Bu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation. **Xinyuan Zhang:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation. **Weixi Chen:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Ian Cunningham:** Writing – review & editing, Methodology, Data curation. **Jeremy Sokolove:** Writing – review & editing, Methodology, Investigation, Data curation. **Lauren Lahey:** Writing – review & editing, Methodology, Investigation, Data curation. **Adriana Heguy:** Writing – review & editing, Supervision, Methodology, Data curation. **Rhina Medina:** Writing – review & editing, Validation, Project administration, Methodology, Data curation. **Carles Ubeda:** Writing – review & editing, Resources, Methodology, Data curation, Conceptualization. **Renuka R. Nayak:** Writing – review & editing, Validation, Methodology. **Jiyuan Hu:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis. **Adam Cantor:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Jakleen Lee:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Frances M.K. Williams:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Data curation. **Jose C. Clemente:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Jose U. Scher:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Acknowledgements

The project was facilitated by the Mid-Atlantic Twin Registry (MATR), administered by the Cohort and Registry Administration (CARA) Core, a Virginia Commonwealth University shared resource. We would like to extend our gratitude to all of the MATR twins who took part in this study.

Funding

This work was supported by National Institutes of Health/National Institute of Arthritis and Musculoskelatal and Skin Diseases Institute (NIH/NIAMS) UC2AR081034 (JUS Principal Investigator [PI], JCC Multiple Principle Investigator [MPI], and RBB), NIH/NIAMS R01AR074500 (JUS [PI], JCC, and RRN), R03 AR072182 (JUS [PI]), the National Center for Advancing Translational Sciences (NCATS), and the National Institutes of Health through Grant Award Number UL1TR001445 (RBB [trainee]); NIH/NIAMS T32AR069515 (JUS [MPI] and RBB [trainee]); NYU Judith and Stewart Colton Center for Autoimmunity (JUS); R03DK138490 (JH [PI]); PID2023-150086OB-I00 from Ministerio de Ciencia, Innovacion y Universidades (MICINN) (Spain) (CU [PI]); NIH K08AR073930 (RRN); VA I01CX002557 (RRN [PI]).

TwinsUK is funded by the Wellcome Trust, Medical Research Council, Versus Arthritis (Grant 21227), European Union

Horizon 2020, Chronic Disease Research Foundation (CDRF), Zoe Ltd, the National Institute for Health Research (NIHR) Clinical Research Network (CRN), and the Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London.

Patient consent for publication

Not applicable

Ethics approval

Institutional Review Board identification S12-00831

Provenance and peer review

Not commissioned, externally peer reviewed.

Data availability statement

A GitHub containing additional results not highlighted in this manuscript and its supplementary figures is available at https://github.com/clemente-lab/twinsra_analyses.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.08.029.

Orcid

Rebecca B. Blank: http://orcid.org/0000-0002-6292-6947

REFERENCES

[1] Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 2014;506(7488):376–81. doi: 10.1038/nature12873.

[2] Smolen JS, Aletaha D, Barton A, Burmester GR, Emery P, Firestein GS, et al. Rheumatoid arthritis. Nat Rev Dis Primers 2018;4(1):18001. doi: 10.1038/nrdp.2018.1.

[3] MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K, et al. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum 2000;43(1):30–7. doi: 10.1002/1529-0131(200001)43:1<30::AID-ANR5>3.0.CO;2-B.

[4] Silman AJ, MacGregor AJ, Thomson W, Holligan S, Carthy D, Farhan A, et al. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. Br J Rheumatol 1993;32(10):903–7. doi: 10.1093/rheumatology/32.10.903.

[5] Aho K, Koskenvuo M, Tuominen J, Kaprio J. Occurrence of rheumatoid arthritis in a nationwide series of twins. J Rheumatol 1986;13(5):899–902.

[6] Maeda Y, Kurakawa T, Umemoto E, Motooka D, Ito Y, Gotoh K, et al. Dysbiosis contributes to arthritis development via activation of autoreactive T cells in the intestine. Arthritis Rheumatol 2016;68(11):2646–61. doi: 10.1002/art.39783.

[7] Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity 2010;32(6):815–27. doi: 10.1016/j.immuni.2010.06.001.

[8] Nayak RR, Alexander M, Deshpande I, Stapleton-Gray K, Rimal B, Patterson AD, et al. Methotrexate impacts conserved pathways in diverse human gut bacteria leading to decreased host immune activation. Cell Host Microbe 2021;29(3):362–77 .e11. doi: 10.1016/j.chom.2020.12.008.

[9] Hitchon CA, Chandad F, Ferucci ED, Willemze A, Ioan-Facsinay A, van der Woude D, et al. Antibodies to *Porphyromonas gingivalis* are associated with anticitrullinated protein antibodies in patients with rheumatoid arthritis and their relatives. J Rheumatol 2010;37(6):1105–12. doi: 10.3899/jrheum.091323.

[10] Mikuls TR, Thiele GM, Deane KD, Payne JB, O'Dell JR, Yu F, et al. *Porphyromonas gingivalis* and disease-related autoantibodies in individuals at increased risk of rheumatoid arthritis. Arthritis Rheum 2012;64(11):3522–30. doi: 10.1002/art.34595.

[11] Zhang X, Zhang D, Jia H, Feng Q, Wang D, Liang D, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med 2015;21(8):895–905. doi: 10.1038/nm.3914.

[12] Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, et al. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. Elife 2013;2:e01202. doi: 10.7554/eLife.01202.

[13] Pianta A, Arvikar S, Strle K, Drouin EE, Wang Q, Costello CE, et al. Evidence of the immune relevance of *Prevotella copri*, a gut microbe, in patients with rheumatoid arthritis. Arthritis Rheumatol 2017;69(5):964–75. doi: 10.1002/art.40003.

[14] Chriswell ME, Lefferts AR, Clay MR, Hsu AR, Seifert J, Feser ML, et al. Clonal IgA and IgG autoantibodies from individuals at risk for rheumatoid arthritis identify an arthritogenic strain of Subdoligranulum. Sci Transl Med 2022;14(668):eabn5166. doi: 10.1126/scitranslmed.abn5166.

[15] Zaiss MM, Joyce Wu HJ, Mauro D, Schett G, Ciccia F. The gut-joint axis in rheumatoid arthritis. Nat Rev Rheumatol 2021;17(4):224–37. doi: 10.1038/s41584-021-00585-3.

[16] Corrêa-Oliveira R, Fachi JL, Vieira A, Sato FT, Vinolo MA. Regulation of immune cell function by short-chain fatty acids. Clin Transl Immunology 2016;5(4):e73. doi: 10.1038/cti.2016.17.

[17] Mizuno M, Noto D, Kaga N, Chiba A, Miyake S. The dual role of short fatty acid chains in the pathogenesis of autoimmune disease models. PLoS One 2017;12(2):e0173032. doi: 10.1371/journal.pone.0173032.

[18] Kim DS, Kwon JE, Lee SH, Kim EK, Ryu JG, Jung KA, et al. Attenuation of rheumatoid inflammation by sodium butyrate through reciprocal targeting of HDAC2 in osteoclasts and HDAC8 in T cells. Front Immunol 2018;9:1525. doi: 10.3389/fimmu.2018.01525.

[19] Martinsson K, Dürholz K, Schett G, Zaiss MM, Kastbom A. Higher serum levels of short-chain fatty acids are associated with non-progression to arthritis in individuals at increased risk of RA. Ann Rheum Dis 2022;81(3):445–7. doi: 10.1136/annrheumdis-2021-221386.

[20] Chang HW, Yan D, Singh R, Bui A, Lee K, Truong A, et al. Multiomic analysis of the gut microbiome in psoriasis reveals distinct host–microbe associations. JID Innov 2022;2(3):100115. doi: 10.1016/j.xjidi.2022.100115.

[21] Yun H, Wang X, Wei C, Liu Q, Li X, Li N, et al. Alterations of the intestinal microbiome and metabolome in women with rheumatoid arthritis. Clin Exp Med 2023;23(8):4695–706. doi: 10.1007/s10238-023-01161-7.

[22] Marazzato M, Iannuccelli C, Guzzo MP, Nencioni L, Lucchino B, Radocchia G, et al. Gut microbiota structure and metabolites, before and after treatment in early rheumatoid arthritis patients: a pilot study. Front Med (Lausanne) 2022;9:921675. doi: 10.3389/fmed.2022.921675.

[23] Artacho A, Isaac S, Nayak R, Flor-Duro A, Alexander M, Koo I, et al. the pretreatment gut microbiome is associated with lack of response to methotrexate in new-onset rheumatoid arthritis. Arthritis Rheumatol 2021;73(6):931–42. doi: 10.1002/art.41622.

[24] Blanco-Míguez A, Beghini F, Cumbo F, McIver LJ, Thompson KN, Zolfo M, et al. Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. Nat Biotechnol 2023;41(11):1633–44. doi: 10.1038/s41587-023-01688-w.

[25] Beghini F, McIver LJ, Blanco-Míguez A, Dubois L, Asnicar F, Maharjan S, et al. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. Elife 2021;10:e65088. doi: 10.7554/eLife.65088.

[26] Visconti A, Martin TC, Falchi M. YAMP: a containerized workflow enabling reproducibility in metagenomics research. Gigascience 2018;7(7) giy072. doi: 10.1093/gigascience/giy072.

[27] Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, GA Al-Ghalith, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37(8):852–7. doi: 10.1038/s41587-019-0209-9.

[28] Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front Psychol 2013;4:863. doi: 10.3389/fpsyg.2013.00863.

[29] Yue M, Kim JH, Evans CR, Kachman M, Erb-Downward JR, D'Souza J, et al. Measurement of short-chain fatty acids in respiratory samples: keep your assay above the water line. Am J Respir Crit Care Med 2020;202(4):610–2. doi: 10.1164/rccm.201909-1840LE.

[30] Paine A, Brookes PS, Bhattacharya S, Li D, De La Luz Garcia-Hernandez M, Tausk F, et al. Dysregulation of bile acids, lipids, and nucleotides in psoriatic arthritis revealed by unbiased profiling of serum metabolites. Arthritis Rheumatol 2023;75(1):53–63. doi: 10.1002/art.42288.

[31] Sokolove J, Bromberg R, Deane KD, Lahey LJ, Derber LA, Chandra PE, et al. Autoantibody epitope spreading in the pre-clinical phase predicts

progression to rheumatoid arthritis. PLoS One 2012;7(5):e35296. doi: 10.1371/journal.pone.0035296.
[32] Kronzer VL, Hayashi K, Yoshida K, Davis 3rd JM, McDermott GC, Huang W, et al. Autoantibodies against citrullinated and native proteins and prediction of rheumatoid arthritis-associated interstitial lung disease: a nested case-control study. Lancet Rheumatol 2023;5(2):e77–87. doi: 10.1016/s2665-9913(22)00380-0.
[33] Assarsson E, Lundberg M, Holmquist G, Björkesten J, Thorsen SB, Ekman D, et al. Homogenous 96-Plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS One 2014;9(4):e95192. doi: 10.1371/journal.pone.0095192.
[34] Bu K, Wallach DS, Wilson Z, Shen N, Segal LN, Bagiella E, et al. Identifying correlations driven by influential observations in large datasets. Brief Bioinform 2022;23(1):bbab482. doi: 10.1093/bib/bbab482.
[35] Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale: L. Erlbaum Associates; 1988.
[36] McKinney W. Data structures for statistical computing in Python. In: SciPy Proceedings. Proceedings of the 9th Python in Science Conference; 2010. p. Austin, TX, USA56–61.
[37] Waskom ML. seaborn: statistical data visualization. J Open Source Softw 2021;6(60):3021. doi: 10.21105/joss.03021.
[38] Team RC. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2021.
[39] Wickham H. ggplot2: elegant graphics for data analysis. Use R!. 2nd ed. Cham: Springer; 2016.
[40] Liu X, Mao B, Gu J, Wu J, Cui S, Wang G, et al. Blautia—a new functional genus with potential probiotic properties? Gut Microbes 2021;13(1):1–12. doi: 10.1080/19490976.2021.1875796.
[41] Verstraeten S, Sencio V, Raise A, Huillet E, Layec S, Deruyter L, et al. Description of a newly isolated *Blautia faecis* strain and its benefit in mouse models of post-influenza secondary enteric and pulmonary infections. Nutrients 2022;14(7):1478. doi: 10.3390/nu14071478.
[42] Ramos MI, Perez SG, Aarrass S, Helder B, Broekstra P, Gerlag DM, et al. FMS-related tyrosine kinase 3 ligand (Flt3L)/CD135 axis in rheumatoid arthritis. Arthritis Res Ther 2013;15(6):R209. doi: 10.1186/ar4403.
[43] Svensson MN, Erlandsson MC, Jonsson IM, Andersson KM, Bokarewa MI. Impaired signaling through the FMS-like tyrosine kinase 3 receptor increases osteoclast formation and bone damage in arthritis. J Leukoc Biol 2016;99(3):413–23. doi: 10.1189/jlb.3HI1114-572RR.
[44] Svensson MN, Andersson SE, Erlandsson MC, Jonsson IM, Ekwall AK, Andersson KM, et al. Fms-like tyrosine kinase 3 ligand controls formation of regulatory T cells in autoimmune arthritis. PLoS One 2013;8(1):e54884. doi: 10.1371/journal.pone.0054884.
[45] Hensvold AH, Frisell T, Magnusson PK, Holmdahl R, Askling J, Catrina AI. How well do ACPA discriminate and predict RA in the general population: a study based on 12 590 population-representative Swedish twins. Ann Rheum Dis 2017;76(1):119–25. doi: 10.1136/annrheumdis-2015-208980.
[46] Picchianti-Diamanti A, Panebianco C, Salemi S, Sorgi ML, Di Rosa R, Tropea A, et al. Analysis of gut microbiota in rheumatoid arthritis patients: disease-related dysbiosis and modifications induced by etanercept. Int J Mol Sci 2018;19(10):2938. doi: 10.3390/ijms19102938.
[47] Andréasson K, Olofsson T, Lagishetty V, Alrawi Z, Klaassens E, Holster S, et al. Treatment for rheumatoid arthritis associated with alterations in the gastrointestinal microbiota. ACR Open Rheumatol 2024;6(7):421–7. doi: 10.1002/acr2.11673.
[48] Zhou B, Dong C, Zhao B, Lin K, Tian Y, Zhang R, et al. *Bacteroides fragilis* participates in the therapeutic effect of methotrexate on arthritis through metabolite regulation. Front Microbiol 2022;13:1015130. doi: 10.3389/fmicb.2022.1015130.
[49] Meng X, Shu Q. Novel primers to identify a wider diversity of butyrate-producing bacteria. World J Microbiol Biotechnol 2024;40(2):76. doi: 10.1007/s11274-023-03872-1.
[50] Zaplana T, Miele S, Tolonen AC. Lachnospiraceae are emerging industrial biocatalysts and biotherapeutics. Front Bioeng Biotechnol 2024;11:1324396. doi: 10.3389/fbioe.2023.1324396.
[51] Muñiz Pedrogo DA, Chen J, Hillmann B, Jeraldo P, Al-Ghalith G, Taneja V, et al. An increased abundance of *Clostridiaceae* characterizes arthritis in inflammatory bowel disease and rheumatoid arthritis: a cross-sectional study. Inflamm Bowel Dis 2019;25(5):902–13. doi: 10.1093/ibd/izy318.
[52] Rogier R, Evans-Marin H, Manasson J, van der Kraan PM, Walgreen B, Helsen MM, et al. Alteration of the intestinal microbiome characterizes preclinical inflammatory arthritis in mice and its modulation attenuates established arthritis. Sci Rep 2017;7(1):15613. doi: 10.1038/s41598-017-15802-x.
[53] Gomez A, Luckey D, Yeoman CJ, Marietta EV, Berg Miller ME, Murray JA, et al. Loss of sex and age driven differences in the gut microbiome characterize arthritis-susceptible 0401 mice but not arthritis-resistant 0402 mice. PLoS One 2012;7(4):e36095. doi: 10.1371/journal.pone.0036095.
[54] Clemente JC, Manasson J, Scher JU. The role of the gut microbiome in systemic inflammatory disease. BMJ 2018;360:j5145. doi: 10.1136/bmj.j5145.
[55] Mäkivuokko H, Tiihonen K, Tynkkynen S, Paulin L, Rautonen N. The effect of age and non-steroidal anti-inflammatory drugs on human intestinal microbiota composition. Br J Nutr 2010;103(2):227–34. doi: 10.1017/s0007114509991553.
[56] Rogers MAM, Aronoff DM. The influence of non-steroidal anti-inflammatory drugs on the gut microbiome. Clin Microbiol Infect 2016;22(2):178.e1–9. doi: 10.1016/j.cmi.2015.10.003.
[57] Prakash A, Peters BA, Cobbs E, Beggs D, Choi H, Li H, et al. Tobacco smoking and the fecal microbiome in a large, multi-ethnic cohort. Cancer Epidemiol Biomarkers Prev 2021;30(7):1328–35. doi: 10.1158/1055-9965.EPI-20-1417.

Ann Rheum Dis 85 (2026) 265–275

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Rheumatoid arthritis

Innate immune cell subsets are enriched in synovial fluid of ACPA-negative rheumatoid arthritis and characterized by distinct type I IFN gene signatures

Alexandra Argyriou[1,2], Marc H. Wadsworth II[3], Joshua Fienman[3], Ana Cristina Gonzalez-Sanchez[1,2], Salim Ghannoum[1,2], Chirag Krishna[3], Christina Gerstner[1,2], Begum Horuluoglu[1,2], Merel Sijbranda[1,2], Lars Rönnblom[4], Maija-Leena Eloranta[4], Marie Wahren-Herlenius[1,2], Aase Hensvold[1,2,5], Sara Turcinov[1,2,6], Aaron Winkler[3], Vivianne Malmström[1,2], Karine Chemin[1,2,*]

[1] *Division of Rheumatology, Department of Medicine, Solna, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden*
[2] *Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden*
[3] *Inflammation & Immunology Research Unit, Pfizer Inc., Cambridge, Massachusetts, United States*
[4] *Department of Medical Sciences, Rheumatology, Uppsala University, Uppsala, Sweden*
[5] *Center for Rheumatology, Academic Specialist Center, Stockholm Health Region, Stockholm, Sweden*
[6] *Theme of Inflammation and Ageing, Medical Unit Gastro, Derma, Rheuma, Karolinska University Hospital, Solna, Sweden*

ARTICLE INFO

Article history:
Received 29 January 2025
Received in revised form 11 July 2025
Accepted 31 July 2025

ABSTRACT

Objectives: Around 30% of patients with rheumatoid arthritis (RA) lack rheumatoid factor and anti-citrullinated protein antibodies (ACPA) complicating diagnosis and potentially delaying treatment. We hypothesised that innate immune mechanisms might be more prominent in ACPA− RA.
Methods: We performed single-cell RNA sequencing of mononuclear cells from peripheral blood (PBMC) and synovial fluid (SFMC) of patients with ACPA− and ACPA+ RA ($n = 4$ per group: discovery cohort; $n = 8$ per group: validation cohort). Dendritic cells and proinflammatory cytokine production were analysed by flow cytometry on SFMC from patients with ACPA− RA, ACPA + RA, and psoriatic arthritis. Interferon (IFN) levels in synovial fluid (SF) and serum were measured in these groups.
Results: Several macrophage subsets and cDC2 were enriched in ACPA− RA SF whereas the frequency of Tph and B cells was increased in ACPA+ RA SF. Type I IFN-stimulated genes were detected in SFMC, but not PBMC, of patients with ACPA− RA. A type I IFN signature was also observed in synovial tissue from two patients with ACPA− RA in an independent dataset. IFN levels were higher in SF than serum but IFN-α/β production did not differ between ACPA+ and ACPA− RA.
Conclusions: This study identifies a distinct innate cell composition and type I IFN gene response in synovial joints, but not in peripheral blood, of patients with ACPA− RA. Similar IFN levels across groups suggest the IFN signature may have been primed before the cells entered the joints. These findings provide a foundation for future research on type I IFN responses in ACPA− RA.

*Correspondence to Dr Karine Chemin.
E-mail address: karine.chemin@ki.se (K. Chemin).
Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.07.029

WHAT IS ALREADY KNOWN ON THIS TOPIC

- The diagnosis of seronegative rheumatoid arthritis (RA) remains challenging, necessitating meticulous clinical and immunological examinations, which may result in delayed diagnosis.
- The weaker *HLA-DRB1* association with seronegative RA suggests that the adaptive immune system may play a less prominent role in the pathogenesis of this subgroup.
- Our hypothesis was that local innate immune responses may contribute to the development of seronegative RA.

WHAT THIS STUDY ADDS

- We undertook this study to deeply characterize immune cells in synovial fluid (SF) and peripheral blood (PB) of clinically well-characterized patients with anti-citrullinated protein antibodies (ACPA)+ and ACPA- RA using single-cell RNA sequencing and flow cytometry. We demonstrate the following:
- A type I interferon (IFN) gene signature in innate immune cells in SF of patients with ACPA− RA. We show elevated expression of type I IFN-stimulated genes, and a higher IFN score in myeloid cells in SF, but not in PB, of patients with ACPA− RA using single-cell transcriptomics.
- Similar levels of IFNs in SF of patients with ACPA+ and ACPA− RA. The levels of type I, type II, and type III IFN were increased in SF compared to serum in both ACPA+ and ACPA− RA. However, there were no differences in IFN levels in SF between ACPA+ and ACPA− RA.
- Using an independent single-cell dataset, we confirm that dendritic cells exhibit an elevated IFN score in synovial biopsies in a subgroup of patients with ACPA- RA.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- This study provides a detailed comparison of the myeloid cell landscape in SF of patients with ACPA+ and ACPA− RA, highlighting the unique involvement of type I IFN responses in ACPA− RA. These findings provide a basis for future research on the initiation of type I IFN responses in the synovial joint and their role in the pathogenesis of ACPA− RA. A better understanding of local dysregulated immune responses in seronegative RA will provide a basis for the development of targeted therapies for this understudied group of patients.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune rheumatic disease that leads to synovial joint damage, causing chronic pain and physical disability [1]. The presence of the antibodies rheumatoid factor (RF) and antibodies against citrullinated proteins (ACPA), which can precede disease onset, is used as a part of the prognostic and diagnostic criteria [2]. Genome-wide association studies (GWAS) have shown that the *HLA-DRB1* shared epitope alleles associate with ACPA+ RA [3] suggesting the involvement of CD4+ T cells in this RA subset. Indeed, several subsets of CD4+ T cells have been identified in the synovial joints of ACPA+ RA [4]. Recent studies have highlighted the subset of $PD1^{hi}HLADR^{+}GPR56^{+}$ CD4+ T cells [5–7] which has a tissue-resident memory signature [7] and is suggested to provide B-cell help through CXCL13 production [6].

However, around 30% of the patients with RA are seronegative for both RF and ACPA (ACPA−) which complicates diagnosis and could delay treatment initiation [8]. Seronegative RA presents with a weaker *HLA-DRB1* association [9,10] and a lower infiltration of $CXCL13^{+}CD4^{+}$ T cells in the synovium [7] compared to ACPA+ RA, suggesting that the adaptive immune system plays a less prominent role in the pathogenesis of ACPA− RA. Innate immunity may have a more central role in ACPA− RA which could explain the lower efficacy of the costimulation blockade, abatacept, in this subgroup, compared to ACPA+ RA [11].

Innate immune cells including macrophages, dendritic cells (DC) and neutrophils, infiltrate the synovial tissue (ST) and synovial fluid (SF) where they may contribute through phagocytosis, antigen presentation and secretion of proinflammatory cytokines including tumour necrosis factor (TNF), interleukin (IL)-6, and IL-1 [12]. Two distinct populations of macrophages are found in the synovial lining: MerTK+CD206+ and MerTK-CD206- populations [13]. The MerTK+CD206+ macrophages populate the ST early during development where they become tissue resident and play a protective role in preserving joint integrity. The MerTK-CD206- macrophages are suggested to be proinflammatory tissue-infiltrating cells derived from circulating monocytes. DCs can be classified into 2 categories: conventional DCs (cDC), and plasmacytoid DCs (pDC). cDCs can be further divided into 4 subtypes: cDC1/DC1 ($CD141^{+}CLEC9A^{+}$), which interact with $CD8^{+}$ T cells; cDC2 ($CLEC10A^{+}$), which include DC2 and DC3 and interact with $CD4^{+}$ T cells; and cDC4/DC4 (CD16+), which have a type I interferon (IFN) signature [14,15]. pDCs ($CD123^{+}CD303^{+}$) are the professional producers of type I IFN upon viral infection [16]. A recent study detected an increase in *CCL13, CCL18*, and *MMP3* expression in ST myeloid cells of ACPA−, compared with ACPA+ RA [17]. Still, our understanding of the functional differences in myeloid cells at the site of inflammation between ACPA+ and ACPA− RA remains incomplete.

Here, we performed single-cell RNA sequencing (scRNA-Seq) to compare the transcriptomic profiles of myeloid cells from SF and peripheral blood (PB) in patients with ACPA+ and ACPA− RA. Our analysis reveals an elevated expression of type I IFN-stimulated genes and a higher IFN score in immune cells in SF of ACPA− RA, which was confirmed in STs from an independent untreated RA cohort [15]. These findings provide a detailed comparison of the myeloid cell landscape in SF across ACPA+ and ACPA− RA and highlight the unique involvement of type I IFN responses in ACPA− RA.

METHODS

Patients and samples

This study was approved by the ethical committee of the Karolinska University Hospital, Sweden, and all patients gave informed consent, according to the Declaration of Helsinki. SF was aspirated from the large, inflamed joints of 18 patients diagnosed with ACPA+ RA, 13 patients with ACPA− RA and 9 patients with psoriatic arthritis (PsA) (Supplementary Table S1). Mononuclear cells were isolated from SF and PB using Ficoll separation and cryopreserved. Cell-free SF and serum were collected and cryopreserved. scRNA-Seq was performed on SFMC and PBMC from 4 patients with ACPA+ and 4 patients with ACPA− RA (discovery cohort, n = 8) and SFMC from 8 patients with ACPA+ and 8 patients with ACPA− RA (validation cohort, n = 16). Full spectrum flow cytometry was done on SFMC from 9 patients with ACPA+, 8 patients with ACPA− and 9 patients with PsA. Bead array and enzyme-linked immunosorbent assay (ELISA) was applied to serum and cell-free SF from 12 ACPA+, 10 ACPA− and 9 patients with PsA (Supplementary Table S1 shows which samples were included in each experiment).

Statistics

Statistical analysis of the full spectrum flow cytometry data was performed on Prism 9 (Dotmatics) and Mann-Whitney test was performed, unless indicated differently on the figure legend. *P* values under 0.05 were considered significant.

RESULTS

Mononuclear myeloid cells in SF and PB of patients with ACPA-positive and ACPA-negative RA

We performed 10× scRNA-Seq on mononuclear cells from PB and SF from 4 ACPA+ and 4 ACPA− patients with RA (Fig 1A,

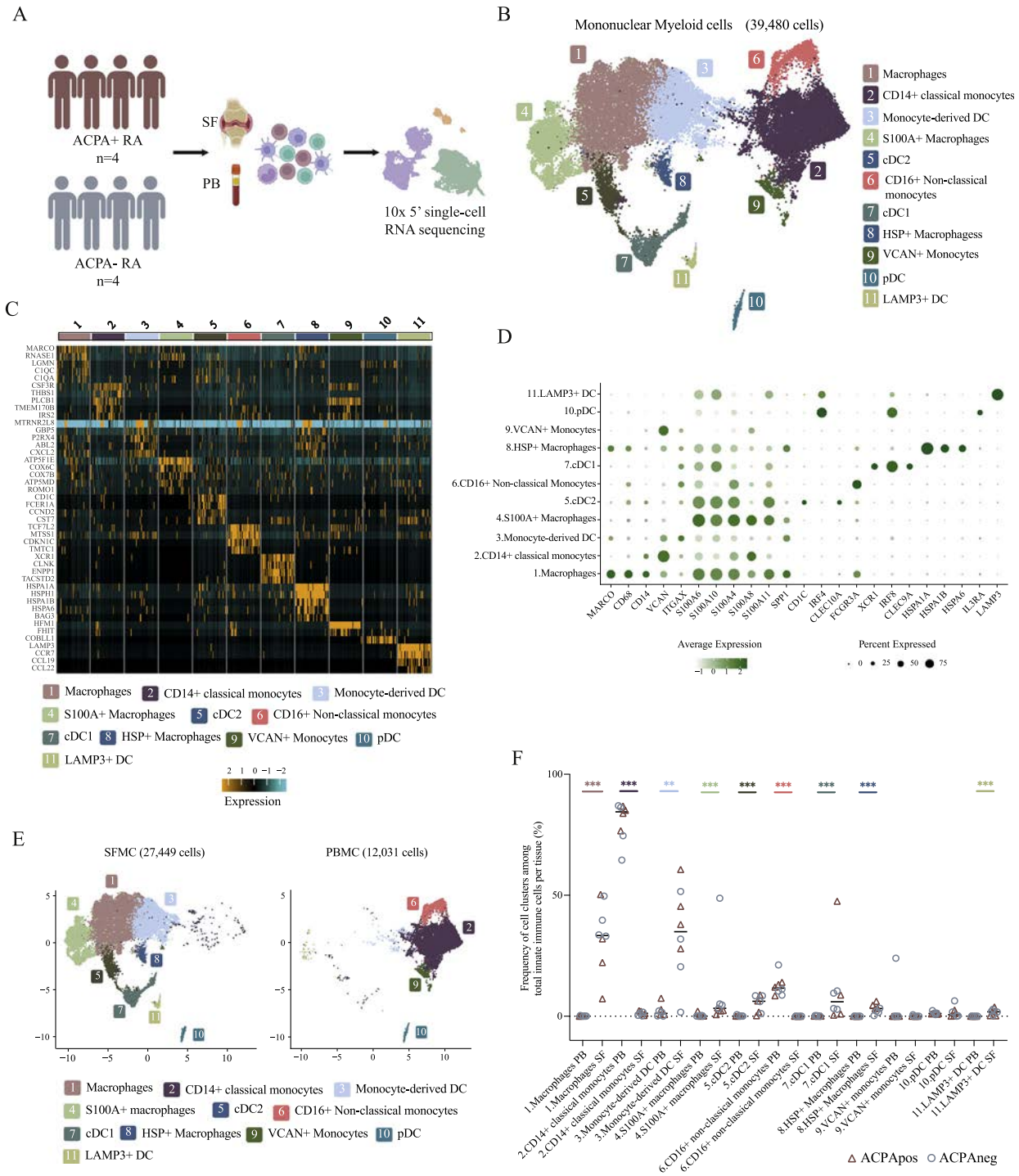


Figure 1. Single-cell RNA sequencing of myeloid cells from synovial fluid (SF) and peripheral blood (PB) of patients with rheumatoid arthritis (RA; discovery cohort). (A) Technical workflow, including 10× 5′ single-cell RNA sequencing on paired SF (n = 4) and PB (n = 4) from 8 patients with RA. (B) UMAP (Uniform Manifold Approximation and Projection) showing subclustering of 39,480 mononuclear myeloid cells in PB and SF of 8 patients with RA (n = 4 anti-citrullinated protein antibodies (ACPA)+, n = 4 ACPA−), coloured by cell type. (C) Heatmap displaying the top 5 most differentially expressed genes in each cluster. (D) Dotplot showing the expression of selected genes in mononuclear myeloid cell clusters in ACPA+ and ACPA− SF and PB. (E) UMAP of mononuclear myeloid cell clusters in SF (left panel, n = 27,449 cells) and PB (right panel, n = 12,031 cells). (F) Frequencies of myeloid cell clusters in PB and SF from n = 4 ACPA+ and n = 4 ACPA− patients with RA. Triangles indicate ACPA+, circles indicate ACPA−, 2-tailed Mann-Whitney *U* test.

Supplementary Table S1, discovery cohort). Following preprocessing (Supplementary Fig S1A), we identified 96,302 immune cells in PB and SF (Supplementary Fig S1B). Unsupervised clustering of the myeloid cells in PB and SF identified 11 clusters, composed of 39,480 myeloid cells (Fig 1B,C, Supplementary Table S2A). In SF (Fig 1D,E, left panel, Supplementary Fig S1C, D), we identified a macrophage cluster (cluster 1) expressing high levels of *MARCO* and *CD68*, a cluster of monocyte-derived DCs (cluster 3) expressing both monocyte and DC genes, including *VCAN* and *ITGAX* [18], a cluster of S100A+ macrophages (cluster 4), expressing *S100A6, S100A10, S100A4, S100A8, S100A11* and *SPP1* [15], a conventional DC 2 cluster (cDC2, cluster 5), expressing *CD1C, IRF4* and *CLEC10A* [19], a conventional DC 1 cluster (cDC1, cluster 7), expressing *XCR1, IRF8*, and *CLEC9A* [19], a heat shock protein macrophage cluster (HSP+ macrophage, cluster 8) expressing *HSPA1A, HSPA1B*, and *HSPA6*, and a cluster of DCs characterized by high expression of lysosome-associated membrane glycoprotein 3 (*LAMP3*, LAMP3+ DC, cluster 11), some of them previously described in RA ST [15,20] as summarised in Supplementary Table S3. In PB (Fig 1E, right panel, Supplementary Fig S1C,D), we mainly detected a cluster of CD14+ classical monocytes (cluster 2) highly expressing *CD14* [21], a cluster of CD16+ non-classical monocytes (cluster 6), expressing *FCG3RA*, and a cluster of VCAN+ monocytes (cluster 9), highly expressing *VCAN, S100A8*, and *S100A9* [22]. A cluster of pDC (cluster 10), expressing *IL3RA, IRF4,* and *IRF8* [19], was present in both compartments. Cell subsets were present in every patient at variable frequencies (Supplementary Fig S1E,F). The clusters of macrophages, monocyte-derived DC, cDC1, cDC2, HSP+ macrophages, and LAMP3+ DC were significantly enriched in SF compared to PB (Fig 1F), as previously described [15,17]. On the contrary, the CD14+ classical and CD16+ nonclassical monocytes were mainly present in PB. Altogether, these data show that several macrophages and DC subsets are enriched in SF and that similar subsets are detected in ACPA+ and ACPA− RA.

A type I IFN gene signature is detected in mononuclear myeloid cells in ACPA-negative RA SF

Differential gene expression analysis of all mononuclear myeloid cells revealed that several ISGs (Interferon-Stimulated Genes) are highly expressed in SF of patients with ACPA− RA, compared to ACPA+ (Fig 2A, Supplementary Table S2B). The type I IFN pathway in ACPA− SF was validated using Enrichr [23] (Supplementary Fig S2A). Among the top 20 DEGs (differentially expressed genes), *IFITM3, LY6E, IFI27, ISG15,* and *IFITM2* were expressed at higher levels and by more mononuclear myeloid cells in SF of ACPA− RA, compared to ACPA+ RA (Fig 2B). A type I IFN gene signature has been identified in PB in other autoimmune rheumatic diseases, such as systemic lupus erythematosus (SLE) [24] and Sjögren's disease [25], where an IFN score has been calculated based on a selected number of type I IFN-related genes [26,27]. We calculated the IFN score of mononuclear myeloid cells in SF using the UCell package (version 2.6.2) [28] and the signature previously described in SLE, which integrates the expression levels of 6 ISGs (*CXCL10, IFI44L, IFIT3, MX1, SERPING1, LY6E)* [29]. This type I IFN score was significantly increased in mononuclear myeloid cells in SF from ACPA−, compared to patients with ACPA+ RA (Fig 2C). Furthermore, 3 genes included in this score (*MX1, SERPING1, LY6E*) were expressed at higher levels and by more cells in ACPA− RA SF, compared to ACPA+ (Fig 2D). Interestingly, the ISG expression was primarily derived from macrophages (cluster 1) and cDC2 (cluster 5); however, all mononuclear myeloid populations had higher IFN score in ACPA−, compared to ACPA+ SF (Fig 2E,F, Supplementary Fig S2B,C). The IFN score was elevated in myeloid cells in SF of all 4 patients with ACPA− RA (Fig 2G). Differential expression analysis of pDC from PB and SF revealed that several ISGs, including *IFI44L, MX1, IFI44,* and *STAT1*, were significantly upregulated in SF (Supplementary Fig S2D). Similarly, macrophages from SF showed significantly higher expression of *IFI6, ISG15, IFI27*, and *LY6E* compared to CD14+ classical monocytes from PB (Supplementary Fig S2E). In PB, no differential ISG expression was detected between ACPA− and ACPA+ RA, but the IFN score was increased in ACPA+ compared to ACPA− RA (Supplementary Fig S2F-G), which is consistent with a type I IFN gene signature previously reported in PB of patients with ACPA+ RA [30]. Finally, we analysed an independent scRNA-Seq dataset derived from RA ST and focused our analysis on immune cells from ACPA− (n = 6) and ACPA+ RA (n = 21), naïve to methotrexate and biologics [15]. We detected a slightly increased IFN score in mononuclear myeloid cells in patients with ACPA− RA (Supplementary Fig S3A,B). The highest IFN score was detected among STAT1 +CXCL10+ (cluster M-6) and CD16+/DC4 clusters (cluster M-11) (Fig S3C). The M-6 cluster corresponds to STAT1+CXCL10 + macrophages that express IFN-response genes [15]. This cluster could represent a subpopulation of our cluster 1 macrophages which expressed the highest IFN gene signature (Supplementary Fig S3E). Indeed, subclustering of cluster 1 identified 2 clusters of cells (C1Q+ and S100A8+) highly expressing IFN-response genes (Supplementary Fig S3E, Supplementary Table S2C). Interestingly, the ISG signature within CD16+/DC4 was driven by 2 ACPA− patients (Supplementary Fig S3D) and this cluster may correspond to a subcluster of cDC2 in our dataset (Supplementary Fig S3F). Altogether, these data indicate an enhanced type I IFN-response gene signature and an increased type I IFN score among synovial innate immune cells in patients with ACPA− RA.

Similar levels of IFNs in SF of ACPA-positive and ACPA-negative RA

We investigated whether this ISG signature could result from an increased type I IFN production in SF of ACPA− patients. No striking difference was observed in the frequency of pDCs in SF between ACPA+ and ACPA− RA in the scRNA-Seq dataset (Supplementary Fig S4A). We further assessed their frequency in SFMC from patients with ACPA+ RA (n = 9), ACPA− RA (n = 8), and PsA (n = 9) using full spectrum flow cytometry (Fig 3A, Supplementary Table S1). The frequencies of pDCs in SF (gated as $CD123^+CD11C^-$ or $CD123^+CD303^+$) were similar in all patient groups (Fig 3B, Supplementary Fig S4B,C). Moreover, pDCs had the same capacity to produce IFN-α and IFN-β upon TLR9 stimulation using CpG ODN (Cytosine-phosphate-Guanine oligonucleotide) in all disease groups (Fig 3C,D). The frequency of IFN-α positive cells was not significantly different among patients with RA receiving corticosteroid, methotrexate, or biologic therapies at the time of sampling (Supplementary Fig S4D). Neither IFN-α nor IFN-β was produced by any other DC subset in SF upon CpG ODN stimulation (Supplementary Fig S4E). Moreover, there were no significant differences in the production of IFN-γ and TNF (Fig 3E) produced by T cells in the 3 disease groups, upon stimulation with the superantigen staphylococcal enterotoxin B (SEB). Finally, the levels of type I (IFN-α, IFN-β), type II (IFN-γ), type III (IFN-λ1) IFN and TNF assessed by

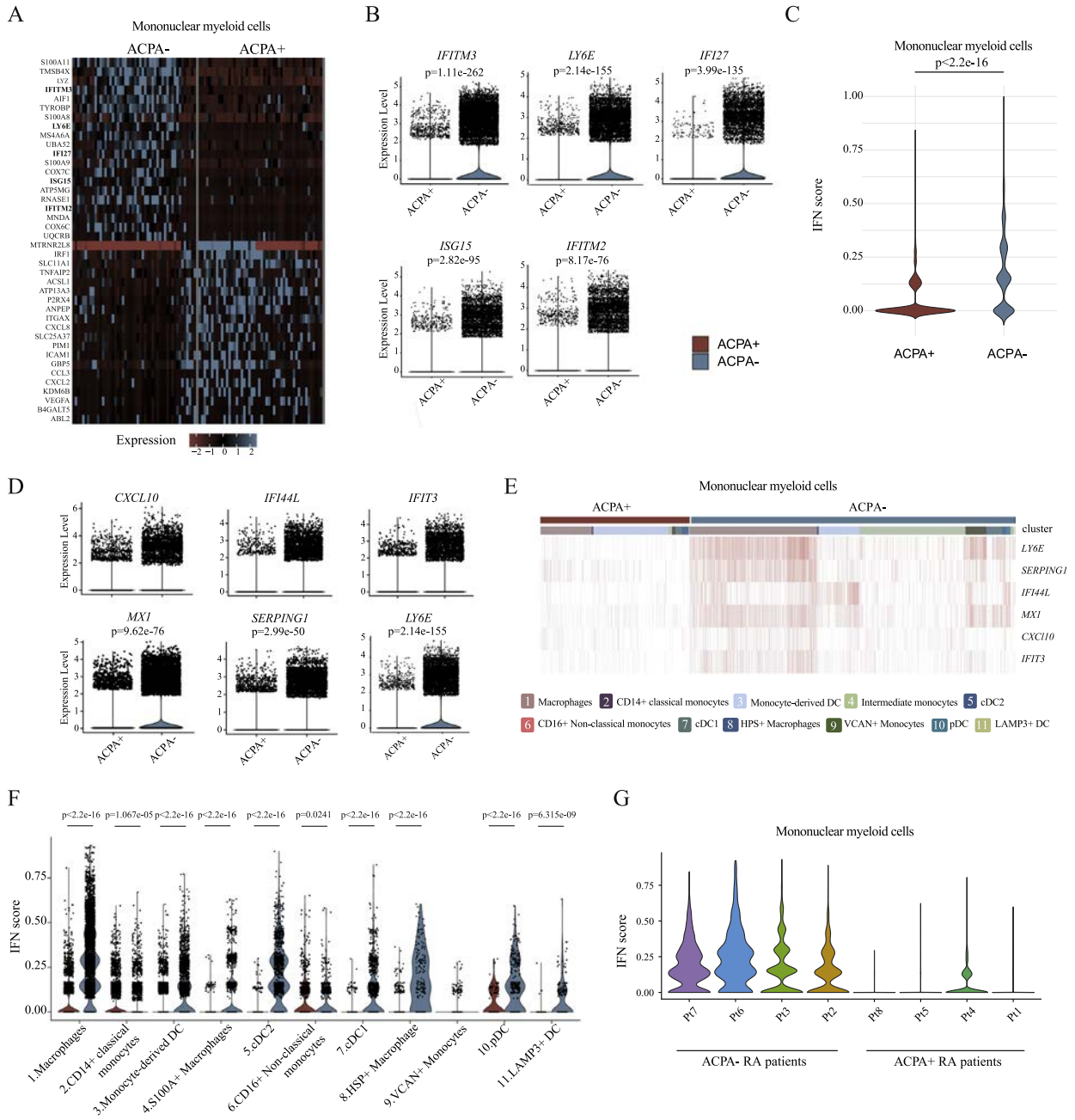


Figure 2. A type I interferon (IFN) signature is detected in mononuclear myeloid cells in synovial fluid (SF) of anti-citrullinated protein antibodies (ACPA)– rheumatoid arthritis (RA) (discovery cohort). (A) Heatmap showing the top 20 differentially expressed genes in SF of ACPA+ (n = 4) and ACPA– (n = 4) RA. (B) Violin plots showing the expression of selected genes in mononuclear myeloid cells from SF in ACPA+ (n = 4) and ACPA– (n = 4) RA. (C) Violin plot showing the IFN score in SF of ACPA+ (n = 4) and ACPA– (n = 4) RA. (D) Violin plots showing the expression of selected genes, used for the IFN score calculation. (E) Heatmap showing the differential gene expression of selected genes in each cluster between ACPA+ and ACPA–. (F) Violin plots showing the IFN score in each mononuclear myeloid subset and split between ACPA+ (n = 4) and ACPA– (n = 4). (G) Violin plots showing the IFN score in each mononuclear myeloid subset in each patient with RA. (C, F, G) Ucell IFN score included *CXCL10, IFI44L, IFIT3, MX1, SERPING1*, and *LY6E*.

bead array and ELISA were increased in SF as compared to serum for all patient groups (Fig 3F, Supplementary Fig S4F). There were no differences in IFN levels in SF between ACPA+ and ACPA– RA; however, the IFN-α levels were higher in serum in ACPA+ RA (Supplementary Fig S4F). We systematically assessed the gene expression of receptors, intracellular signalling molecules, and transcription factors related to the type I IFN pathway (Supplementary Table S4). For most of these genes, we could not detect significant differences in expression between ACPA– and ACPA+ mononuclear myeloid cells. Interestingly, *STAT1* was significantly increased in macrophages, cDC1 and cDC2 in ACPA– RA SF compared to ACPA+ RA SF (Supplementary Fig S4G). On the contrary, *IRF1* was expressed at higher levels in macrophages and monocyte-derived DC in ACPA+ RA, compared to ACPA– SF (Supplementary Fig S4H). Altogether, these findings suggest that the elevated IFN score in myeloid cells in SF of ACPA– RA is not driven by an excess of local type I IFN production in the synovial joint.

SF of ACPA-negative RA is enriched in mature cDC2

Since cDC2 was one of the clusters that had the highest IFN score among the mononuclear myeloid subsets in ACPA– RA, we further investigated its profile. At steady state, in SF, the frequency of cDC2s among CD11c+ DCs was increased in ACPA–, as compared to ACPA+ RA or PsA (Supplementary Fig S4B,

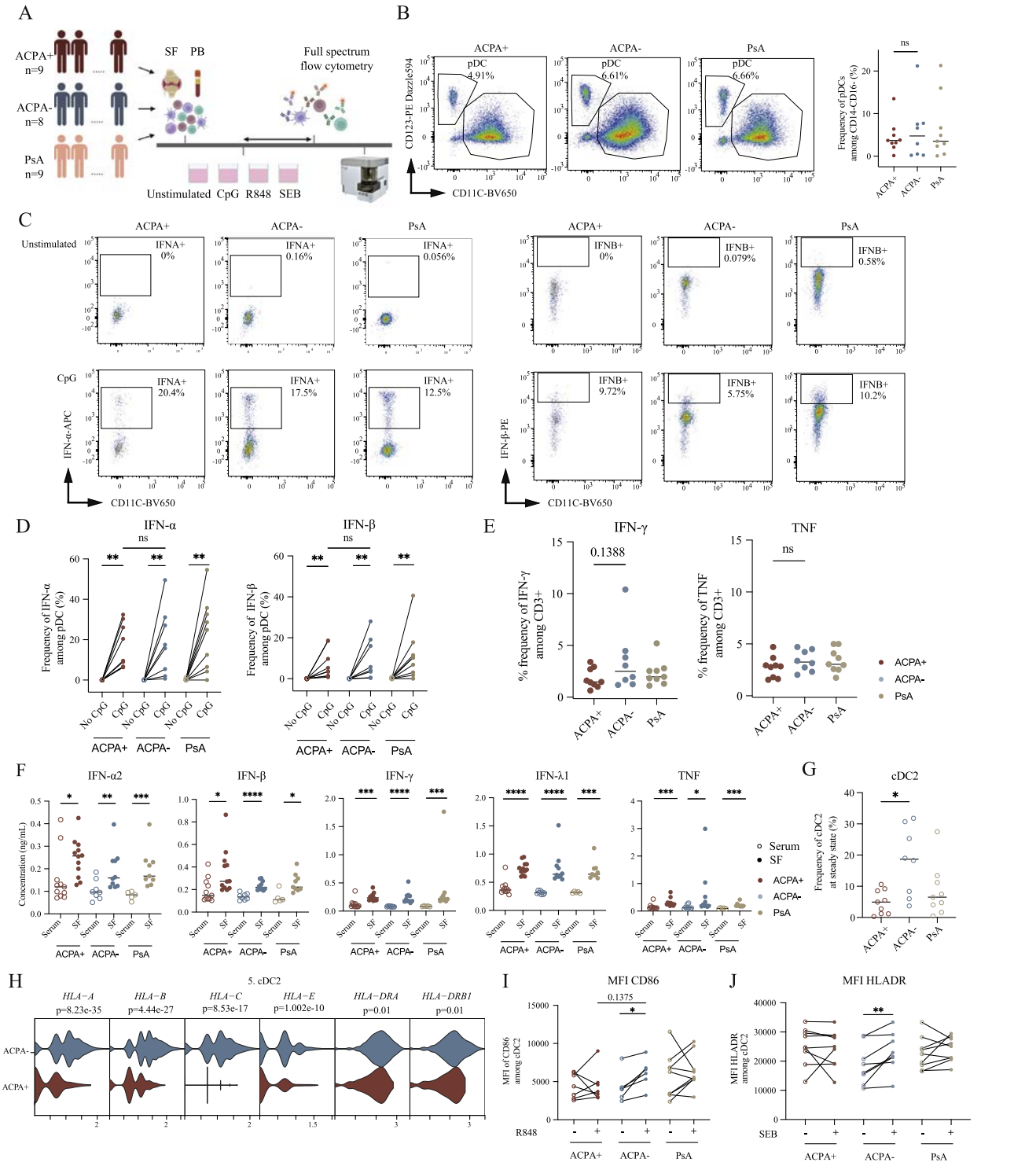


Figure 3. Profile of cDC2 in synovial fluid of anti-citrullinated protein antibodies (ACPA)+ and ACPA− rheumatoid arthritis (RA). (A) Full spectrum flow cytometry workflow. (B) Representative flow cytometry dotplots of pDC in ACPA+ (left panel), ACPA− (middle panel) and psoriatic arthritis (PsA; right panel), quantified in $n = 9$ ACPA+, $n = 8$ ACPA− and $n = 9$ PsA. (C) Representative flow cytometry dotplots showing the expression of interferon (IFN)-α and IFN-β in ACPA+, ACPA−, and PsA before (upper panels) and after CpG ODN (Cytosine-phosphate-Guanine oligonucleotide) stimulation (lower panels). (D) Frequency of IFN-α (left panel) and IFN-β (right panel) positive cells among pDC in $n = 9$ ACPA+, $n = 8$ ACPA− and $n = 9$ PsA upon CpG ODN stimulation. (E) Frequency of IFN-γ and TNF-positive cells among CD3+ cells upon SEB stimulation. (F) Levels of IFN-α, IFN-β, IFN-γ, IFN-λ1 and TNF in $n = 12$ ACPA+, $n = 10$ ACPA− and $n = 9$ PsA detected with bead array. (G) Frequency of cDC2 among CD11c+ cells in RA ($n = 9$ ACPA+, $n = 8$ ACPA−) and PsA ($n = 9$) at steady state. (H) Violin plots showing the expression of selected genes on cDC2 in $n = 8$ RA (4 ACPA+, 4 ACPA−). (I) Mean fluorescent intensity (MFI) of CD86 in cDC2 before and after stimulation with R848 in RA ($n = 9$ ACPA+, $n = 8$ ACPA−) and PsA ($n = 9$). (J) MFI of HLA-DR in cDC2 before and after stimulation with SEB in RA ($n = 9$ ACPA+, $n = 8$ ACPA−) and PsA ($n = 9$). Red colour indicates ACPA+, blue colour ACPA−, and yellow colour PsA. (C, D, E, F, G) Line represents median. Two-tailed Mann-Whitney U test. SEB, superantigen B.

Fig 3G). cDC2 cells expressed significantly higher levels of *HLA-I* and *HLA-II* genes in ACPA− compared to ACPA+ RA SF (Fig 3H, Supplementary Fig S5A, Supplementary Table S2D), suggesting an increased maturation level. Additionally, a significantly higher expression of *FCGR3A* (encoding CD16) was detected in cDC2s in ACPA− SF (Supplementary Fig S5B,C). Upon TLR7/8 stimulation with R848, cDC2s upregulated CD86 and tended to upregulate HLA-DR in ACPA− RA, but not in the other patient groups (Fig 3I, Supplementary Fig S5F). At steady state, in all patient groups, cDC2s presented a more mature state than cDC1s, as indicated by higher expression of CD86 and HLA-DR (Supplementary Fig S5D,E). Following SEB stimulation, triggering polyclonal T-cell activation, cDC2s upregulated HLA-DR in ACPA− RA, but not in the other disease groups (Fig 3J). cDC2s also produced higher levels of IL-8 and IL-6 upon CpG ODN and R848 stimulation, respectively, compared to cDC1s or pDCs without differences between the disease groups (Supplementary Fig S5G). Altogether, these data show that mature cDC2s are enriched in SF of patients with ACPA− RA.

ACPA-negative RA is enriched in innate immune cells that display distinct type I IFN gene signatures

To further investigate immune cell types in ACPA− RA SF, we performed scRNA-Seq on SFMC from a separate validation cohort composed of 8 patients with ACPA+ and 8 patients with ACPA− RA; of note, 1 patient with ACPA+ RA (#8) was already included in the discovery cohort (Table S1). We identified mononuclear myeloid cells, T cells and B cells (Supplementary Fig S6A, Fig 4A). Briefly, mononuclear myeloid cells included CD14+ macrophages (cluster 2), cDC2 (cluster 5), S100A+ macrophages (cluster 6), cDC1 (cluster 7), C1Q+ macrophages (cluster 8), SPP1+ macrophages (cluster 9), FN1+ macrophages (cluster 11), HSP+ macrophages (cluster 15), CXCL8+ macrophages (cluster 16), pDC (cluster 17), and LAMP3+ DC (cluster 18) (Fig 4A, Supplementary Fig S6B,C, Supplementary Table S2E). C1Q+ macrophages also expressed *APOE, CD163*, and *TREM2* and might correspond to TREM2high TIMD4pos resident macrophages as previously described (Supplementary Fig S6D, Supplementary Table S3) [13]. Higher frequencies of Tph and B cells were detected in SF of patients with ACPA+ RA, whereas ACPA− RA SF tended to show an increased frequency of the mononuclear myeloid cells: C1Q+, S100A+, CD14+ macrophages and cDC2 (Fig 4B, Supplementary Fig S6F). We then evaluated the expression of several ISGs among the different mononuclear myeloid cell subsets across the 2 cohorts. Interestingly, in both cohorts, we identified specific ISG characterizing distinct synovial mononuclear myeloid subsets (Fig 4C,D). CD14+ macrophages (cluster 1 in discovery and 2 in validation cohort) were characterized by high expression of *IFI6, ISG15, IFI27,* and *LY6E*. cDC2 (cluster 5 in both cohorts) showed high expression of *IFITM2, IFITM3*, and *LY6E*. pDC (cluster 10 in discovery and 17 in validation cohort) highly expressed *MX1* and *IFI44L* (Fig 4E,F). Since macrophages showed high IFN score in the discovery cohort (Fig 2F), we compared CD14+ macrophages (cluster 2) from ACPA+ and ACPA− RA and found several ISG (including *IFI30, SIGLEC1, ISG15,* and *IFI6*) upregulated in ACPA-RA (Fig 4G, left panel). This signature was observed in 6 out of 8 patients with ACPA− RA, and 2 out of 8 patients with ACPA+ RA (Fig 4G, right panel). Similarly, macrophages from ACPA− RA in the discovery cohort (cluster 1) upregulated the same genes, compared to ACPA+ RA (Fig 4H). Of note, we did not detect significant differences in the IFN score among total mononuclear myeloid cells between patients who were or were not receiving corticosteroids, biologics or methotrexate at the time of sampling, nor any correlation of IFN score with age and disease duration (Supplementary Fig S6G). Altogether, these data highlight distinct type I IFN gene signatures in synovial innate immune cells. The elevated type I IFN gene signature observed in ACPA− RA SF may result from either enhanced expression of IFN-stimulated genes or an increased frequency of synovial cell subsets expressing these genes.

DISCUSSION

In this study, we compared the transcriptomic profile of immune cells in SF and PB of patients with ACPA− and ACPA+ RA, using single-cell RNA sequencing. We demonstrated increased ISG expression in mononuclear myeloid cells from SF, but not PB, of ACPA− RA, compared to ACPA+, accompanied by a higher IFN score. However, this was neither a consequence of enhanced IFN levels in SF of ACPA−, nor of an increased frequency of pDCs, the main producers of type I IFN [16]. Instead, other innate immune cells were enriched in ACPA− SF. For instance, cDC2s were more frequent in SF of ACPA− RA and showed an increased level of maturation compared to cDC1s and pDCs. The elevated type I IFN signature in ACPA− RA SF may stem from both increased expression of IFN-stimulated genes and a higher abundance of cells showing this signature such as cDC2s or C1Q+ macrophages.

Type I IFN pathways have been implicated in the pathogenesis of several autoimmune rheumatic diseases [31] including SLE [24], Sjögren's disease [25], RA [32], and myositis [33–35]. An elevated IFN gene signature has been detected in PB in early ACPA+ RA compared to patients at a later stage of the disease [36]. In these patients, IFN-α correlated with the ISG signature, higher disease activity, and worse clinical response [30,32]. Moreover, increased expression of IFN-β has been identified in ST of RA, compared to osteoarthritis or reactive arthritis [37]. We also detected increased IFN-α levels in the serum of ACPA+ compared to patients with ACPA− RA. So far, it was unclear how this signature differs in SF between ACPA+ and ACPA− RA. We observed an enrichment of all tested IFNs in SF compared to PB, regardless of the disease subset. However, we did not detect any significant differences in the levels of the tested IFNs between ACPA+ and ACPA− SF, despite the increased ISG expression in myeloid cells in ACPA− SF. We cannot rule out that IFN consumption contributes to this discrepancy. Considering the broad spectrum of the type I IFN family [38], we also cannot exclude the possibility of differential production of other type I IFNs between ACPA+ and ACPA− RA. While the type I IFN signature was detected in immune cells from all ACPA− RA SF samples, only 2 STs showed elevated type I IFN score. This difference may reflect the heterogeneity within the ACPA− group, the disease stages, or variations between SF and ST compartments. We found no significant association between the IFN score and treatment at the time of sampling, age or disease duration; however, heterogeneity within the ACPA-negative group may still impact our findings. Nevertheless, these data suggest that, in a subset of ACPA− RA, the priming of the type I IFN signature may occur in a localised niche within the synovial joint, in the lymph nodes or at a different stage of the disease progression not captured in our analysis.

What drives IFN production and as a result, the expression of ISGs in autoimmune diseases remains unknown. Several potential mechanisms have been proposed, including the formation of immune complexes composed by autoantibodies and nuclear

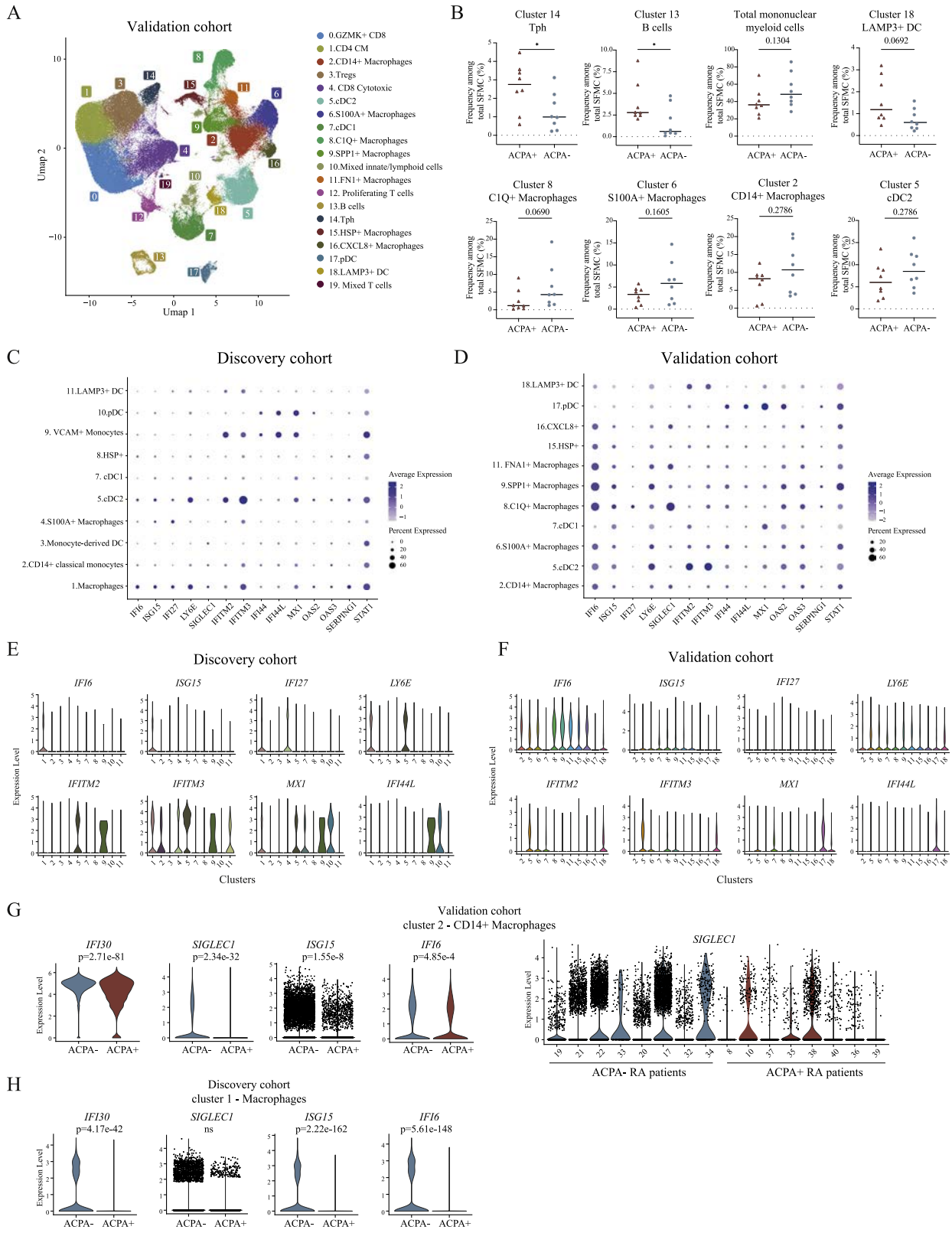


Figure 4. Single-cell RNA sequencing of immune cells from synovial fluid (SF) of patients with rheumatoid arthritis (RA; validation cohort). (A) UMAP (Uniform Manifold Approximation and Projection) displaying the 20 clusters of SF mononuclear cells of 16 patients with RA (n = 8 anti-citrullinated protein antibodies (ACPA)+ and n = 8 ACPA−). (B) Frequencies of selected clusters from n = 8 ACPA+ and n = 8 ACPA− RA SF. Triangles indicate ACPA+, circles indicate ACPA−, 2-tailed Mann-Whitney U test. (C) Dotplot showing the expression levels of selected genes in mononuclear myeloid cell subsets in SF in the discovery cohort and (D) in the validation cohort. (E) Violin plots showing the expression of selected genes among SF mononuclear myeloid subsets in the discovery cohort and (F) in the validation cohort. (G) Violin plots showing the expression of selected interferon-stimulated genes in CD14+ macrophages (cluster 2) from ACPA+ (n = 8) and ACPA− RA (n = 8) from the validation cohort (left panel) and expression of a selected interferon-stimulated gene (*SIGLEC1*) split by individual patient samples (right panel). (H) Violin plots showing the expression of selected interferon-stimulated genes in macrophages (cluster 1) from ACPA+ and ACPA− RA from the discovery cohort. (I) Violin plots showing the expression of *FCGR3A* (CD16) in cDC2 (cluster 5) from ACPA+ and ACPA− RA from the validation cohort. (A, B, D, F, G, I) Validation cohort consisted of n = 8 ACPA− and n = 8 ACPA+ RA samples. (C, E, H) Discovery cohort consisted of n = 4 ACPA− and n = 4 ACPA+ RA samples. (H) ns, nonsignificant.

DNA or RNA that derive from viral infections, NETosis, or apoptotic cells [32,39]. The type I IFN signature in PB of ACPA+ RA [32] may be related to a more systemic activation of the immune system, that is, through immune complexes involving ACPAs. Of note, 1 patient with ACPA− RA presented with anti-double-stranded DNA antibodies (Supplementary Table S1), which could contribute to this signature. It should be noted that an enrichment of specific innate immune cell types, such as cDC2 cells, which exhibit a type I IFN signature, may contribute to an increase total IFN score. Recently, increased ISG expression and elevated IFN score were also observed in synovial, but not peripheral blood, CD8+ T cells in patients with immune checkpoint inhibitor-induced inflammatory arthritis (ICI-IA), compared to ACPA+ RA and PsA [40]. This suggests common pathogenic mechanisms between ICI-IA and ACPA− arthritis, involving localised innate immune reactions in the synovial joint. GWAS have identified single-nucleotide polymorphisms (SNPs) in the IFN regulatory factor 5 (*IRF5*) locus which associate with ACPA− RA and SLE [41,42], leading to increased transcription of *IRF5* and increased type I IFN production [43]. We did not detect any differences in *IRF5* expression but additional SNPs in the type I IFN pathway may contribute to this increased type I IFN signature, where the initial trigger remains to be identified.

The frequency of CXCL13+ Tph cells and B cells was increased among ACPA+ synovial mononuclear cells consistent with a lymphocyte-driven inflammation in ACPA+ RA. In contrast, the frequency of C1Q+, S100A+, and CD14+ macrophages and cDC2 tended to be increased in ACPA− RA, suggesting a predominance of innate immune responses. C1Q+ macrophages likely correspond to TREM2high TIMD4pos resident macrophages which are suggested to be involved in homeostatic functions, including the clearance of debris [13]. Conversely, in treatment naive and resistant active RA, increased frequencies of S100A12+ MerTK- macrophages have been detected, suggesting a population of pathogenic, proinflammatory cells that contribute to sustained synovial inflammation [13]. Similarly, the frequency of CD9+ CLEC10A+ cells, which likely correspond to cDC2 in our dataset, was also increased in active RA [13]. cDC2s were more frequent in SF of ACPA−, compared to ACPA+ RA and they highly expressed *HLA-I* and *HLA-II* alleles, suggesting that cDC2s have been actively primed, possibly through exposure to type I IFN [44] as suggested by their elevated IFN-induced gene signature (Fig 2F). Indeed, type I IFN drives cDC2 maturation upon viral infection [45]. We also found that cDC2s have a more mature phenotype (including the production of proinflammatory cytokines IL-8, IL-6, and TNF), compared to cDC1s, as previously shown in healthy human lymph nodes and in RA ST [46,47]. ACPA− cDC2 also expressed higher levels of *FCGR3A*/CD16 (Supplementary Figs S5B and S6E) which may correspond to the previously described DC4 cells [14,15]. Interestingly, following SEB stimulation, cDC2s upregulated *HLA-DR* in ACPA− RA, but not in the other patient groups (Fig 3J), suggesting an enhanced licensing by CD4+ T cells, which are known to interact with cDC2s [48]. The consequences of the increased cDC2 frequency in ACPA− SF on T-cell priming remain to be explored but our recent data showing clonal expansion of regulatory T cells in SF of ACPA− RA [7], makes them potential candidates for interacting with cDC2 in the ACPA− RA synovial joint.

Innate immune mechanisms have been suspected to contribute to the pathogenesis of seronegative RA. In this study, we demonstrate a distinct type I IFN gene response signature in immune cells within the synovial joint, but not in PB, of ACPA− RA. These findings provide a foundation for future research to explore the initiation of type I IFN responses and their role in the pathogenesis of ACPA− RA.

Limitation of the study

Our study was based on a limited sample size from patients with longstanding disease and receiving immunosuppressive treatments, which could affect gene signatures. Moreover, the use of SF mononuclear cells, typically obtained during disease flares, may introduce a bias and may not fully capture the complexity of immune cells within the joint tissue. However, we identified a comparable set of innate immune populations to those previously reported in ST [13] and we confirmed the type I IFN signature in ST of ACPA− RA, driven by a subset of patients with ACPA− RA, in a treatment-naïve cohort. Since this ST cohort was not our own, we had limited access to clinical data, including information on viral infections or genetic background. We also cannot rule out that these 6 patients are not true seronegative RA cases, as in the absence of serologic markers, other forms of arthritis may be misdiagnosed as ACPA− RA in the early stages [49]. Finally, we identified an increased ISG signature in immune cells in SF of ACPA− RA but found no differences in the production or levels of IFN-α and IFN-β. We cannot exclude the possibility that the type I IFN signature is driven by another IFN cytokine, as the type I IFN-α family consists of 13 cytokines, for which some reagents are still unavailable.

Competing interests

AW reports a relationship with Pfizer Inc that includes: employment. MHW reports a relationship with Pfizer Inc that includes: employment. CK reports a relationship with Pfizer Inc that includes: employment. JF reports a relationship with Pfizer Inc that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Alexandra Argyriou: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marc H. Wadsworth:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Joshua Fienman:** Methodology, Writing – review & editing. **Ana Cristina Gonzalez-Sanchez:** Visualization, Formal analysis, Writing – review & editing. **Salim Ghannoum:** Formal analysis, Writing – review & editing. **Chirag Krishna:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Christina Gerstner:** Writing – review & editing, Methodology, Formal analysis. **Begum Horuluoglu:** Writing – review & editing, Methodology, Formal analysis. **Merel Sijbranda:** Writing – review & editing, Formal analysis. **Lars Rönnblom:** Writing – review & editing, Validation, Methodology. **Maija-Leena Eloranta:** Writing – review & editing, Validation, Methodology. **Marie Wahren-Herlenius:** Writing – review & editing, Validation, Methodology. **Aase Hensvold:** Validation, Methodology, Formal analysis. **Sara Turcinov:** Formal analysis, Investigation, Writing – review & editing. **Aaron Winkler:** Writing – review & editing, Project administration, Investigation. **Vivianne Malmström:** Writing – review & editing, Project

administration, Investigation. **Karine Chemin:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Acknowledgements

We thank the patients who donated samples and the medical staff at Karolinska University Hospital and the Center for Rheumatology and Academic Special Center in Stockholm. We thank Julia Norkko for cryopreserving and organizing biological samples. We thank Annika van Vollenhoven and the Flow Cytometry Core at the Center for Molecular Medicine. We thank Monika Hansson for her technical assistance with the measurement of ACPA levels. We thank Prof. Soumya Raychaudhuri, Harvard Medical School for sharing his dataset [15].

Funding

This study was supported by grants from the Swedish Research Council (2023-02439), the Swedish Rheumatism Association (R-993204), the Olle Enqvist fund (222-0028), the Gustav V80 Year Fund (FAI-2024-1086), the Nanna Svartz Fund (2022-00466), the Margaretha Nilsson Fund, and the Karolinska Institutet Fund (2024-02471).

Patient consent for publication

Not applicable.

Ethics approval

This study was approved by the ethical committee of the Karolinska University Hospital, Sweden (Dnr: 03-138, Dnr: 2019-04308, Dnr 2021-04971). All patients gave informed consent, according to the Declaration of Helsinki.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Single cell RNA sequencing data have been deposited at the European Genome-phenome Archive (EGA), which is hosted by the EBI and the CRG, under accession number EGAS50000001260.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.07.029.

Orcid

Alexandra Argyriou: http://orcid.org/0000-0002-4181-927X
Marc H. WadsworthII: http://orcid.org/0000-0001-9479-8516
Christina Gerstner: http://orcid.org/0009-0003-4342-4241
Begum Horuluoglu: http://orcid.org/0000-0003-2241-5170
Merel Sijbranda: http://orcid.org/0009-0001-0848-4594
Lars Rönnblom: http://orcid.org/0000-0001-9403-6503
Maija-Leena Eloranta: http://orcid.org/0000-0002-8454-1351
Marie Wahren-Herlenius: http://orcid.org/0000-0002-0915-7245
Aase Hensvold: http://orcid.org/0000-0002-7669-3777
Sara Turcinov: http://orcid.org/0000-0002-7476-1019
Aaron Winkler: http://orcid.org/0000-0001-7365-795X
Vivianne Malmström: http://orcid.org/0000-0001-9251-8082
Karine Chemin: http://orcid.org/0000-0002-2597-7809

REFERENCES

[1] Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham 3rd CO, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;62(9):2569–81.
[2] Raposo B, Klareskog L, Robinson WH, Malmström V, Grönwall C. The peculiar features, diversity and impact of citrulline-reactive autoantibodies. Nat Rev Rheumatol 2024;20(7):399–416.
[3] van der Helm-van Mil AH, Verpoort KN, Breedveld FC, Huizinga TW. Toes RE, de Vries RR. The HLA-DRB1 shared epitope alleles are primarily a risk factor for anti-cyclic citrullinated peptide antibodies and are not an independent risk factor for development of rheumatoid arthritis. Arthritis Rheum 2006;54(4):1117–21.
[4] Chemin K, Gerstner C, Malmström V. Effector functions of CD4+ T cells at the site of local autoimmune inflammation-lessons from rheumatoid arthritis. Front Immunol 2019;10:353.
[5] Kobayashi S, Murata K, Shibuya H, Morita M, Ishikawa M, Furu M, et al. A distinct human CD4+ T cell subset that secretes CXCL13 in rheumatoid synovium. Arthritis Rheum 2013;65(12):3063–72.
[6] Rao DA, Gurish MF, Marshall JL, Slowikowski K, Fonseka CY, Liu Y, et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. Nature 2017;542(7639):110–4.
[7] Argyriou A, Wadsworth 2nd MH, Lendvai A, Christensen SM, Hensvold AH, Gerstner C, et al. Single cell sequencing identifies clonally expanded synovial CD4(+) T(PH) cells expressing GPR56 in rheumatoid arthritis. Nat Commun 2022;13(1):4046.
[8] Bugatti S, De Stefano L, Gandolfo S, Ciccia F, Montecucco C. Autoantibody-negative rheumatoid arthritis: still a challenge for the rheumatologist. Lancet Rheumatol 2023;5(12):e743–55.
[9] Ding B, Padyukov L, Lundström E, Seielstad M, Plenge RM, Oksenberg JR, et al. Different patterns of associations with anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis in the extended major histocompatibility complex region. Arthritis Rheum 2009;60(1):30–8.
[10] Ohmura K, Terao C, Maruya E, Katayama M, Matoba K, Shimada K, et al. Anti-citrullinated peptide antibody-negative RA is a genetically distinct subset: a definitive study using only bone-erosive ACPA-negative rheumatoid arthritis. Rheumatology (Oxford) 2010;49(12):2298–304.
[11] Sokolove J, Schiff M, Fleischmann R, Weinblatt ME, Connolly SE, Johnsen A, et al. Impact of baseline anti-cyclic citrullinated peptide-2 antibody concentration on efficacy outcomes following treatment with subcutaneous abatacept or adalimumab: 2-year results from the AMPLE trial. Ann Rheum Dis 2016;75(4):709–14.
[12] McInnes IB, Buckley CD, Isaacs JD. Cytokines in rheumatoid arthritis—shaping the immunological landscape. Nat Rev Rheumatol 2016;12(1):63–8.
[13] Alivernini S, MacDonald L, Elmesmari A, Finlay S, Tolusso B, Gigante MR, et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. Nat Med 2020;26(8):1295–306.
[14] Villani AC, Satija R, Reynolds G, Sarkizova S, Shekhar K, Fletcher J, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science 2017;356(6335):eaah4573.
[15] Zhang F, Jonsson AH, Nathan A, Millard N, Curtis M, Xiao Q, et al. Deconstruction of rheumatoid arthritis synovium defines inflammatory subtypes. Nature 2023;623(7987):616–24.
[16] Fitzgerald-Bocarsly P, Feng D. The role of type I interferon production by dendritic cells in host defense. Biochimie 2007;89(6-7):843–55.
[17] Wu X, Liu Y, Jin S, Wang M, Jiao Y, Yang B, et al. Single-cell sequencing of immune cells from anticitrullinated peptide antibody positive and negative rheumatoid arthritis. Nat Commun 2021;12(1):4977.
[18] Tang-Huau TL, Gueguen P, Goudot C, Durand M, Bohec M, Baulande S, et al. Human in vivo-generated monocyte-derived dendritic cells and macrophages

cross-present antigens through a vacuolar pathway. Nat Commun 2018;9 (1):2570.
[19] Mildner A, Jung S. Development and function of dendritic cell subsets. Immunity 2014;40(5):642–56.
[20] MacDonald L, Elmesmari A, Somma D, Frew J, Di Mario C, Madhu R, et al. Synovial tissue myeloid dendritic cell subsets exhibit distinct tissue-niche localization and function in health and rheumatoid arthritis. Immunity 2024;57(12) 2843-62.e12.
[21] Guilliams M, Mildner A, Yona S. Developmental and functional heterogeneity of monocytes. Immunity 2018;49(4):595–613.
[22] Hillman H, Khan N, Singhania A, Dubelko P, Soldevila F, Tippalagama R, et al. Single-cell profiling reveals distinct subsets of CD14+ monocytes drive blood immune signatures of active tuberculosis. Front Immunol 2022; 13:1087010.
[23] Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics 2013;14:128.
[24] Rönnblom L, Pascual V. The innate immune system in SLE: type I interferons and dendritic cells. Lupus 2008;17(5):394–9.
[25] Yao Y, Liu Z, Jallal B, Shen N, Rönnblom L. Type I interferons in Sjögren's syndrome. Autoimmun Rev 2013;12(5):558–66.
[26] Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci U S A. 2003;100 (5):2610–5.
[27] Nezos A, Gravani F, Tassidou A, Kapsogeorgou EK, Voulgarelis M, Koutsilieris M, et al. Type I and II interferon signatures in Sjogren's syndrome pathogenesis: contributions in distinct clinical phenotypes and Sjogren's related lymphomagenesis. J Autoimmun 2015;63:47–58.
[28] Andreatta M, Carmona SJ. UCell: robust and scalable single-cell gene signature scoring. Comput Struct Biotechnol J 2021;19:3796–8.
[29] Chiche L, Jourde-Chiche N, Whalen E, Presnell S, Gersuk V, Dang K, et al. Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. Arthritis Rheumatol 2014;66(6):1583–95.
[30] Cooles FAH, Tarn J, Lendrem DW, Naamane N, Lin CM, Millar B, et al. Interferon-α-mediated therapeutic resistance in early rheumatoid arthritis implicates epigenetic reprogramming. Ann Rheum Dis 2022;81 (9):1214–23.
[31] Muskardin TL, Niewold TB. Type I interferon in rheumatic diseases. Nat Rev Rheumatol 2018;14(4):214–28.
[32] Lin CMA, Isaacs JD, Cooles FAH. Role of IFN-α in rheumatoid arthritis. Curr Rheumatol Rep 2024;26(2):37–52.
[33] Salajegheh M, Kong SW, Pinkus JL, Walsh RJ, Liao A, Nazareno R, et al. Interferon-stimulated gene 15 (ISG15) conjugates proteins in dermatomyositis muscle with perifascicular atrophy. Ann Neurol 2010;67(1):53–63.
[34] Greenberg SA. Type 1 interferons and myositis. Arthritis Res Ther 2010;12 (Suppl 1):S4.
[35] Argyriou A, Horuluoglu B, Galindo-Feria AS, Diaz-Boada JS, Sijbranda M, Notarnicola A, et al. Single-cell profiling of muscle-infiltrating T cells in idiopathic inflammatory myopathies. EMBO Mol Med 2023;15(10):e17240.
[36] Cooles FA, Anderson AE, Lendrem DW, Norris J, Pratt AG, Hilkens CMU, et al. The interferon gene signature is increased in patients with early treatment-naive rheumatoid arthritis and predicts a poorer response to initial therapy. J Allergy Clin Immunol 2018;141(1) 445-8.e4.
[37] van Holten J, Smeets TJ, Blankert P, Tak PP. Expression of interferon beta in synovial tissue from patients with rheumatoid arthritis: comparison with patients with osteoarthritis and reactive arthritis. Ann Rheum Dis 2005;64 (12):1780–2.
[38] McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. Nat Rev Immunol 2015;15(2):87–103.
[39] Rönnblom L, Leonard D. Interferon pathway in SLE: one key to unlocking the mystery of the disease. Lupus Sci Med 2019;6(1):e000270.
[40] Wang R, Singaraju A, Marks KE, Shakib L, Dunlap G, Adejoorin I, et al. Clonally expanded CD38(hi) cytotoxic CD8 T cells define the T cell infiltrate in checkpoint inhibitor-associated arthritis. Sci Immunol 2023;8(85): eadd1591.
[41] Sigurdsson S, Padyukov L, Kurreeman FA, Liljedahl U, Wiman AC, Alfredsson L, et al. Association of a haplotype in the promoter region of the interferon regulatory factor 5 gene with rheumatoid arthritis. Arthritis Rheum 2007;56 (7):2202–10.
[42] Graham RR, Kyogoku C, Sigurdsson S, Vlasova IA, Davies LR, Baechler EC, et al. Three functional variants of IFN regulatory factor 5 (IRF5) define risk and protective haplotypes for human lupus. Proc Natl Acad Sci U S A. 2007;104(16):6758–63.
[43] Rullo OJ, Woo JM, Wu H, Hoftman AD, Maranian P, Brahn BA, et al. Association of IRF5 polymorphisms with activation of the interferon alpha pathway. Ann Rheum Dis 2010;69(3):611–7.
[44] Baccala R, Hoebe K, Kono DH, Beutler B, Theofilopoulos AN. TLR-dependent and TLR-independent pathways of type I interferon induction in systemic autoimmunity. Nat Med 2007;13(5):543–51.
[45] Bosteels C, Neyt K, Vanheerswynghels M, van Helden MJ, Sichien D, Debeuf N, et al. Inflammatory type 2 cDCs acquire features of cDC1s and macrophages to orchestrate immunity to respiratory virus infection. Immunity 2020;52(6) 1039-56.e9.
[46] Canavan M, Marzaioli V, Bhargava V, Nagpal S, Gallagher P, Hurson C, et al. Functionally mature CD1c(+) dendritic cells preferentially accumulate in the inflammatory arthritis synovium. Front Immunol 2021;12:745226.
[47] Granot T, Senda T, Carpenter DJ, Matsuoka N, Weiner J, Gordon CL, et al. Dendritic cells display subset and tissue-specific maturation dynamics over human life. Immunity 2017;46(3):504–55.
[48] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392(6673):245–52.
[49] Paalanen K, Puolakka K, Nikiphorou E, Hannonen P, Sokka T. Is seronegative rheumatoid arthritis true rheumatoid arthritis? A nationwide cohort study. Rheumatology (Oxford) 2021;60(5):2391–5.

Ann Rheum Dis 85 (2026) 276–284

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Psoriatic arthritis

Understanding the drivers of BASDAI and back pain scores in psoriatic arthritis

Pankti Mehta[1,2,3], Fadi Kharouf[1,2], Virginia Carrizo Abarza[1,2], Shangyi Gao[1], Dafna D Gladman[1,2,3], Vinod Chandran[1,2,3,4], Denis Poddubnyy[1,2,3,*]

[1] *Gladman Krembil Psoriatic Arthritis Program, Centre for Prognosis Studies in the Rheumatic Diseases, Schroeder Arthritis Institute, Krembil Research Institute, University Health Network, Toronto, ON, Canada*
[2] *Division of Rheumatology, Department of Medicine, University of Toronto, Toronto, ON, Canada*
[3] *Institute of Medical Science, University of Toronto, Toronto, ON, Canada*
[4] *Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada*

ARTICLE INFO

Article history:
Received 21 July 2025
Received in revised form 8 September 2025
Accepted 29 September 2025

ABSTRACT

Objectives: The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) is widely used to assess axial disease activity in psoriatic arthritis (PsA). However, 5 of its 6 questions reflect general disease activity rather than axial-specific symptoms. We aimed to evaluate the performance of BASDAI and its back pain subscore in assessing axial disease in PsA.

Methods: Patients with BASDAI scores were identified from a longitudinal PsA cohort initiated in 1978. Axial disease was defined radiographically. Trends in BASDAI and back pain scores were compared between patients with and without axial involvement. Associations of total BASDAI and back pain subscore with axial disease were assessed using univariable and multivariable linear mixed models in the entire cohort and stratified subgroups.

Results: Of 1059 patients, 449 (42.4%) had axial and 610 (57.6%) had peripheral disease only. The mean age was 44.4 years (SD 12.8), and 55.9% were male. No difference in the BASDAI and back pain trends was observed between the axial and peripheral disease groups. Axial involvement was not associated with total BASDAI scores (β = −0.14, 95% CI −0.05 to 0.33). However, it was associated with a small, yet significant increase in back pain subscore (0.30, 0.06-0.55). Both BASDAI and back pain scores were associated with active peripheral joints, enthesitis, dactylitis, age, Psoriasis Area and Severity Index, and inversely with male sex. These associations were consistent across axial and peripheral disease subgroups.

Conclusions: BASDAI and its back pain subscore are influenced by peripheral musculoskeletal and skin disease activity in PsA, limiting their utility for assessing axial activity.

INTRODUCTION

Psoriatic arthritis (PsA) is a chronic inflammatory musculoskeletal disease spanning multiple domains, including the joints, skin, nails, and entheses [1]. Axial involvement in PsA (axPsA) is reported in 12.5% to 78% of patients, depending on the criteria and definitions applied [2].

Historically, our understanding of axPsA has been extrapolated from axial spondyloarthritis (axSpA) [3]. However, recent insights have highlighted clinically relevant differences between these 2 conditions [4–6]. Notably, isolated axial disease remains rare in PsA, occurring in only 2% to 5% of cases. Classical inflammatory back pain is also less commonly observed in axPsA [2]. Differences in demographic profiles, imaging characteristics, and genetic associations further call attention to the uniqueness of axPsA [2]. Patients with axPsA tend to be older at diagnosis, less likely to be male, with a lower frequency of human leukocyte antigen (HLA)-B*27 positivity as compared to

*Correspondence to Dr Denis Poddubnyy, Division of Rheumatology, Department of Medicine, Temerty Faculty of Medicine, University of Toronto, Toronto, ON, Canada.

E-mail address: Denis.Poddubnyy@uhn.ca (D. Poddubnyy).

Handling editor Josef S Smolen

https://doi.org/10.1016/j.ard.2025.09.016

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) is commonly used to assess axial disease activity in psoriatic arthritis (PsA). However, its items largely reflect general inflammatory symptoms and may be influenced by almost always coexistent peripheral disease in PsA. Evidence on the performance of BASDAI in distinguishing axial from peripheral disease activity in PsA is limited and mostly cross-sectional.

WHAT THIS STUDY ADDS

- In this large longitudinal PsA cohort, BASDAI and its back pain subscore did not differ between patients with and without axial involvement. Both scores were influenced by peripheral arthritis, enthesitis, dactylitis, and skin involvement, regardless of axial disease status. Axial involvement was only modestly associated with increased back pain scores, not with total BASDAI.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- BASDAI and its back pain subscore may not be reliable tools for evaluating axial disease activity in PsA. There is a need for PsA-specific measures to assess axial involvement along with magnetic resonance imaging to reliably estimate axial activity. These findings may guide outcome measure selection in clinical trials and routine practice for axial PsA.

axSpA [5,7–9]. Efforts are currently underway through multicentre collaborations to establish specific criteria for defining axial PsA [10].

Despite our understanding of these differences and recognition of a subset of axPsA as a distinct entity, outcome measures used for axPsA are still predominantly borrowed from axSpA literature [11]. The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Axial Spondyloarthritis Disease Activity Score (ASDAS) are commonly used in axPsA, showing moderate to good correlation with overall disease activity [12]. However, even in axSpA, BASDAI scores tend to be higher in patients with concomitant peripheral disease, reflecting higher overall activity but also a disproportionate contribution of peripheral symptoms to the overall score [13]. In PsA, the almost always coexistent peripheral disease and higher likelihood of concomitant degenerative disc disease due to older age further challenge the validity of axial disease activity assessment using these measures. Indeed, smaller cross-sectional studies suggest that BASDAI does not effectively distinguish between axial and peripheral manifestations in PsA [14,15]. Longitudinal studies evaluating the performance of BASDAI and its back pain subscore in PsA are lacking. The factors driving BASDAI and back pain subscores in PsA remain unclear, an important limitation given that clinical trials of axPsA frequently rely on BASDAI to define the target population and assess outcomes [16].

This study aims to evaluate the utility of BASDAI and its back pain subscore in assessing axial disease activity among individuals with axPsA.

METHODS

Setting

It was an observational cohort study conducted at the Gladman Krembil Psoriatic Arthritis Program, Schroeder Arthritis Institute, Toronto, Canada [17]. Clinical, radiographic, and laboratory data from patients with PsA is prospectively collected at 6- to 12-month intervals since 1978 using a standardised protocol. This protocol includes a comprehensive history, physical examination, disease activity assessment, patient-reported outcomes, and laboratory evaluations. Radiographs of the hands, feet, pelvis, cervical spine, and lumbar spine are obtained every 2 years. Data are stored in an electronic database. Over 99% of patients in the cohort meet the Classification Criteria for Psoriatic Arthritis [18]. BASDAI, other outcome measures, laboratory assessments (including HLA testing), and radiographs are performed for all patients, regardless of the presence or absence of axial disease. All radiographs are reviewed in separate consensus reading sessions by at least 2 experienced readers (DDG, VC, and DP), independent of clinical assessments.

Patients

All patients enrolled in the cohort after 1999 and up to 30 May 2025 with at least 1 available BASDAI score were included for this study.

Assessment

Demographic characteristics (age, sex, and disease duration), body weight, comorbidities (Charlson Comorbidity Index), clinical features (swollen joint count [SJC], tender joint count [TJC], enthesitis, dactylitis, and nail involvement), disease activity (Psoriasis Area and Severity Index [PASI], clinical Disease Activity index for Psoriatic Arthritis) [19], patient-reported outcomes (BASDAI, Health Assessment Questionnaire [HAQ]) [20,21], laboratory values (erythrocyte sedimentation rate, C reactive protein, HLA-B-*27 and C-*06), radiographic features (number of joints with erosions, sacroiliitis, syndesmophytes, modified Steinbrocker score [mSS]) and treatment (nonsteroidal anti-inflammatory drugs [NSAIDs], conventional synthetic [cs] disease-modifying antirheumatic drugs [DMARDs], biologic [b] DMARDs and Janus kinase inhibitors [JAKi]) were retrieved from the database.

Patients were classified into peripheral and axial groups. The peripheral group had patients with pure peripheral joint involvement without axial disease over the follow-up duration. Axial disease was defined radiographically using the modified New York criteria for sacroiliitis and/or the presence of syndesmophytes at any point during their follow-up [22], irrespective of the presence of peripheral manifestations. Pure axial disease was defined as patients with axial disease without any evidence of peripheral involvement over their entire follow-up duration.

Statistical analysis

Baseline details at the time of enrolment were described using descriptive statistics for peripheral and axial groups. Quantitative variables were described as mean ± SD or median (IQR) based on tests for normality. Qualitative variables were expressed as numbers and percentages.

We studied trends of the total BASDAI and back pain subscore (BASDAI question 2) for axial and peripheral subgroups. The BASDAI and back pain scores were plotted on the y-axis and disease duration from enrolment on the x-axis. The smoothed trajectories of the scores were outlined. The associations of BASDAI and back pain with axial vs peripheral disease, adjusted for disease duration from enrolment, were analysed using linear mixed models (LMM) with random effects.

The association of the presence of axial disease with BASDAI and back pain was studied using univariable and multivariable LMM with individualised random effects in the total cohort. Variables for the multivariable model were selected using a directed acyclic graph (Supplementary Fig), which included axial vs peripheral disease, age, sex, SJC (0-66), TJC (0-68), dactylitis, enthesitis, PASI score (0-72), nail disease, mSS (0-168), and treatment used (NSAID, csDMARD, bDMARD, and JAKi).

Factors associated with BASDAI and BASDAI back pain were also studied in the axial and peripheral subgroups separately using univariable and multivariable LMM with random effects. Covariates studied were age, sex, SJC (0-66), TJC (0-68), dactylitis, enthesitis, PASI score (0-72), nail disease, mSS (0-168), and treatment used (NSAID, csDMARD, bDMARD, and JAKi). The associations with remaining BASDAI questions (questions 1, 3-6) were also analysed using univariable and multivariable LMM using the same variables.

Statistical analyses were conducted using SAS version 9.3 (SAS Institute) and R version 4.2.3. The outcomes of the LMM analysis were expressed as beta (β) estimates with 95% CIs.

Consent and ethics

We obtained ethics approval from the University Health Network Research Ethics Board (08-0630). All patients provided informed consent at enrolment.

RESULTS

Baseline characteristics

We included 1059 patients with available BASDAI scores, of whom 610 (57.6%) had pure peripheral PsA, and 449 (42.4%) had axial involvement over follow-up. Of 449 with axial involvement, only 6 patients (6 of 1059, 0.57%) had pure axial disease. The median number of BASDAI records per patient was 6.00 (3.00, 11.00) in the total cohort. Compared to the peripheral group, patients in the axial subgroup were more often male and had a younger age at PsA onset. The SJC and TJC were similar between groups, but the axial group had a higher prevalence of dactylitis and nail disease and a lower prevalence of enthesitis. The mSS was higher in the axial group. HLA-B*27 positivity was also more common in the axial subgroup. There were no significant differences in PASI, HAQ, disease activity in psoriatic arthritis, or BASDAI scores between the 2 groups (Table 1).

Table 1
Baseline characteristics of the peripheral and axial subgroups at enrolment into the cohort

Characteristics	Peripheral disease only, 610	Axial disease ever, 449
Demographics		
Age at onset of PsA (y), median (IQR)	41.00 (30.00-51.00)	35.00 (27.00-46.00)
Sex, male (%)	301 (49.4)	292 (65.0)
Duration of PsA (y), median (IQR)	1.75 (0.48-5.61)	3.48 (0.88-9.56)
Follow-up duration from enrolment (y), median (IQR)	9.70 (4.90-15.89)	17.09 (10.53-27.16)
CCI, 0-37, mean (SD)	1.02 (0.98)	1.12 (0.75)
Clinical features		
SJC, 0-66, median (IQR)	2.00 (0.00-4.00)	2.00 (0.00-5.00)
TJC, 0-68, median (IQR)	3.00 (1.00-9.00)	4.00 (1.00-11.00)
Dactylitis, n (%)	126 (20.8)	130 (29.1)
Enthesitis, n (%)	162 (26.9)	83 (18.6)
PASI score, 0-72, median (IQR)	1.80 (0.10-4.07)	1.60 (0.00-4.60)
Nail disease, n (%)	343 (56.3)	336 (74.8)
Radiographic damage		
Modified Steinbrocker score, 0-168, mean (SD)	5.02 (12.42)	13.93 (26.01)
Syndesmophytes	0 (0.0)	104 (23.2)
Sacroiliitis	0 (0.0)	222 (49.6)
Patient-reported outcomes		
HAQ, 0-3, median (IQR)	0.50 (0.12-1.12)	0.50 (0.12-1.12)
BASDAI, 0-10, median (IQR)	4.00 (2.00-5.97)	3.50 (1.80-5.80)
Laboratory features		
Abnormal ESR	196 (32.6)	220 (49.2)
Abnormal CRP	269 (45.4)	226 (51.7)
HLA-B*27	47 (10.2)	87 (21.3)
HLA-C*06	123 (26.7)	104 (25.5)
Disease activity		
DAPSA, 0-30, median (IQR)	17.00 (9.80-28.00)	16.10 (8.85-31.35)
Treatment		
NSAIDs	358 (58.7)	308 (68.6)
csDMARDs	225 (36.9)	173 (38.5)
bDMARDs	84 (13.8)	47 (10.5)
tsDMARDs	7 (1.1)	1 (0.2)

b, biologic; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CCI, Charlson Comorbidity Index; CRP, C reactive protein; cs, conventional synthetic; DAPSA, disease activity in psoriatic arthritis; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; HAQ, health assessment questionnaire; HLA, human leukocyte antigen; NSAID, nonsteroidal anti-inflammatory drug; PASI, Psoriasis Area and Severity Index; PsA, psoriatic arthritis; SJC, swollen joint count; TJC, tender joint count.

Trends of BASDAI and BASDAI back pain

BASDAI

Figure 1A shows the trends in BASDAI scores over time for the axial and peripheral subgroups. After adjusting for disease duration, there was no significant difference between the groups (axial disease: $\beta = -0.14$ [95% CI −0.38 to 0.09]).

BASDAI back pain

Similarly, Figure 1B illustrates the trends in BASDAI back pain subscores over time. No significant difference was observed after adjusting for disease duration (axial disease: $\beta = -0.13$ [95% CI −0.42 to 0.17]).

Association with BASDAI and BASDAI back pain, total cohort

BASDAI

In the univariable analysis, BASDAI was significantly associated with age, higher SJC, TJC, and PASI scores. It was also positively associated with the presence of enthesitis, dactylitis, nail disease, and NSAID use. It was negatively associated with male sex and bDMARD use. No association was observed with the presence of axial disease ($\beta = -0.14$, 95% CI −0.32 to 0.04), csDMARD, mSS, or JAKi use (Table 2).

In the multivariable analysis, BASDAI remained significantly associated with age, higher SJC, TJC, and PASI score. Significant associations were also seen with the presence of enthesitis, dactylitis, and NSAID use. It was inversely associated with male sex, csDMARD, and bDMARD use. Axial disease ($\beta = 0.14$, 95% CI −0.05 to 0.33), nail disease, mSS, and JAKi use were not significantly associated with BASDAI scores (Fig. 2A).

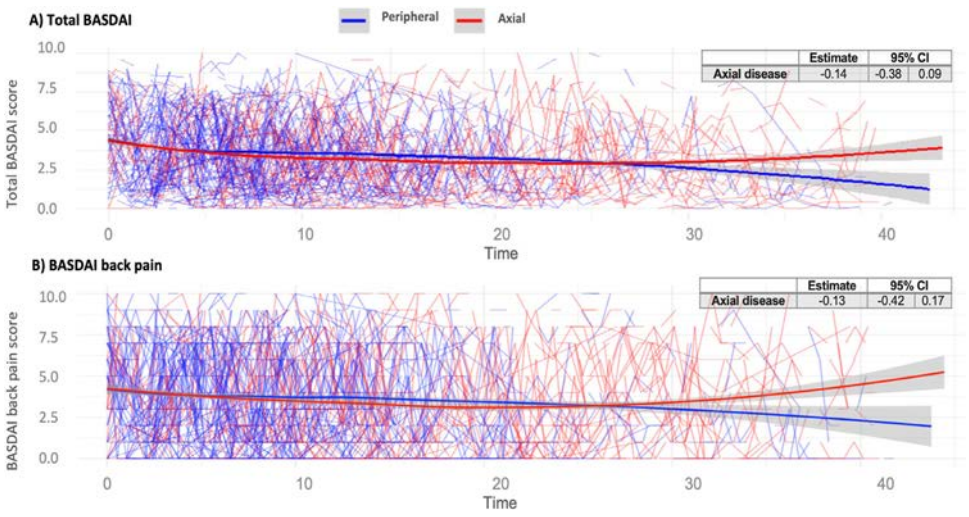


Figure 1. Trends of BASDAI (A) and BASDAI back pain (B) plotted over disease duration for axial and peripheral subgroups with their smoothed trajectories. BASDAI, Bath Ankylosing Spondylitis Disease Activity Index.

BASDAI back pain

In the univariable analysis, it showed significant associations with higher SJC, TJC, and PASI scores. It was also associated with the presence of dactylitis and NSAID use. BASDAI back pain was negatively associated with age, male sex, and csDMARD use. No significant associations were found with axial disease (β = 0.16, 95% CI −0.07 to 0.39), nail disease, mSS, bDMARDs, or JAKi use (Table 2).

In the multivariable analysis, BASDAI back pain was significantly associated with the presence of axial disease, older age, higher SJC, TJC, and PASI scores. It was also associated with the presence of enthesitis and NSAID use. A significant inverse association was seen with male sex. No significant associations were observed with dactylitis, nail disease, mSS, csDMARD, bDMARD, or JAKi use (Fig. 2B).

Association with BASDAI and BASDAI back pain, axial subgroup

BASDAI

In univariable analysis, BASDAI scores were significantly associated with higher SJC, TJC, and PASI score. It was also associated with the presence of enthesitis, dactylitis, PASI score, nail disease, and NSAID use. Being male and bDMARD use were associated with lower BASDAI scores. There was no significant association with age, mSS, csDMARD, or JAKi use (Supplementary Table S1).

In the multivariable model, higher SJC, TJC, enthesitis, and dactylitis were significantly associated with higher BASDAI scores. Male sex was associated with lower BASDAI scores. NSAID use was positively associated, while bDMARD use was negatively associated with BASDAI. Age, PASI score, nail disease, mSS, csDMARD, and JAKi use were not statistically significant (Fig 3A).

BASDAI back pain

In univariable analysis, BASDAI back pain was associated with older age higher SJC, TJC, and PASI score. It was positively associated with the presence of enthesitis, dactylitis, NSAID, and JAKi use. Male sex was again associated with significantly lower BASDAI back pain scores along with csDMARD and bDMARD use. No significant associations were found with nail disease and mSS (Supplementary Table S1).

In the multivariable model for BASDAI back pain, TJC, enthesitis, and older age were significantly associated with higher scores. Male sex remained strongly associated with lower BASDAI back pain scores. NSAID use and JAKi use were positively associated whereas csDMARD use was negatively associated

Table 2
Univariable LMM with BASDAI and BASDAI back pain score as outcomes for the total cohort

	Univariable LMM with BASDAI as the outcome			Univariable LMM with BASDAI back pain score as the outcome		
Variables	Estimate	95% CI		Estimate	95% CI	
Axial disease	−0.14	−0.32	0.04	0.16	−0.07	0.39
SJC, 0-66	0.16	0.15	0.18	0.08	0.07	0.10
TJC, 0-68	0.09	0.08	0.10	0.06	0.05	0.07
Enthesitis	0.66	0.55	0.78	0.65	0.50	0.80
Dactylitis	1.04	0.85	1.23	0.48	0.24	0.73
PASI score, 0-72	0.04	0.03	0.06	0.03	0.01	0.04
Nail disease	0.24	0.14	0.33	0.02	−0.10	0.13
mSteinbrocker score, 0-168	−0.0003	−0.004	0.003	0.00	−0.005	0.004
Age (y)	−0.01	−0.01	−0.0005	0.02	0.01	0.03
Sex, male	−0.97	−1.21	−0.73	−1.09	−1.38	−0.80
NSAIDs	0.44	0.34	0.54	0.40	0.27	0.52
csDMARDs	−0.02	−0.13	0.10	−0.15	−0.29	−0.01
bDMARDs	−0.55	−0.66	−0.44	0.02	−0.10	0.13
JAKi	0.01	−0.46	0.49	0.40	0.27	0.52

b, biologic; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; cs, conventional synthetic; DMARD, disease-modifying antirheumatic drug; JAKi, Janus kinase inhibitor; LMM, linear mixed model; m, modified; NSAID, nonsteroidal anti-inflammatory drug; PASI, Psoriasis Area and Severity Index; SJC, swollen joint count; TJC, tender joint count.

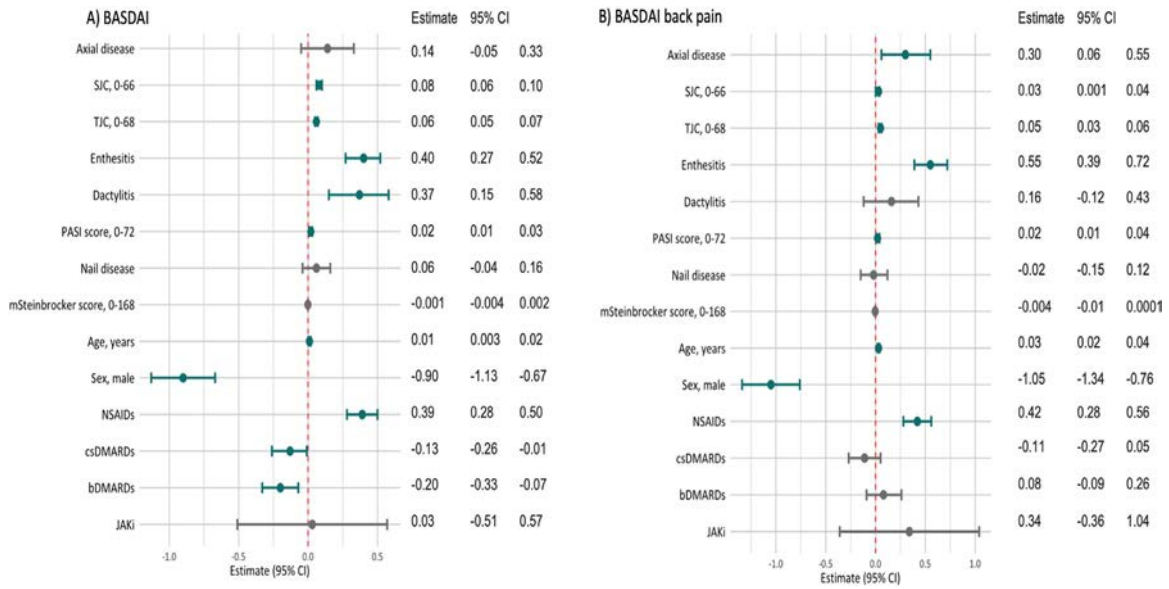


Figure 2. Associations with BASDAI (A) and BASDAI back pain (B) in the total cohort in the multivariable LMM. Numbers represent parameter estimates with 95% CIs. b, biologic; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; cs, conventional synthetic; DMARD, disease-modifying antirheumatic drug; JAKi, Janus kinase inhibitor; LMM, linear mixed model; m, modified; NSAID, nonsteroidal anti-inflammatory drug; PASI, Psoriasis Area and Severity Index; SJC, swollen joint count; TJC, tender joint count.

with BASDAI back pain scores. SJC, PASI score, nail disease, mSS score, dactylitis, and bDMARD use were not significantly associated (Fig 3B).

Association with BASDAI and BASDAI back pain, peripheral subgroup

BASDAI

In univariable analysis, BASDAI was significantly associated with SJC, TJC, PASI scores, dactylitis, enthesitis, nail disease, and NSAID use. It was inversely associated with male sex and bDMARD use. It was not associated with age, mSS, csDMARD, or JAKi use (Supplementary Table S2).

In multivariable analysis, BASDAI was associated with older age, higher SJC, TJC, enthesitis, PASI score, and NSAID use. Negative association with male sex was persistent. There was no significant association between BASDAI and nail disease, dactylitis, mSS, csDMARD, or JAKi use (Fig 4A).

BASDAI back pain

In univariable analysis, BASDAI back pain scores were significantly associated with older age, higher SJC, TJC, PASI, enthesitis, NSAIDs, and csDMARD. It was negatively associated with male sex. It was not associated with dactylitis, mSS, nail disease, csDMARD, bDMARD, and JAKi use (Supplementary Table S2).

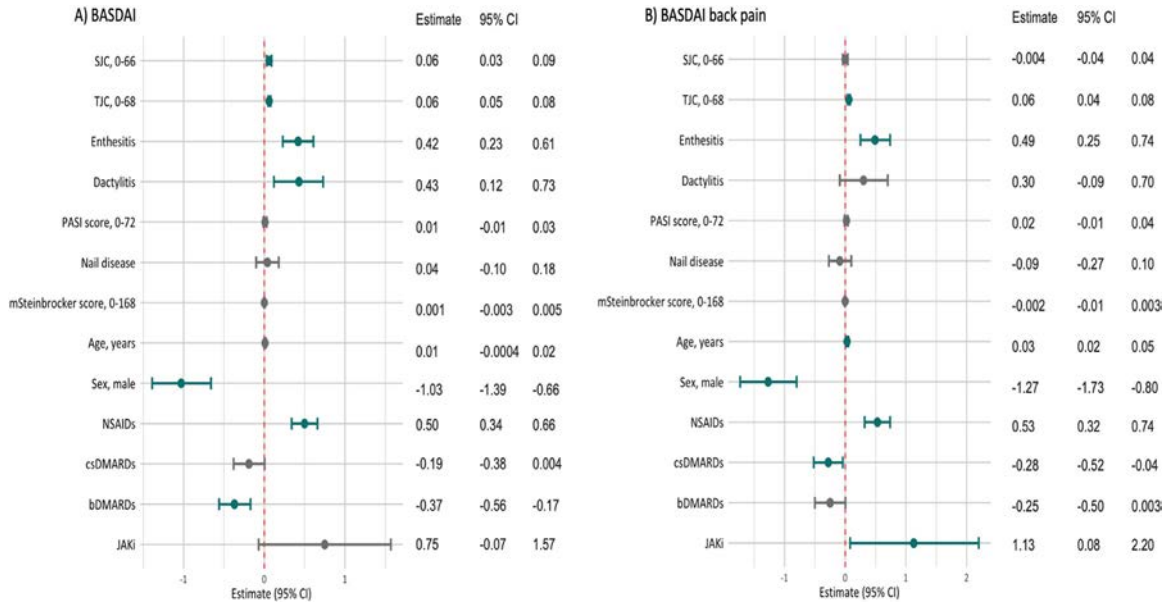


Figure 3. Associations with BASDAI(A) and BASDAI back pain(B) score in the axial cohort in the multivariable LMM. Numbers represent parameter estimates with 95% CIs. b, biologic; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; cs, conventional synthetic; DMARD, disease-modifying antirheumatic drug; JAKi, Janus kinase inhibitor; LMM, linear mixed model; m, modified; NSAID, nonsteroidal anti-inflammatory drug; PASI, Psoriasis Area and Severity Index; SJC, swollen joint count; TJC, tender joint count.

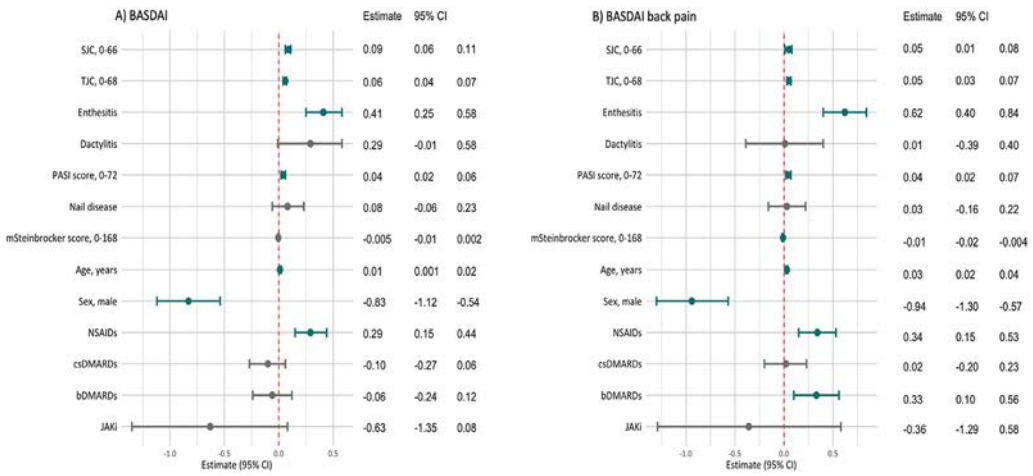


Figure 4. Associations with BASDAI(A) and BASDAI back pain(B) score in the peripheral group in the multivariable LMM. Numbers represent parameter estimates with 95% CIs. b, biologic; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; cs, conventional synthetic; DMARD, disease-modifying antirheumatic drug; JAKi, Janus kinase inhibitor; LMM, linear mixed model; m, modified; NSAID, nonsteroidal anti-inflammatory drug; PASI, Psoriasis Area and Severity Index; SJC, swollen joint count; TJC, tender joint count.

In the multivariable analysis, BASDAI back pain was associated with older age, male sex, SJC, TJC, enthesitis, PASI score, NSAID, and bDMARD use. The mSS score showed a small but statistically significant negative association (β = −0.01, 95% CI −0.02 to −0.004, P = .007). Dactylitis, nail disease, csDMARD, and JAKi were not significantly associated with BASDAI back pain (Fig 4B).

Association with other BASDAI components (question 1, questions 3-6)

In univariable analysis, axial disease was significantly associated with peripheral joint pain (question 3, β = −0.23, 95% CI −0.45 to −0.02), entheseal pains (β = −0.29, 95% CI −0.51 to −0.07), early morning stiffness (EMS) severity (question 5, β = −0.29, 95% CI −0.51 to −0.07) and duration (question 6, β = −0.36, 95% CI −0.57 to −0.15). It was not significantly associated with fatigue (question 1, β = 0.01, 95% CI −0.20 to −0.23) (Supplementary Tables S3-S7).

In multivariable analysis, axial disease was not associated with fatigue (β = 0.11, 95% CI −0.13 to 0.35), peripheral joint pain (β = 0.07, 95% CI −0.16 to 0.29), entheseal pain (β = 0.08, 95% CI −0.15 to 0.31), EMS severity (β = −0.05, 95% CI −0.29 to 0.19) or duration (β = −0.12, 95% CI −0.36 to 0.11) (Supplementary Tables S3-S7).

DISCUSSION

In our study, we did not observe significant differences in the longitudinal trends of BASDAI or its back pain subscore between patients with axial and peripheral PsA. In the whole cohort, BASDAI was significantly associated with peripheral joint disease activity, PASI score, age, and sex, but not with the presence of axial disease. Similarly, the BASDAI back pain subscore was influenced by the same variables and showed a modest association with axial disease. When stratified by axial and peripheral subgroups, peripheral joint involvement, age, and sex continued to be consistent drivers of BASDAI and back pain scores. Notably, PASI was associated with both BASDAI and back pain subscore in the peripheral group only. Axial disease was not associated with other individual BASDAI components on multivariable analysis.

We did not find significant differences in the trajectories of BASDAI and BASDAI back pain scores between axial and peripheral disease subgroups in our study when assessed longitudinally after adjusting for the disease duration. These findings are in line with prior work by Taylor and Harrison [14], who assessed BASDAI in 133 patients with PsA classified clinically into axial or peripheral subtypes. Their study found that BASDAI correlated with patient-perceived disease activity, independent of axial involvement. Subscores analysed in 86 patients revealed higher BASDAI back pain scores in those with axial symptoms, though the study was limited by small size, cross-sectional design, and use of clinical criteria to define axial disease [14].

Similar results were observed by Reddy et al [15], who evaluated 119 PsA patients from the Psoriatic Arthritis Research Consortium. Axial disease was defined by Assessment of Spondyloarthritis International Society criteria and/or imaging. The mean BASDAI and change in BASDAI over follow-up were comparable between axial and peripheral subgroups, despite slightly higher back pain scores in the axial group at baseline [15]. A cross-sectional study by Fernández-Sueiro et al [23] also reported no significant differentiation by BASDAI between axial and peripheral PsA. While these studies consistently suggest that BASDAI back pain scores may be higher in axPsA, limitations such as small sample sizes, absence of longitudinal data, and heterogeneity in axial disease definitions limit the generalisability of their findings.

In contrast, Baraliakos et al [24] conducted a pooled analysis of the two phase three trials of guselkumab in PsA (DISCOVER-1 and -2 trials) in 436 patients and reported a statistically significant association between axial disease and higher BASDAI scores over 52 weeks. Axial disease in their study was defined based on physician discretion, supported by imaging features that might have been a reason for the observed findings, with imaging more likely performed in patients with back pain. Although axial disease was associated with a higher BASDAI estimate (β = 0.5) over 52 weeks of follow-up, the baseline BASDAI demonstrated poor discriminatory ability (AUC = 0.57; 95% CI 0.50-0.63) between axial and peripheral disease, likely reflecting issues with case definition and imaging variability [24].

Importantly, most prior studies did not adjust for relevant confounders that may influence BASDAI. In our multivariable model, axial disease did not significantly impact BASDAI overall. A small but significant association was observed with

BASDAI back pain (β = 0.30), but both BASDAI and its back pain subscore were more strongly associated with SJC, TJC, dactylitis, enthesitis, PASI, age, and sex. NSAID use was also associated with both scores, likely reflecting confounding by indication. Upon subgroup analysis of axial and peripheral groups, the same variables were consistently associated with BASDAI and back pain scores, with PASI losing significance in the axial group but showing similar effect estimates. Male sex was consistently associated with lower BASDAI and back pain scores across all models, consistent with prior observations of sex-based differences in symptom reporting and pain perception in axPsA and axSpA cohorts [25–27].

Fatigue was not significantly associated with axial disease. While peripheral joint pain, enthesitis, and EMS (intensity and duration) were negatively associated with axial disease in the univariable analysis, these associations did not persist in multivariable models. Other cross-sectional studies have similarly reported a lack of differentiation between axial and peripheral subgroups based on BASDAI component analysis [14,15].

Overall, this points to the poor reliability of BASDAI in both identifying axial disease and assessing axial activity in PsA. This limitation is particularly relevant when evaluating the differential impact of therapies on axial manifestations in PsA, given that BASDAI continues to be used as a key endpoint in clinical trials to assess axPsA. Post hoc analyses of clinical trials have often applied screening and outcome criteria developed for axSpA, which may not be appropriate for PsA [16]. For example, in the post hoc analyses of the phase three trials of Ustekinumab in PsA (PSUMMIT 1 and 2 trials) of ustekinumab, physician-reported spondylitis was used to identify patients with axial disease, while BASDAI back pain, modified BASDAI, and ASDAS were applied to assess treatment response [16,28]. Similarly, post hoc analyses of the DISCOVER 1 and 2 trials of guselkumab relied on radiographic evidence to classify patients, but outcomes were again measured using modified BASDAI and ASDAS [29]. A prospective phase four randomised controlled trial of guselkumab in axial PsA (STAR trial) is currently underway, evaluating guselkumab in patients with PsA and magnetic resonance imaging (MRI)-confirmed axial involvement. The primary endpoint of the study is BASDAI; however, as all participants also have active peripheral arthritis, this substantially challenges the validity of this outcome, particularly in light of the data presented here. Importantly, MRI inflammation scores are included as key secondary outcomes, providing an objective measure of axial disease activity [30].

Thus, reliance on BASDAI to identify and assess activity in axPsA in clinical trials is problematic, as it is strongly influenced by peripheral joint disease and degenerative/mechanical changes in the axial skeleton. In the view of the authors, MRI-based inflammation endpoints should instead serve as the primary means of assessing axial disease activity in axPsA, as they provide a direct and objective measure of axial involvement. Further data are needed to determine to what extent symptoms reflecting disease burden in the axial domain, such as back pain and the intensity and duration of morning stiffness, can be appropriately used in patients with MRI-confirmed active axPsA. This is particularly important for future trial design and interpretation of treatment efficacy in axPsA.

A key strength of our study is the use of a large, prospectively collected database comprising well-characterised patients with PsA who were followed longitudinally. The analyses incorporated repeated BASDAI measurements over time and were adjusted for potential confounders, enhancing the robustness of the observed associations. The main limitation lies in the lack of a universally accepted definition for axial disease in PsA with lack of MRI imaging. In our study, axial disease was defined radiographically which implies the presence of objective signs of structural damage in the axial skeleton attributable to PsA. While we acknowledge that early cases may have been missed using this approach, the observed prevalence of approximately 42% aligns with previously reported estimates. Furthermore, the use of repeated radiographs for all patients in the cohort likely minimised the chance of misclassification.

In conclusion, BASDAI and back pain scores appear to be a useful tool for monitoring disease activity in patients. However, it is not reliable in assessing axial activity. Both scores tend to be impacted by inflammatory activity in other PsA domains as well as by age and sex. This is particularly relevant while interpreting clinical trials using BASDAI to evaluate treatment efficacy in patients with axPsA.

Competing interests

Denis Poddubnyy reports a relationship with AbbVie Inc that includes consulting or advisory and funding grants, a relationship with Eli Lilly and Company that includes consulting or advisory and funding grants, a relationship with Janssen Pharmaceuticals Inc that includes consulting or advisory and funding grants, a relationship with Novartis Pharmaceuticals Corporation that includes consulting or advisory and funding grants, a relationship with Pfizer Inc that includes consulting or advisory and funding grants, a relationship with UCB that includes consulting or advisory and funding grants, a relationship with BIOCAD that includes consulting or advisory, a relationship with Bristol Myers Squibb Co that includes consulting or advisory, and a relationship with MoonLake Immunotherapeutics Ltd that includes consulting or advisory. Dafna Gladman reports a relationship with AbbVie Inc that includes consulting or advisory and funding grants, a relationship with Amgen Inc that includes consulting or advisory and funding grants, a relationship with AstraZeneca Canada Inc that includes consulting or advisory and funding grants, a relationship with Bristol Myers Squibb Co that includes consulting or advisory and funding grants, a relationship with Eli Lilly and Company that includes consulting or advisory and funding grants, a relationship with Fresenius Kabi USA LLC that includes consulting or advisory and funding grants, a relationship with Janssen Pharmaceuticals Inc that includes consulting or advisory and funding grants, a relationship with Novartis Pharmaceuticals Corporation that includes consulting or advisory and funding grants, and a relationship with UCB that includes consulting or advisory and funding grants. Vinod Chandran reports a relationship with AbbVie Inc that includes consulting or advisory and funding grants, a relationship with Amgen Inc that includes consulting or advisory and funding grants, a relationship with Eli Lilly and Company that includes consulting or advisory and funding grants, a relationship with Bristol-Myers Squibb Company that includes consulting or advisory, a relationship with Fresenius Kabi USA LLC that includes consulting or advisory, a relationship with Janssen Pharmaceuticals Inc that includes consulting or advisory, a relationship with Novartis Pharmaceuticals Corporation that includes consulting or advisory, a relationship with Pfizer Inc that includes consulting or advisory, a relationship with UCB that includes consulting or advisory. Dr Vinod Chandran's spouse is an employee of AstraZeneca. PM, VCA, FK, and SG have no conflicts of interest.

CRediT authorship contribution statement

Pankti Mehta: Writing – review & editing, Writing – original draft, Visualization, Methodology, Data curation, Conceptualization. **Fadi Kharouf:** Writing – review & editing, Methodology, Data curation. **Virginia Carrizo Abarza:** Writing – review & editing, Methodology, Data curation. **Shangyi Gao:** Writing – review & editing, Methodology, Formal analysis. **Dafna D Gladman:** Writing – review & editing, Methodology, Data curation. **Vinod Chandran:** Writing – review & editing, Methodology, Data curation. **Denis Poddubnyy:** Writing – review & editing, Visualization, Supervision, Methodology, Data curation, Conceptualization.

Acknowledgements

The Gladman Krembil Psoriatic Arthritis Research Program is funded by a grant from the Krembil Foundation and the Schroeder Arthritis Institute.

DP and PM contributed to the conceptualisation. PM, DP, SG, FK and VCA contributed to the study design and acquisition, analysis or interpretation of data. All authors contributed to data acquisition, analysis and interpretation. All authors contributed to manuscript preparation or revision and approved the final version prior to publication. All authors agreed to be accountable for all aspects of the work

Funding

No specific funding was received for this work.

Patient consent for publication

All patients provided informed consent at enrollment.

Ethics approval

We obtained ethics approval from the University Health Network Research Ethics Board (08-0630).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

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.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.09.016.

Orcid

Pankti Mehta: http://orcid.org/0000-0001-9134-0999
Denis Poddubnyy: http://orcid.org/0000-0002-4537-6015

REFERENCES

[1] Ritchlin CT, Colbert RA, Gladman DD. Psoriatic arthritis. N Engl J Med 2017;376(10):957–70.
[2] Poddubnyy D, Jadon DR, Van den Bosch F, Mease PJ, Gladman DD. Axial involvement in psoriatic arthritis: an update for rheumatologists. Semin Arthritis Rheum 2021;51(4):880–7.
[3] Helliwell PS, Hickling P, Wright V. Do the radiological changes of classic ankylosing spondylitis differ from the changes found in the spondylitis associated with inflammatory bowel disease, psoriasis, and reactive arthritis? Ann Rheum Dis 1998 Mar;57(3):135–40.
[4] Helliwell PS. Axial disease in psoriatic arthritis. Rheumatology (Oxford) 2020 Jun 1;59(6):1193–5.
[5] Feld J, Chandran V, Haroon N, Inman R, Gladman D. Axial disease in psoriatic arthritis and ankylosing spondylitis: a critical comparison. Nat Rev Rheumatol 2018;14(6):363–71.
[6] Gladman DD. Axial psoriatic arthritis. Curr Rheumatol Rep 2021;23(6):35.
[7] Michelena X, López-Medina C, Erra A, Juanola X, Font-Ugalde P, Collantes E, et al. Characterising the axial phenotype of psoriatic arthritis: a study comparing axial psoriatic arthritis and ankylosing spondylitis with psoriasis from the REGISPONSER registry. RMD Open 2022;8(2):e002513.
[8] Queiro R, Sarasqueta C, Belzunegui J, Gonzalez C, Figueroa M, Torre-Alonso JC. Psoriatic spondyloarthropathy: a comparative study between HLA-B27 positive and HLA-B27 negative disease. Semin Arthritis Rheum 2002 Jun 1;31(6):413–8.
[9] Jadon DR, Sengupta R, Nightingale A, Lindsay M, Korendowych E, Robinson G, et al. Axial disease in psoriatic arthritis study: defining the clinical and radiographic phenotype of psoriatic spondyloarthritis. Ann Rheum Dis 2017 Apr 1;76(4):701–7.
[10] Poddubnyy D, Baraliakos X, Van den Bosch F, Braun J, Coates LC, Chandran V, et al. Axial involvement in psoriatic arthritis cohort (AXIS): the protocol of a joint project of the Assessment of Spondyloarthritis International Society (ASAS) and the group for research and assessment of psoriasis and psoriatic arthritis (GRAPPA). Ther Adv Musculoskelet Dis 2021 Dec 18;13 1759720X211057975.
[11] Helliwell PS. Axial involvement in psoriatic arthritis: is it unique? Rheumatology. (Oxford). 2024 Dec 1;63(suppl 2):ii15–9.
[12] Eder L, Chandran V, Shen H, Cook RJ, Gladman DD. Is ASDAS better than BASDAI as a measure of disease activity in axial psoriatic arthritis? Ann Rheum Dis 2010 Dec 1;69(12):2160–4.
[13] Heuft-Dorenbosch L, van Tubergen A, Spoorenberg A, Landewé R, Dougados M, Mielants H, et al. The influence of peripheral arthritis on disease activity in ankylosing spondylitis patients as measured with the Bath Ankylosing Spondylitis Disease Activity Index. Arthritis Rheum 2004 Apr 15;51(2):154–9.
[14] Taylor WJ, Harrison AA. Could the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) be a valid measure of disease activity in patients with psoriatic arthritis? Arthritis Rheum 2004 Jun 15;51(3):311–5.
[15] Reddy SM, Xue K, Husni ME, Scher JU, Stephens-Shields AJ, Goel N, et al. Use of the Bath Ankylosing Spondylitis Disease Activity Index in patients with psoriatic arthritis with and without axial disease. J Rheumatol 2024;51 (2):139–43.
[16] Braun J, Landewé RB. No efficacy of anti-IL-23 therapy for axial spondyloarthritis in randomised controlled trials but in post-hoc analyses of psoriatic arthritis-related 'physician-reported spondylitis'? Ann Rheum Dis 2022;81 (4):466–8.
[17] Gladman DD, Chandran V. Observational cohort studies: lessons learnt from the University of Toronto psoriatic arthritis program. Rheumatology (Oxford) 2011 Jan;50(1):25–31.
[18] Taylor W, Gladman D, Helliwell P, Marchesoni A, Mease P, Mielants H, et al. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis Rheum 2006;54(8):2665–73.
[19] Schoels M, Aletaha D, Funovits J, Kavanaugh A, Baker D, Smolen JS. Application of the DAREA/DAPSA score for assessment of disease activity in psoriatic arthritis. Ann Rheum Dis 2010 Aug;69(8):1441–7.
[20] Blackmore MG, Gladman DD, Husted J, Long JA, Farewell VT. Measuring health status in psoriatic arthritis: the Health Assessment Questionnaire and its modification. J Rheumatol 1995;22(5):886–93.
[21] Husted JA, Gladman DD, Farewell VT, Long JA, Cook RJ. Validating the SF-36 health survey questionnaire in patients with psoriatic arthritis. J Rheumatol 1997;24(3):511–7.
[22] van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984 Apr 1;27(4):361–8.
[23] Fernández-Sueiro JL, Willisch A, Pértega-Díaz S, Tasende JAP, Fernández-López JC, Villar NO, et al. Validity of the bath ankylosing spondylitis disease activity index for the evaluation of disease activity in axial psoriatic arthritis. Arthritis Care Res (Hoboken) 2010 Jan 15;62(1):78–85.

[24] Baraliakos X, Gladman DD, Chakravarty SD, Gong C, Shawi M, Rampakakis E, et al. BASDAI versus ASDAS in evaluating axial involvement in patients with psoriatic arthritis: a pooled analysis of two phase 3 studies. Rheumatol Adv Pract 2024 Mar 14;8(2):rkae058.
[25] Mease PJ, McLean RR, Dube B, Liu M, Rebello S, Glynn M, et al. Comparison of men and women with axial spondyloarthritis in the US-based corrona psoriatic arthritis/spondyloarthritis registry. J Rheumatol 2021 Oct 1;48(10):1528–36.
[26] Coates LC, van der Horst-Bruinsma IE, Lubrano E, Beaver S, Drane E, Ufuktepe B, et al. Sex-specific differences in patients with psoriatic arthritis: a systematic review. J Rheumatol 2023 Apr;50(4):488–96.
[27] Wright GC, Kaine J, Deodhar A. Understanding differences between men and women with axial spondyloarthritis. Semin Arthritis Rheum 2020 Aug;50(4):687–94.
[28] Helliwell PS, Gladman DD, Chakravarty SD, Kafka S, Karyekar CS, You Y, et al. Effects of ustekinumab on spondylitis-associated endpoints in TNFi-naïve active psoriatic arthritis patients with physician-reported spondylitis: pooled results from two phase 3, randomised, controlled trials. RMD Open 2020 Feb 10;6(1):e001149.
[29] Mease PJ, Helliwell PS, Gladman DD, Poddubnyy D, Baraliakos X, Chakravarty SD, et al. Efficacy of guselkumab on axial involvement in patients with active psoriatic arthritis and sacroiliitis: a post-hoc analysis of the phase 3 DISCOVER-1 and DISCOVER-2 studies. Lancet Rheumatol 2021 Oct;3(10):e715–23.
[30] Gladman DD, Mease PJ, Bird P, Soriano ER, Chakravarty SD, Shawi M, et al. Efficacy and safety of guselkumab in biologic-naïve patients with active axial psoriatic arthritis: study protocol for STAR, a phase 4, randomized, double-blinded, placebo-controlled trial. Trials 2022 Sep 5;23(1):743.

Ann Rheum Dis 85 (2026) 285–296

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Psoriatic arthritis

Entheseal tissue signature in response to IL-17A inhibition in psoriatic arthritis: results from the EBIO entheseal biopsy study

Maria Gabriella Raimondo[1,2], Hashem Mohammadian[1,2], Simon Rauber[1,2], Stefano Alivernini[3,4,5], Vladyslav Fedorchenko[1], Aleix Rius Rigau[1,2], Mario Raphael Angeli[1,2], Filippo Fagni[1,2], Giulia Corte[1,2], Koray Tascilar[1,2], Hannah Labinsky[1,2], Alina M. Ramming[1,2], Frank Roemer[6,7], Juergen Rech[1,2], Lars Braeuer[8], Maria Antonietta D'Agostino[3,4,5], Georg Schett[1,2], Milena Pachowsky[1,2,9], Arnd Kleyer[1,2,10], Andreas Ramming[1,2,*]

[1] *Department of Medicine 3 – Rheumatology and Immunology, Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg and Universitaetsklinikum Erlangen, Erlangen, Germany*
[2] *Deutsches Zentrum Immuntherapie (DZI), Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg and Universitaetsklinikum Erlangen, Erlangen, Germany*
[3] *Immunology Research Core Facility, Gemelli Science and Technology Park, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*
[4] *Division of Rheumatology and Clinical Immunology- Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*
[5] *Università Cattolica del Sacro Cuore, Rome, Italy*
[6] *Institute of Radiology, Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg and Universitätsklinikum Erlangen, Erlangen, Germany*
[7] *Department of Radiology, Chobanian & Avedisian School of Medicine, Boston University, Boston, MA, USA*
[8] *Institute of Functional and Clinical Anatomy, Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Erlangen, Germany*
[9] *Department of Orthopedic and Trauma Surgery, Waldkrankenhaus St. Marien, Erlangen, Germany*
[10] *Department of Rheumatology and Clinical Immunology, Charité – Universtitaetsmedizin Berlin, Berlin, Germany*

ARTICLE INFO

Article history:
Received 25 June 2025
Received in revised form 17 August 2025
Accepted 31 August 2025

ABSTRACT

Objectives: Enthesitis, a hallmark of psoriatic arthritis (PsA), reflects the interplay between mechanical stress and immune dysregulation at tendon-bone interfaces. This study investigates the cellular and molecular responses in entheseal tissues following interleukin (IL)-17A inhibition in patients with active PsA.

Methods: In this prospective, interventional phase 4 trial, we enrolled 10 patients with enthesitis of the lateral epicondyle, performed entheseal biopsies, and analysed tissues by imaging mass cytometry (IMC) and spatial transcriptomics before and after treatment.

Results: Following 24 weeks of IL-17A inhibition, 9/10 patients of the cohort clinically responded to treatment as assessed by Disease Activity in Psoriatic Arthritis (DAPSA), Spondyloarthritis research consortium of canada (SPARCC) score, and power Doppler sonography. IMC analysis showed significant reductions of enthesitis-related immune cell populations, in particular IL-17-producing CD4, CD8, $CD4^{neg}/CD8^{neg}$ T cells, granulocytes, and innate lymphoid cells

*Correspondence to Dr Andreas Ramming.
E-mail address: andreas.ramming@uk-erlangen.de (A. Ramming).
Handling editor Josef S. Smolen.
Arnd Kleyer and Andreas Ramming are senior authors.

https://doi.org/10.1016/j.ard.2025.08.032

type 3. Spatial transcriptomics revealed that CD200 + DKK3 + fibroblasts and innate lymphoid cells type 2 expanded and formed an anti-inflammatory niche upon treatment. Notably, IL-17A inhibition led to decreased osteoblast differentiation markers, suggesting a potential mechanism to inhibit pathological bone formation.

Conclusions: These findings underline the pivotal role of IL-17A in enthesitis, showing that IL-17A inhibition profoundly modulates the tissue microenvironment of entheses in PsA.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Enthesitis is a key feature of psoriatic arthritis (PsA); however, only occasional reports have addressed the cellular composition of active enthesitis. Existing data are primarily derived from animal models, cadaveric studies, and surgical samples from patients without inflammatory diseases. Only 1 study to date has examined a limited number of patients with spondyloarthritis, without providing a comprehensive characterisation of the cellular composition. As a result, the pathogenesis of enthesitis remains poorly understood.

WHAT THIS STUDY ADDS

- This is the first clinical study to use a validated, minimally invasive, standardised entheseal biopsy approach to investigate the tissue signature of active enthesitis in patients with PsA. It also enables the monitoring of tissue-specific cellular changes in response to interleukin (IL)-17A blockade.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- This study strengthens the understanding of enthesitis as a core feature in PsA and highlights IL-17A as a key driver of local inflammation. It supports IL-17A blockade as a targeted strategy to modulate the entheseal microenvironment, with potential implications for precision therapy in PsA and related conditions.

INTRODUCTION

Enthesitis is characterised by inflammation of the key musculoskeletal structures that connect tendons and ligaments with bone [1,2]. This process is increasingly recognised as a potential primary event in the pathogenesis of psoriatic disease and related conditions such as spondyloarthritis [3]. Patients with psoriatic arthritis (PsA) exhibit high susceptibility to mechanical stress, which may be influenced by genetic factors, leading to pathological inflammatory responses even after physiological mechanical loading [3,4]. Despite its clinical significance, the intricate molecular and cellular processes underlying enthesitis remain poorly understood. Current insights are mostly derived from animal models, cadaveric studies, or surgical specimens taken from patients without inflammatory conditions, with the exception of 1 cross-sectional study on biopsies at the plantar fascia and patellar tendon insertion sites of patients with spondyloarthritis [4–7]. No longitudinal studies assessing entheseal tissues in conjunction with immune interventions have been done to date.

The importance of enthesitis in PsA is underscored by its incorporation into the core requirements of the Classification Criteria for Psoriatic Arthritis (CASPAR) [8]. While existing therapies for psoriatic disease, such as interleukin (IL)-17 inhibitors, have shown efficacy in alleviating the clinical symptoms of enthesitis [9–12], the specific cellular sources of inflammatory cytokines and the consequent changes following IL-17 targeting at the cellular level and the entheseal microenvironment are still unclear. Moreover, enthesitis precedes new bone formation, resulting in bony spurs that contribute to irreversible structural changes and functional impairment in PsA [13].

Entheses are considered as specialised ‘organs’ due to their unique microenvironment, which facilitates the presence and activity of resident mesenchymal cells (MSCs) and specific immune cell populations, such as $\gamma\delta$ T cells and type 3 innate lymphoid cells (ILC3s) [2,6,7]. Despite indications that these cells may be involved in initiating inflammatory processes in the context of enthesitis, little is known about their exact roles and how they function within this setting in human disease.

In this study, we present the findings from the Entheseal BIOpsy Study (EBIO), a pioneering investigation that examines the entheseal tissue signature in response to IL-17A blockade in patients with active PsA. Through the implementation of a refined methodological approach, including ultrasound-assisted tissue collection, we unveil the interplay between the immune microenvironment and enthesitis pathology in this study.

METHODS

Study design and participants

This trial has been designed as investigator-initiated prospective, open-label, single-arm interventional study with a treatment phase with the anti-IL17A monoclonal antibody secukinumab (Novartis) for a period of 6 months. The trial was conducted as monocentric study at the Department of Internal Medicine 3 of the Universitätsklinikum Erlangen (Erlangen, Germany). Sample size was based on the exploratory nature of the study, and a sample size of 10 patients was chosen for practicability reasons. After the screening visit (week 2), study visits were done at baseline (week 0), 12 weeks follow-up, and 24 weeks follow-up (= end of treatment). Safety assessment across the study was performed at the end of the study (week 28). The maximum total length of the study was 7 months (Fig 1A, Supplementary Fig S1A). Secukinumab was provided by Novartis for the duration of the trial from baseline to the end of treatment as a subcutaneous injection with a dosage of 300 mg at weeks 0, 1, 2, 3, then every 4 weeks, according to the treatment dose regimen for psoriasis and PsA. Entheseal biopsy of the common extensor tendon enthesis was performed at baseline (week 0), before treatment initiation, and at the end of treatment (week 24). Ultrasound assessment was performed at screening (week −2) and at the end of the treatment (week 24).

Key inclusion criteria comprised (i) diagnosis of PsA according to the CASPAR classification criteria, (ii) a patients’ age over 18 years at the time of consent, (iii) active enthesitis of the common extensor tendons’ enthesis based tenderness and positive power Doppler (PD) signal in the ultrasound with symptom duration for at least 6 weeks prior inclusion, and (iv) failure, intolerance or contraindication to conventional synthetic disease-modifying antirheumatic drug (csDMARD) therapy, such as methotrexate, leflunomide and sulfasalazine. Patients also had to be naive for biologic or targeted synthetic. All inclusion and exclusion criteria are reported in the Supplementary Table S1.

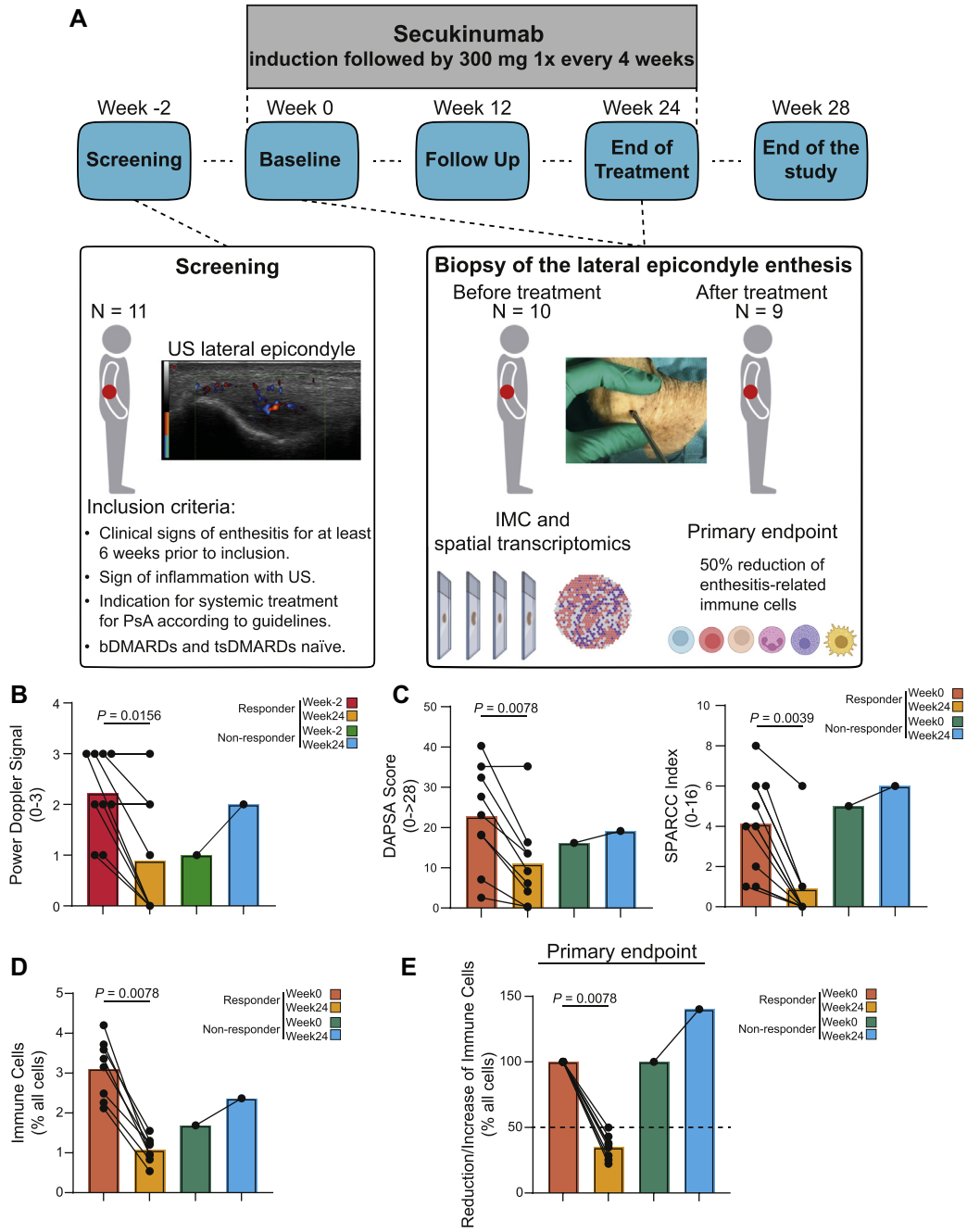


Figure 1. Study design and primary endpoint. (A) Graphical representation of the study design. At the screening visit (week −2), ultrasound (US) of the lateral epicondyle was performed to assess enthesitis in 11 participants (1 of whom was a screening failure). Minimally invasive, US-guided biopsies of the lateral epicondyle enthesis were then conducted at baseline (week 0) and at the end-of-treatment visit (week 24) for further analysis, including imaging mass cytometry (IMC) and spatial transcriptomics. Biopsies were obtained from 10 patients before treatment initiation and from 9 patients after 12 weeks of therapy. During the study, participants received secukinumab (anti-IL-17A) at a dose of 300 mg per month. The total study duration for each patient was 28 weeks (Created in BioRender. Ramming, A. (2025) https://BioRender.com/icriz58). (B) Power Doppler signal assessed by ultrasound evaluation of the lateral epicondyle at the screening visit (week −2) and at the end-of-treatment visit (week 24), distinguishing between responder (N = 9) and nonresponder patients (N = 1). Individual values represented in a before-and-after plot. Paired nonparametric Wilcoxon test was performed, and $P < .05$ was considered significant. (C) Disease activity in psoriatic arthritis (DAPSA) score and Spondyloarthritis Research Consortium of Canada (SPARCC) score at baseline visit (week 0) and at the end-of-treatment visit (week 24), distinguishing between responder (N = 9) and nonresponder patients (N = 1). Individual values represented in a before-and-after plot. Nonparametric Wilcoxon test was performed, and $P < .05$ was considered significant. (D) Immune cell quantification based on imaging mass cytometry analysis (following the gating strategy described in the Supplementary Fig S1A) at baseline visit (week 0) and at the end-of-treatment visit (week 24), distinguishing between responder (N = 8) and nonresponder patients (N = 1). Individual values represented in a before-and-after plot. Nonparametric Wilcoxon test was performed, and $P < .05$ was considered significant. (E) Primary endpoint: reduction or increase of the immune cells for each individual patient at the end-of-treatment visit (week 24) normalised towards the corresponding baseline visit (week 0), distinguishing between responder (N = 8) and nonresponder patients (N = 1). Individual values represented in a before-and-after plot. A 2-sided nonparametric Wilcoxon test was performed and $P < .05$ was considered significant. Dashed bar identifies a 50% reduction. bDMARD, biologic disease-modifying antirheumatic drug; IL, interleukin; PsA, psoriatic arthritis; tsDMARD, targeted synthetic disease-modifying antirheumatic drug.

The first patient was recruited on the May 26, 2020. The end of the study was defined as the last visit of the last patient, which was on March 29, 2022.

Study protocols were developed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Ethical approval from the local institutional review board (University of Erlangen-Nürnberg, #30_19B) and written informed consent were obtained from all participants. Applicable institutional review board and independent ethics committee approval were obtained before study initiation, and all patients provided written informed consent before participation. The trial is registered with ClinicalTrials.gov (NCT04887597).

Procedures

Entheseal biopsy of the extensor common tendon's enthesis was performed as previously described [14]. Briefly, biopsies were done by Blakesley forceps in 60° flexed position after identifying the lateral epicondyle, the olecranon, and the radial head by ultrasound. In patients with PsA, 2 mL of 1% mepivacaine was injected before biopsy, followed by skin incision. The instrument was inserted until bone contact, then slightly withdrawn to obtain the biopsy (5 mm) from the extensor tendon insertions of the lateral epicondyle. The skin incision was closed by 2 stitches with nonabsorbable suture material after biopsy.

Entheseal tissues were either prepared for single-cell suspension for single-cell RNA sequencing (scRNA-seq) (Supplementary Materials) or fixed in phosphate buffered saline containing 4 % (w/v) formaldehyde for 16-24 hours before embedding and sectioning. Tissues were dehydrated, infiltrated, and embedded in paraffin, cut into 5 µm thin sections and mounted on standard histological slides. Thin sections were deparaffinised by heating the slides at 65°C for 30 min, washed in HistoClear (National Diagnostics) and rehydrated through a series of 100% (v/v) ethanol, 95% (v/v) ethanol, 80% (v/v) ethanol, 60% (v/v) ethanol, and water. Mayer's haematoxylin solution (Merck) was applied for 10 minutes. Excess haematoxylin was washed off, and the stain was blued by rinsing the slides for 10 minutes in tap water. Eosin counterstain (0.3% (wt/v) Eosin Y (Sigma), 0.01% (v/v) acetic acid) was applied for 3 minutes and excess stain was washed off with deionised water. The sections were again dehydrated in isopropanol and mounted with Roti Mount mounting medium (Carl Roth). Slides were digitised with a NanoZoomer S60 (Hamamatsu), and regions of interest were exported as *.TIFF files. Imaging mass cytometry (IMC) and digital spatial transcriptomics were adopted for deep cellular characterisation of the tissues (Supplementary Material). All key resources are listed in the Supplementary Table S2.

Outcomes

The primary endpoint of the study was defined as the percentage of patients with a reduction of enthesitis-related immune cells by at least 50% in the entheseal tissue after 24 weeks of treatment with secukinumab. Demographic data such as age, gender (male/female), and disease duration were collected (Table 1). The extent of clinical joint disease in PsA (tenderness/swelling) was assessed by the disease activity in psoriatic arthritis (DAPSA) score and disease activity score-28 (DAS-28) based on erythrocyte sedimentation rate and C-reactive protein. Extent of clinical entheseal disease in PsA was assessed by Spondyloarthritis Research Consortium of Canada (SPARCC) score, Leeds enthesitis index (LEI) and Maastricht ankylosing spondylitis enthesitis score (MASES). The extent of clinical skin disease in PsA was assessed by psoriasis area and severity index (PASI). In addition, minimal disease activity status was assessed. The impact of disease and physical function was assessed by the 12-item psoriatic arthritis impact of disease (PSAID-12) and the health assessment questionnaire (HAQ), respectively. In addition, safety data were collected at each visit.

Table 1
Baseline characteristics of the patients with psoriatic arthritis

Patients' characteristics	N = 10
Gender (females), N (%)	4 (40)
Age (y); median ± SD	52.9 ± 9.5
Disease duration (y); mean ± SD	8.6 (13.3)
Autoantibodies	
RF; n (%)	1 (10)
ACPA; n (%)	0 (0)
Previous medications[a]	
csDMARDs; N (%)	9 (90)
Glucocorticoids; N (%)	1 (10)
NSAIDs; N (%)	9 (90)
Concomitant medications[a]	
csDMARDs; n (%)	3 (30)
Glucocorticoid; n (%)	1 (10)
NSAIDs; n (%)	6 (60)

ACPA, anticitrullinated protein antibody; csDMARD, conventional synthetic disease-modifying antirheumatic drug (methotrexate, leflunomide, sulfasalazine); NSAID, nonsteroidal anti-inflammatory drug; RF, rheumatoid factor.
[a] None of the patients received biological or targeted synthetic disease-modifying antirheumatic drugs before or during the study (except for the study medication secukinumab).

Statistical analysis

Statistical analysis of nonsequencing data was performed using Prism 9. Unless otherwise stated, all data are reported as mean and individual values in *before-after* plots. Nonparametric 2-sided paired Wilcoxon test was used where appropriate. *P* values were corrected for multiple testing using the Benjamini-Hochberg method. Linear regression and Pearson's correlation coefficient (r) were used to assess correlations. Differences were considered significant when $P < .05$.

Additional methods are provided in the Supplementary Material.

RESULTS

Patients' characteristics

Patient characteristics are summarised in Table 1. Eleven patients with active PsA and enthesitis of the lateral epicondyle were screened, and 10 patients were enrolled. Four patients were female. At screening, the mean DAPSA score was 22.8 ± 13.3 (mean ± SD), indicating moderate disease activity. On average, patients exhibited 9.6 ± 6.83 tender joints and 2.8 ± 4.24 swollen joints (mean ± SD; Table 2). Their mean entheseal score based on the SPARCC was 4.30 ± 2.75 (mean ± SD; Table 2). Three patients had both axial and peripheral involvement. Of the 10 patients with PsA, 9 failed csDMARDs, while 1 patient was csDMARD naive due to predominant active axial involvement (Table 1).

The study included 5 visits: screening (week −2), baseline (week 0), treatment week 12, end-of-treatment week 24 and end-of-study week 28 (Fig 1A). During screening, inclusion and

Table 2
Clinical disease activity and composite scores of patients with PsA across the study

Outcome	Screening (wk −2)	Baseline (wk 0)	Follow-up (wk 12)	End of treatment (wk 24)	End of study (wk 28)
Swollen joint count (66)	2.8 (4.24)	2.40 (3.63)	1.40 (2.72)	1.10 (2.85)	0.90 (1.66)
Tender joint count (68)	9.6 (6.83)	7.80 (6.55)	2.80 (3.05)	3.00 (6.50)	3.20 (6.03)
Patient global (VAS)	50.7 (28.46)	56.5 (21.92)	27.80 (19.81)	36.50 (27.30)	32.60 (25.59)
Physician global (VAS)	53.8 (28.57)	49.40 (21.76)	16.70 (11.26)	16.10 (21.40)	21.50 (22.84)
Pain (VAS)	48.9 (26.80)	51.00 (28.69)	31.90 (23.60)	37.90 (27.27)	34.90 (25.18)
DAS28-ESR	3.90 (1.16)	3.59 (1.30)	2.61 (1.31)	2.49 (1.00)	2.28 (1.21)
DAS28-CRP	3.75 (1.00)	3.90 (0.95)	2.62 (1.23)	2.35 (0.99)	2.53 (1.16)
DAPSA	22.84 (13.27)	22.07 (12.07)	10.68 (8.81)	11.76 (10.49)	10.96 (10.73)
LEI	2 (1.25)	1.70 (1.06)	1.20 (1.48)	0.80 (1.14)	0.70 (1.25)
MASES	2.80 (2.44)	1.20 (1.62)	1.00 (1.25)	1.00 (1.89)	0.60 (1.07)
SPARCC	4.30 (2.75)	4.20 (2.30)	2.20 (2.25)	1.40 (2.46)	1.00 (1.56)
PASI	8.12 (9.27)	8.38 (9.19)	1.56 (2.30)	3.10 (5.64)	0.62 (0.75)
HAQ-DI	0.83 (0.73)	0.74 (0.73)	0.62 (0.52)	0.84 (0.63)	0.72 (0.65)
PSAID-12	4.26 (2.09)	4.37 (2.05)	3.02 (1.91)	3.87 (2.70)	3.05 (2.24)
MDA[a]	0 (100)	2 (20)	4 (40)	3 (30)	4 (40)

DAPSA, disease activity in psoriatic arthritis; DAS28-CRP, disease activity score-28 based on C-reactive protein; DAS28-ESR, disease activity score-28 based on erythrocyte sedimentation rate; HAQ-DI, health assessment questionnaire disability index; LEI, Leeds enthesitis index; MASES, Maastricht ankylosing spondylitis enthesitis score; MDA, minimal disease activity; PASI, psoriasis area and severity index; PRO, patient report outcome; PSAID-12, 12-item psoriatic arthritis impact of disease; SPARCC, Spondyloarthritis Research Consortium of Canada; VAS, visual analogue scale.
Data are expressed as mean (± SD)
[a] Data are shown as number of patients (%).

exclusion criteria (Supplementary Table S1) were evaluated. Active enthesitis was assessed by ultrasound according to the Outcome Measures in Rheumatology initiative (OMERACT) definition [15] (Supplementary Material). All patients had to show a positive PD signal in the epicondylar entheseal region at screening (Fig 1B). At baseline, all patients underwent a minimally invasive ultrasound-guided biopsy of the epicondylar extensor tendon enthesis [14] before initiating treatment with secukinumab at a dose of 300 mg, following a standard therapeutic regimen [16] (Fig 1A). Throughout the study, no patients discontinued or switched treatments. At the end-of-treatment visit, follow-up biopsies were conducted for 9 of 10 patients. One patient declined the second biopsy, though not experiencing an adverse event after the first biopsy. The patients, however, also completed the study through week 28. All but 1 patient responded positively to treatment. No safety issues related to the biopsies were identified; in particular, there were no cases of prolonged bleeding or neuronal damage. Six months after treatment, PD signals were reduced compared to baseline ($P = .0156$), except for the single nonresponder (Fig. 1B). Clinical assessment using DAPSA and SPARCC scores demonstrated significant reductions after 24 weeks of treatment: DAPSA decreased to a mean ± SD of 11.8 ± 10.5 units ($P = .0078$) and SPARCC to 1.4 ± 2.5 units ($P = .0039$), with the 1 nonresponder showing increased disease activity and entheseal score (Fig 1C). Also, other composite scores related to activity of psoriatic disease in the joints, the entheses and in the skin, such as DAS-28, LEI, MASES, PASI, PSAID-12, and HAQ decreased with IL-17A inhibition (Tables 2 and 3). No serious adverse events were reported: mild adverse events occurred in 5 of 10 patients, including infections (SARS-CoV-2 infection, external otitis, asymptomatic bacteriuria), erythema, mucositis, and gastritis, for which concomitant medications were prescribed. One patient experienced a moderate adverse event consisting in a bone fracture, not related to the treatment with secukinumab. Overall, 60% of patients used nonsteroidal anti-inflammatory drugs (NSAIDs) on demand (Table 1).

IL-17A inhibition changes entheseal immune cell composition

To assess changes in immune cell composition within entheseal tissues before and after IL-17A inhibition, we employed IMC to quantify immune cell populations (Fig. 2A,B). A significant reduction in the enthesitis-related immune cell population was observed in 8 out of 9 patients who underwent both biopsies (Fig 1D), with responder patients exhibiting reductions greater than 50% (Fig 1E), thereby meeting the primary endpoint. Further analysis of immune cell subpopulations, excluding fibroblasts and endothelial cells, identified significant reductions in granulocytes, T cells, and ILC3s posttreatment ($P = .0156$ for all), while the nonresponder exhibited an increase across these populations (Fig 2C). Analysis of T cells indicated a significant decrease in CD4+ T cells and double-negative (CD4−, CD8−) T cells, which include $\gamma\delta$ T cells, among

Table 3
Changes in clinical parameters and composite scores from baseline to end of treatment

Outcome	n	Change from baseline, mean (SD)	*P*
Swollen joint count (66)	10	−1.30 (2.05)	.125
Tender joint count (68)	10	−4.80 (6.71)	.063
Patient global (VAS)	10	−20.00 (24.90)	.049
Physician global (VAS)	10	−33.30 (26.37)	.012
Pain (VAS)	10	−13.10 (21.15)	.053
DAS28-ESR	10	−1.11 (1.28)	.029
DAS28-CRP	10	−1.55 (1.00)	.004
DAPSA	10	−10.31 (8.40)	.014
LEI	10	−0.90 (1.30)	.061
MASES	10	−0.20 (1.89)	.344
SPARCC	10	−2.80 (1.99)	.008
PASI	10	−5.28 (5.33)	.006
HAQ-DI	10	0.10 (0.46)	.344
PSAID12	10	−0.50 (2.14)	.625

DAPSA, disease activity in psoriatic arthritis; DAS28-CRP, disease activity score-28 based on C-reactive protein; DAS28-ESR, disease activity score-28 based on erythrocyte sedimentation rate; HAQ, health assessment questionnaire; LEI, Leeds enthesitis index; MASES, Maastricht ankylosing spondylitis enthesitis score; PASI, psoriasis area and severity index; PSAID-12, 12-item psoriatic arthritis impact of disease; SPARCC, Spondyloarthritis Research Consortium of Canada; VAS, visual analogue scale.

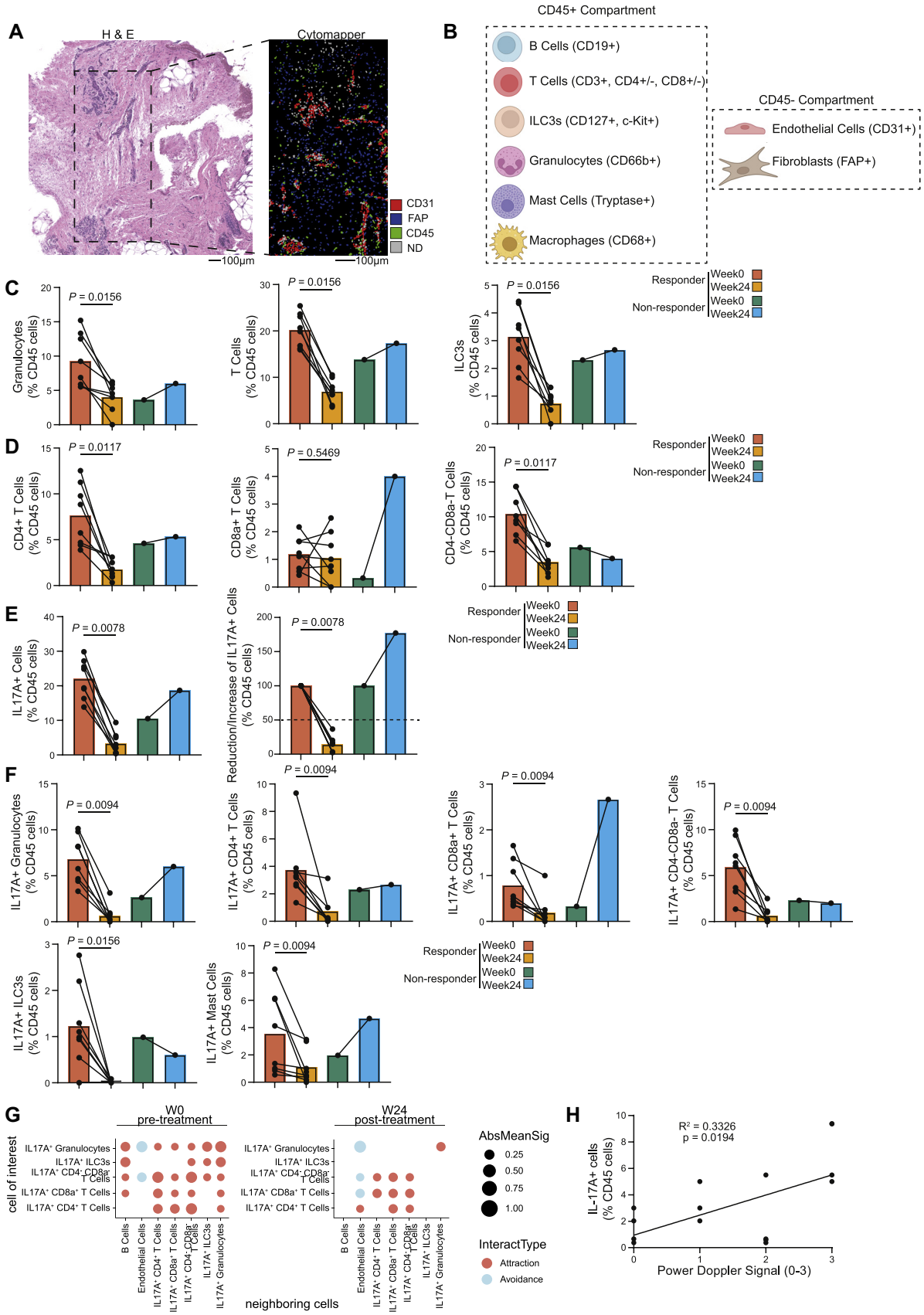


Figure 2. Changes in the entheseal immune cell composition upon IL-17A inhibition. (A) Representative haematoxylin and eosin (H&E) staining of an entheseal biopsy of the lateral epicondyle and of the corresponding cytomapper imaging showing CD45+, CD31+, and FAP+ cells. (B) Schematic representation of immune (CD45+) and nonimmune (CD45-) cells identified in the study, based on the expression of specific positive markers (Created in BioRender. Ramming, A. (2025) https://BioRender.com/icriz58). (C) Quantification of granulocytes, T cells, innate lymphoid cells type 3 (ILC3s) at baseline visit (week 0) and at the end-of-treatment visit (week 24), distinguishing between responder (N = 8) and nonresponder patients (N = 1). Individual values represented in a before-and-after plot. A 2-sided paired Wilcoxon test was performed and corrected for multiple testing using Benjamini-Hochberg (BH) method. $P < .05$ was considered significant. (D) Quantification of T-cell subclusters at baseline visit (week 0) and at the end-of-treatment visit (week 24), distinguishing between responder (N = 8) and nonresponder patients (N = 1). Individual values represented in a before-and-after plot. A 2-sided paired Wilcoxon test was performed and corrected for multiple testing using BH method. $P < .05$ was considered

responder patients ($P = .0177$; Fig 2D). In contrast, CD8+ T cells remained unchanged in responders, whereas the nonresponder demonstrated an increase in CD8+ T cells (Fig 2D). Other immune cell populations, including B cells, macrophages, and mast cells, showed no changes following therapy (Supplementary Fig S1B). Notably, the nonresponder demonstrated an overall increase in immune cells (Fig 1C, Supplementary Fig S1B).

IL-17A production in the identified immune cells was assessed, after confirming that secukinumab treatment did not mask the IL-17A epitope also recognised by the detection antibody (Supplementary Fig S1C). Total IL-17A+ cells significantly decreased by over 50% 6 months after treatment (Fig 2E, Supplementary Fig S1D), with an opposite trend in the nonresponder. Significant reductions in IL-17A+ cells were observed in granulocytes, T cells, ILC3s, and mast cells in responder patients. Interestingly, IL17A+ CD8+ T cells and mast cells exhibited similar trends among responders (Fig 2F, Supplementary Fig S1E). We then examined whether these changes were accompanied by alterations in the spatial distribution of IL-17A-producing cells within the entheseal tissue. Before treatment, these cells exhibited significant mutual attraction, a spatial association that disappeared after treatment (Fig 2G). Interestingly, IL17A+ cells in responder patients significantly correlated with the PD signal of the lateral epicondyle's enthesis at the time of the biopsies ($R^2 = 0.3326$, $P = .0194$) (Fig 2H).

Assessment of the nonimmune cell compartment, such as endothelial cells and FAP+ fibroblasts, showed no significant changes in the responder cohort but increased in the nonresponder patient (Supplementary Fig S1F).

IL-17A inhibition induces an entheseal niche switch

To investigate changes in the mesenchymal and nonmesenchymal compartments pre- and posttreatment, we performed GeoMx spatial transcriptomics on CD45+ and vimentin+ cells (Figs 3A and 4A). After preprocessing, we estimated the cellular composition of the spatial transcriptomic dataset using spatial deconvolution [17]. To generate a reference for the deconvolution, we performed scRNA-seq on inflamed and noninflamed entheseal tissues and integrated the resulting datasets with existing synovial tissue datasets from healthy donors (E-MTAB-14339) and patients with PsA (E-MTAB-11791) [18] (Fig 3A), allowing the definition of cellular identities within the entheseal tissue (Fig 3B). Analysis of the resulting scRNA-seq dataset revealed distinct mesenchymal, myeloid, and lymphoid subclusters (Fig 3C,D).

Comparing pre- and posttreatment, significant shifts were observed from proinflammatory cells, including IL-17A+ T cells and SPP1+ macrophages, to predominantly anti-inflammatory cells posttreatment, including CD200+ DKK3+ MSCs, ILC2s, and conventional dendritic cells type 2 (Fig 4B). Therefore, pro- or anti-inflammatory niches were defined based on the colocalisation of these cell types, classified as inflammatory or resolving according to literature data [19–21]. To further strengthen these results, differential gene expression analysis was performed between the 2 main niches identified and an upregulation of proinflammatory genes (manually selected or from the downstream IL-17 pathway in Kyoto Encyclopedia of Genes and Genomes: hsa04657) was observed in the proinflammatory niche, while an upregulation of anti-inflammatory genes (related to $CD200^+$ $DKK3^+$ fibroblasts and ILC2 proresolving signatures) was described in the anti-inflammatory niche (Supplementary Fig S2A). Moreover, to assess shift in niche frequencies, a beta regression model was applied, and a significant increase in anti-inflammatory niche frequency after treatment was observed (Supplementary Fig S2B), further reinforcing the niche differentiation and transition from a predominantly proinflammatory environment to an anti-inflammatory environment following treatment.

By examining correlations in abundance between cell types across regions of interest followed by hierarchical clustering, we identified clusters of cells with similar spatial distributions, suggesting shared tissue niches. Namely, the posttreatment anti-inflammatory cells, as well as pretreatment proinflammatory cells, colocalised in their distinct respective niches (Fig 4C). Notably, the anti-inflammatory niche also contained CCL21+ lymphatic endothelial cells, which are involved in dendritic cell transmigration, and IBSP+ MSCs, indicative of osteoblast progenitors (Fig 4B,C). Other niches exhibited colocalisation between T regulatory cells and MERK+ macrophages (Fig 4C), although no significant changes in the frequency of these populations were observed (Fig 4B).

To further evaluate the impact of anti-IL17A treatment on osteoblast differentiation, which is responsible for structural changes in the entheses, we conducted differential gene expression comparing pre- and posttherapy samples. This was followed by gene set enrichment analysis using all subterms from gene ontology biological process (GOBP) 'ossification GO:0001503'. Among the ossification-related GOBP terms, 'osteoblast differentiation GO:0001649' was significantly downregulated (adjusted $P = .0203$), indicating a decrease in transcriptional activity associated with osteoblast differentiation after treatment. Other ossification-related processes, including bone mineralisation and ligamentous ossification, did not show significant enrichment (Fig 4D,E). These findings suggest that the treatment may modulate pathways involved in pathological bone formation, particularly by reducing osteoblast-driven activity and therefore inhibiting bony spur formation.

DISCUSSION

Our study presents pivotal insights into the dynamic immunological and cellular landscape of enthesitis in patients with PsA. The efficacy of the IL-17A-neutralising monoclonal antibody secukinumab in improving the disease state and modulating the entheseal immune microenvironment underscores the importance of IL-17A as a critical player in PsA. Our findings illuminate the mechanistic underpinnings of enthesitis and raise

significant. (E) Quantification of IL-17A+ cells and percentage of reduction or increase for each individual patient at baseline visit (week 0) and at the end-of-treatment visit (week 24) (N = 8 responder and N = 1 nonresponder patients). Individual values represented in a before-and-after plot. Paired nonparametric Wilcoxon test was performed, and $P < .05$ was considered significant. (F) Quantification of IL-17A+ fraction within granulocytes, different subset of T cells, ILC3s and mast cells at baseline visit (week 0) and at the end-of-treatment visit (week 24), distinguishing between responder (N = 8) and nonresponder patients (N = 1). Individual values represented in a before-and-after plot. A 2-sided paired Wilcoxon test was performed and corrected for multiple testing using the BH method. $P < .05$ was considered significant. (G) Pairwise interaction test of enthesitis-related IL-17A-producing cells in the imaging mass cytometry dataset before and after treatment (N = 9). (H) Correlation between IL17A+ cells and power Doppler signal in responder patients (N = 8). Linear regression and Pearson's correlation coefficient (r) were used to assess correlation. IL, interleukin.

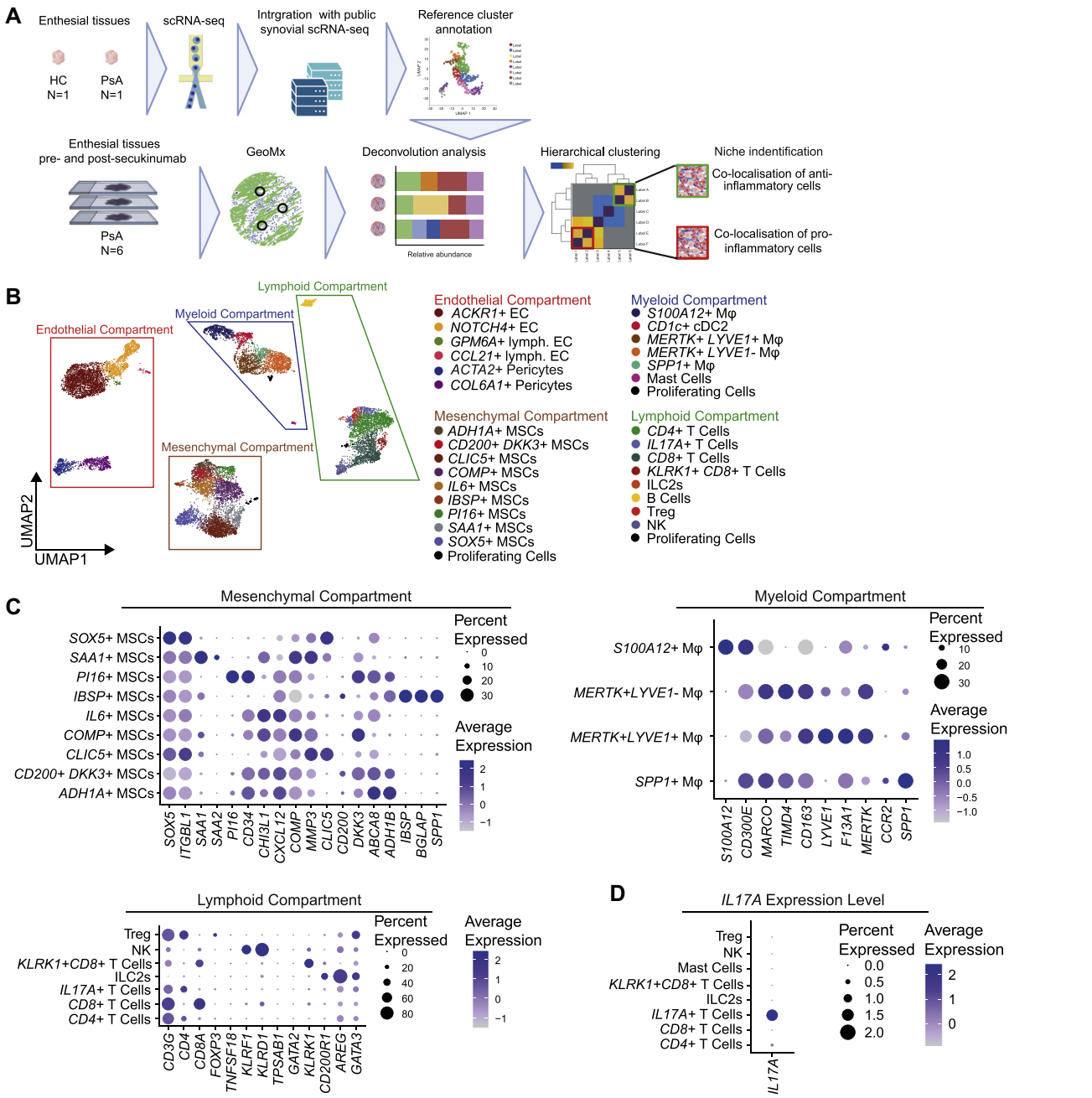


Figure 3. Cluster annotation based on scRNA-seq analysis of entheseal and synovial tissue. (A) Graphical representation of the sequencing workflow (Created in BioRender. Ramming, A. (2025) https://BioRender.com/211vw2w). (B) Uniform manifold approximation and projection (UMAP) plot of cells identified in the scRNA-seq dataset of inflammatory (N = 1) and noninflammatory (N = 1) entheseal tissues integrated with scRNA-seq from synovial tissues of patients with psoriatic arthritis (E-MTAB-11791) (N = 5) and healthy donors (N = 3) (E-MTAB-14339). Detailed annotation with Seurat-identified clusters is shown. (C) Expression of the most relevant marker genes among mesenchymal, myeloid, and lymphoid clusters from the scRNA-seq dataset of entheseal and synovial tissue. (D) Expression of *IL17A* across different cell clusters in the scRNA-seq dataset of entheseal and synovial tissue. IL, interleukin; scRNA-seq, single cell RNA sequencing.

important questions regarding the long-term implications of targeting IL-17A in PsA.

First, the significant reduction in total immune cell populations following IL-17A blockade reinforces the hypothesis that IL-17A drives the inflammatory processes at the enthesis. The observed decrease in granulocytes, T cells, and especially $\gamma\delta$ T cells aligns with previous literature [22–25] implicating these cells in the pathogenesis of both PsA and enthesitis. The unique response profiles observed in the nonresponder patient provide a contrasting perspective that highlights the complexity of immune interactions and individual patient variations, emphasising the necessity for personalised approaches in treatment. In light of our results, it becomes imperative to investigate the cellular and molecular mechanisms that differentiate responders from nonresponders, potentially opening avenues for tailored therapeutic interventions.

The transition from a proinflammatory to an anti-inflammatory niche within the entheseal tissue in conjunction with IL-17 inhibition is particularly noteworthy. Our spatial transcriptomics data indicate that IL-17 inhibition not only reduces proinflammatory cell types but also facilitates the expansion of anti-inflammatory populations, such as CD200+ DKK3+ MSCs and ILC2s [19,21]. This finding suggests a potential cellular reprogramming effect initiated by IL-17 inhibition, hinting at the

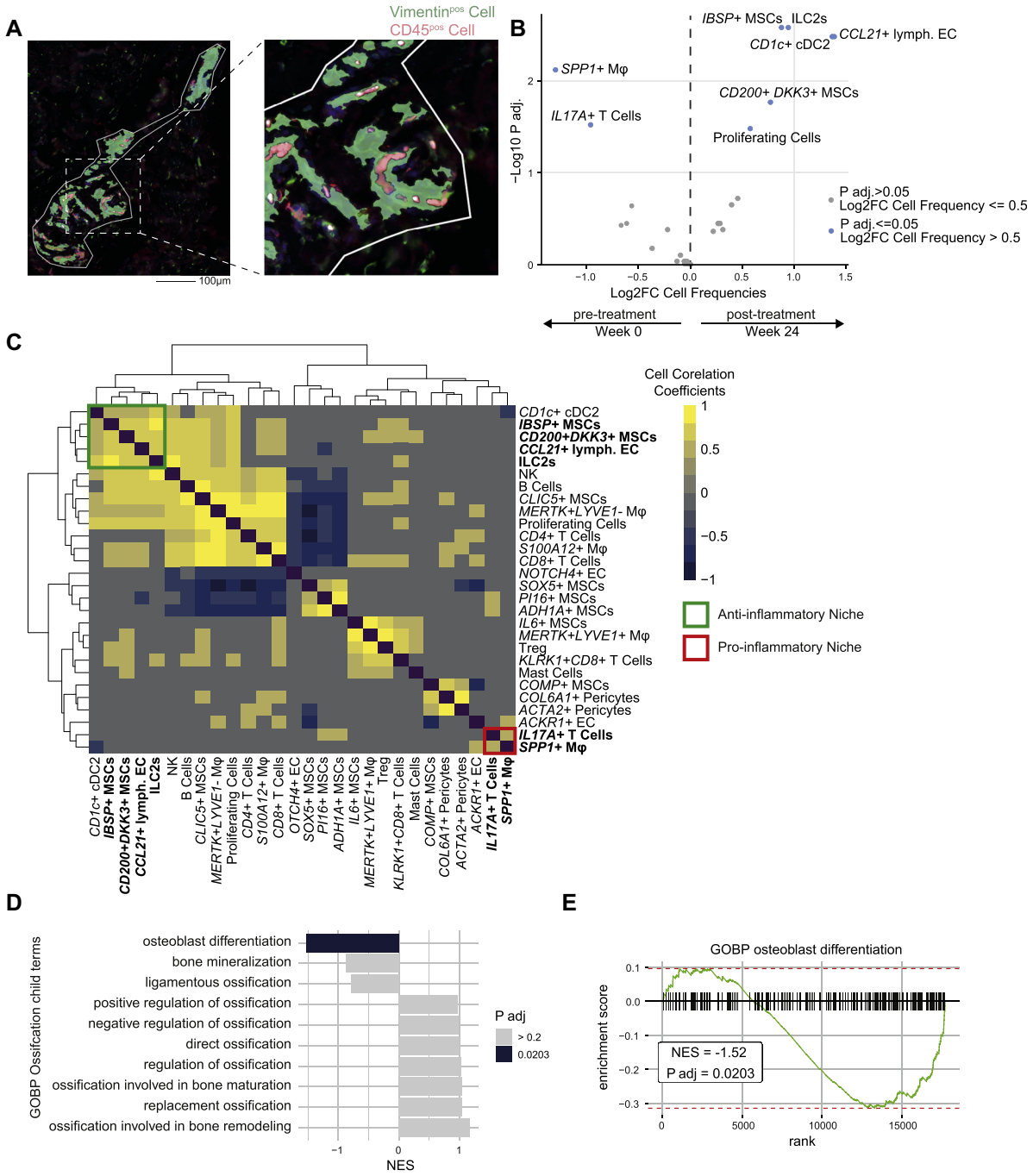


Figure 4. IL-17A inhibition induces entheseal niche switch. (A) Representative image of a region of interest (ROI) selected with GeoMx Digital Spatial Profiler (NanoString) in the entheseal biopsy based on CD45 + (red) and vimentin + (green) cell-masks. (B) Volcano plot showing the result of differential abundance analysis of cluster frequencies between before (week 0) and after (week 24) treatment. Blue dots indicate cell types that are significantly changed (adjusted $P \leq .05$). (C) Heatmap of pairwise correlation coefficients of cell type frequencies across ROIs with hierarchical clustering. Niches of pro- (red) and anti-inflammatory (green) cells are highlighted. In bold are cell types that have significantly increased or decreased within entheseal tissues after treatment. (D) Gene set enrichment analysis (GSEA) of all subterms of 'ossification GO:0001503' from gene ontology biological process (GOBP). Normalised enrichment scores (NES) are shown. Bars represent the direction and magnitude of enrichment. Colour indices adjusted *P*. (E) GSEA enrichment plot for 'osteoblast differentiation GO:0001649' pathway in the ranked list of genes sorted by differential expression comparing pre and post treatment. Green line indicates running sum of enrichment score. Black vertical lines indicate positions of genes from the 'osteoblast differentiation' pathway in the ranked list. cDC2, conventional dendritic cells type 2; M, macrophage; MSC, mesenchymal cell.

possibility of reconstructing a homeostatic microenvironment in chronic inflammatory conditions and protecting from inflammation despite repeated mechanical loading of entheses as a potential trigger for further immune cell infiltration.

A critical methodological aspect of our study is the focus on entheseal biopsies from the elbow region. While this may present a limitation, emphasising accessibility in sampling is crucial. The elbow offers a viable option for assessing functionally relevant entheseal niches over time, while potential alternatives like the Achilles tendon pose more anatomical limitations and challenges [26,27]. Also, by concentrating on a defined anatomical site, we ensured a higher quality of sampling for our analyses. Nevertheless, it is essential to consider how our findings in the elbow might compare to other anatomical regions, such as the lower extremity, where differential responses could manifest and should be investigated in future studies.

Another intriguing aspect of our findings is the potential impact of IL-17A blockade on osteoblast-related activities. Our results indicate a significant downregulation of genes associated with osteoblast differentiation posttreatment, suggesting that targeting IL-17A might mitigate pathological local bone formation at affected sites in PsA. These data align with previous findings in both mice and humans, supporting a pro-osteogenic role for IL-17A through its regulation of osteoblast activity [28–30]. Given the relationship between chronic inflammation, enthesitis, and the development of bony spurs and ankylosis, these insights could inform new strategies targeting structural damage mechanisms in PsA. The delicate balance between inflammation and bone remodelling highlights the need for treatments that not only alleviate symptoms but also address structural changes contributing to functional impairment.

Cellular and molecular assessment of entheses during targeted therapeutic interventions presents methodological challenges due to the specific anatomical locations and the need for precise sampling techniques. However, we observed a moderate correlation between IL-17A+ cells and the PD signal, suggesting that ultrasound may capture only a fraction of the IL-17A-producing pathogenic cells. This finding supports the added value of enthesial biopsy in more accurately characterising the local inflammatory milieu at the enthesis. We have established a high-quality biopsy technique, as detailed in our previous work [14], which includes ultrasound-guided procedures to identify the entheseal region, enhancing tissue sampling accuracy and quality. This approach facilitates the collection of biologically relevant entheseal tissue, enabling a detailed investigation of immune cell composition and inflammatory profiles in response to treatment, which can be used in future studies with other therapeutic modalities such as IL-23, Janus kinase, and tumor necrosis factor-inhibitors, which are used for the treatment of enthesitis. Such an approach allows us to define a specific cellular and molecular pattern associated with different treatments.

While our study provides pivotal insights, we recognise its limitations. The rather small sample size, reflecting the inherent challenges of studying human entheseal tissues, constrains the generalisability of our findings. The impact of previous or concomitant medications, such as cDMARDs and NSAIDs, could not be assessed due to the small size of the cohort. However, the fact that all patients had active enthesitis despite previous exposure to cDMARDs and NSAIDs supports the homogeneity of this population. Further studies with other treatment modalities will be important to validate and expand these observations. In particular, larger-scale studies including a greater number of nonresponder patients are needed to enable a more robust assessment of cellular differences in the enthesial microenvironment in response to anticytokine strategies. Additionally, such longitudinal assessments of immune cell dynamics and entheseal pathology after treatment could clarify the temporal relationship between immune reconstitution and clinical outcomes and thereby provide a deeper insight into the tissue effects of cytokine inhibition.

In conclusion, our findings show the profound molecular and cellular remodelling in the entheseal microenvironment upon IL-17A inhibition in patients with PsA. Understanding the changes in functional cell populations offers a promising avenue for further research into targeted therapy of PsA. Future investigations should continue to dissect the cellular interactions within the enthesis and explore other targeted therapies in order to understand their differential functional impact on the diseased tissue.

Acknowledgements

We thank M. Rose, C. Pfaff, and K-T. Yang for excellent technical assistance. The IMC analysis was supported by the de.NBI Cloud within the German Network for Bioinformatics Infrastructure (de.NBI) and ELIXIR-DE (Forschungszentrum Jülich and W-de.NBI-001, W-de.NBI-004, W-de.NBI-008, W-de.NBI-010, W-de.NBI-013, W-de.NBI-014, W-de.NBI-016, W-de.NBI-022).

Contributors

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, AR (Andreas.ramming@uk-erlangen.de). AK and AR share senior authorship. AR, AK, MP, and JR contributed to the design of the study. MGR, HM, VF, SA, ARR, and FR contributed to the acquisition of data. MGR, HM, SR, SA, VF, ARR, KT, FR, SR, GS, MP, AK, and AR contributed to the interpretation of data. MGR, SA, MP, FF, GC, JR, ARR, DS, HL, AMR, KT, LB, SR, FR, MADA, and GS contributed to support of material. MGR, HM, GS, AK, and AR contributed to manuscript preparation.

Funding

The work was supported by the German Research Foundation (DFG) to AR (project numbers: 320379231 [RA 2506/4-1, RA 2506/4-2], 423477573 [RA 2506/6-1], 493624887 [RA 2506/7-1]), to MGR, GC, and FF (project number: 493624887 Clinician Scientist Program NOTICE) and to AMR (project number: 422621469 [SO 1735/2-1]); CRC1181 (project number: 261193037) to GS (projects A01/Z03) and AR (project C06); TRR369 (project number: 501752319) to GS (projects C06/Z01) and AR (project A04) and FOR 2886/2 Project Z 405969122 to MP; Gottfried Wilhelm Leibniz Prize 2023 to GS; Major research instrumentation funding for light CyTOF (DFG project number 424726560). The work was supported by the European Research Council (project number 853508—BARRIER BREAK) to AR and (project number 810316—4-D nanoScope) to GS. The work was supported by the Federal Ministry of Education and Research (BMBF, 01EC1903A—MASCARA). This work was supported by the Innovative Medicines Initiative projects RTCure (777357). This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement no 101007757 (HIPPOCRATES). The JU receives support from the European Union's Horizon 2020 research and innovation program and EFPIA. Any dissemination of results must indicate that it reflects only the author's view and that the JU is not responsible for any use that may be made of the information it contains. The work was supported by the PARTNER Fellowship program to MGR, SA, and MADA. The work was supported by Novartis Pharma to AR. The work was supported by the Interdisciplinary Centre for Clinical Research (IZKF) Erlangen (D034 to AR, P049 and J106 to MGR, J107 to SR). The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Competing interests

AR reports financial support was provided by Novartis. AR reports a relationship with Bristol-Myers Squibb (BMS), Novartis, Janssen, Boehringer Ingelheim and UCB that includes: speaking and lecture fees. GS reports a relationship with BMS, Cabaletta, Janssen, Kyverna, Miltenyi, and Novartis that includes: speaking and lecture fees. MADA reports a relationship

with Amgen, AbbVie, UCB, Pfizer, J&J, Galapagos, Novartis, BMS, Biogen, MSD, Lilly that includes: consulting or advisory and speaking and lecture fees. MGR reports a relationship with UCB and AbbVie that includes: speaking and lecture fees. The other authors declare they have no competing interests. The present work was performed in (partial) fulfillment of the requirements for obtaining the degree "Dr. rer. biol. hum." at the Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU).

Patient consent for publication

Not applicable.

Ethics approval

Ethical approval from the local institutional review board (University of Erlangen-Nürnberg, #30_19B) and written informed consent was obtained from all participants.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Clinical data have been collected and stored in the Department of Medicine 3, Rheumatology and Immunology, Study outpatient clinic. Single cell RNA-seq and GeoMx spatial transcriptomic datasets of entheseal tissues have been deposited in Array Express and are available with accession numbers: E-MTAB-15133 and E-MTAB-15134 respectively. Publicly available single cell RNA-seq dataset from synovial tissue reanalysed in this study are available from Array Express with accession numbers: E-MTAB-11791 and E-MTAB-14339. The study was conducted at the Study Outpatient Clinic of the Medicine 3, Rheumatology and Immunology Department, Universitätsklinikum Erlangen, Germany, where the data have been deposited.

Code availability

All the methods and algorithms used in this manuscript are from previously published studies and are cited in the methods section. Selection criteria, thresholds, and other essential parameters are stated in the methods section. No new method for data analysis was developed.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.08.032.

Orcid

Maria Gabriella Raimondo: http://orcid.org/0000-0003-2020-6711

Andreas Ramming: http://orcid.org/0000-0002-7003-501X

REFERENCES

[1] Schett G, Lories RJ, D'Agostino MA, Elewaut D, Kirkham B, Soriano ER, et al. Enthesitis: from pathophysiology to treatment. Nat Rev Rheumatol 2017;13(12):731–41.

[2] Benjamin M, McGonagle D. The entheseal organ concept and its relevance to the spondyloarthropathies. Adv Exp Med Biol 2009;649:57–70.

[3] Gracey E, Burssens A, Cambré I, Schett G, Lories R, McInnes IB, et al. Tendon and ligament mechanical loading in the pathogenesis of inflammatory arthritis. Nat Rev Rheumatol 2020;16(4):193–207.

[4] Jacques P, Lambrecht S, Verheugen E, Pauwels E, Kollias G, Armaka M, et al. Proof of concept: enthesitis and new bone formation in spondyloarthritis are driven by mechanical strain and stromal cells. Ann Rheum Dis 2014;73(2):437–45.

[5] McGonagle D, Marzo-Ortega H, O'Connor P, Gibbon W, Hawkey P, Henshaw K, et al. Histological assessment of the early enthesitis lesion in spondyloarthropathy. Ann Rheum Dis 2002;61(6):534–7.

[6] Cuthbert RJ, Fragkakis EM, Dunsmuir R, Li Z, Coles M, Marzo-Ortega H, et al. Brief report: group 3 innate lymphoid cells in human enthesis. Arthritis Rheumatol 2017;69(9):1816–22.

[7] Sherlock JP, Joyce-Shaikh B, Turner SP, Chao CC, Sathe M, Grein J, et al. IL-23 induces spondyloarthropathy by acting on ROR-γt+ CD3+CD4-CD8- entheseal resident T cells. Nat Med 2012;18(7):1069–76.

[8] Taylor W, Gladman D, Helliwell P, Marchesoni A, Mease P, Mielants H, et al. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. Arthritis Rheum 2006;54(8):2665–73.

[9] Kaeley GS, Schett G, Conaghan PG, McGonagle D, Behrens F, Goupille P, et al. Enthesitis in patients with psoriatic arthritis treated with secukinumab or adalimumab: a post hoc analysis of the EXCEED study. Rheumatology (Oxford) 2024;63(1):41–9.

[10] Coates LC, Wallman JK, McGonagle D, Schett GA, McInnes IB, Mease PJ, et al. Secukinumab efficacy on resolution of enthesitis in psoriatic arthritis: pooled analysis of two phase 3 studies. Arthritis Res Ther 2019;21(1):266.

[11] Behrens F, Sewerin P, de Miguel E, Patel Y, Batalov A, Dokoupilova E, et al. Efficacy and safety of secukinumab in patients with spondyloarthritis and enthesitis at the Achilles tendon: results from a phase 3b trial. Rheumatology (Oxford) 2022;61(7):2856–66.

[12] Carron P, Varkas G, Cypers H, Van Praet L, Elewaut D, Van den Bosch F, et al. Anti-TNF-induced remission in very early peripheral spondyloarthritis: the CRESPA study. Ann Rheum Dis 2017;76(8):1389–95.

[13] Simon D, Kleyer A, Faustini F, Englbrecht M, Haschka J, Berlin A, et al. Simultaneous quantification of bone erosions and enthesiophytes in the joints of patients with psoriasis or psoriatic arthritis - effects of age and disease duration. Arthritis Res Ther 2018;20(1):203.

[14] Pachowsky ML, Raimondo MG, Xu C, Rauber S, Tascilar K, Labinsky H, et al. Concise report: a minimal-invasive method to retrieve and identify entheseal tissue from psoriatic arthritis patients. Ann Rheum Dis 2022;81(8):1131–5.

[15] Balint PV, Terslev L, Aegerter P, Bruyn GAW, Chary-Valckenaere I, Gandjbakhch F, et al. Reliability of a consensus-based ultrasound definition and scoring for enthesitis in spondyloarthritis and psoriatic arthritis: an OMERACT US initiative. Ann Rheum Dis 2018;77(12):1730–5.

[16] Mease P, van der Heijde D, Landewé R, Mpofu S, Rahman P, Tahir H, et al. Secukinumab improves active psoriatic arthritis symptoms and inhibits radiographic progression: primary results from the randomised, double-blind, phase III FUTURE 5 study. Ann Rheum Dis 2018;77(6):890–7.

[17] Danaher P, Kim Y, Nelson B, Griswold M, Yang Z, Piazza E, et al. Advances in mixed cell deconvolution enable quantification of cell types in spatial transcriptomic data. Nat Commun 2022;13(1):385.

[18] Edalat SG, Gerber R, Houtman M, Lückgen J, Teixeira RL, Palacios Cisneros MDP, et al. Molecular maps of synovial cells in inflammatory arthritis using an optimized synovial tissue dissociation protocol. iScience 2024;27(6):109707.

[19] Rauber S, Mohammadian H, Schmidkonz C, Atzinger A, Soare A, Treutlein C, et al. $CD200^+$ fibroblasts form a pro-resolving mesenchymal network in arthritis. Nat Immunol 2024;25(4):682–92.

[20] Alivernini S, MacDonald L, Elmesmari A, Finlay S, Tolusso B, Gigante MR, et al. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. Nat Med 2020;26(8):1295–306.

[21] Rauber S, Luber M, Weber S, Maul L, Soare A, Wohlfahrt T, et al. Resolution of inflammation by interleukin-9-producing type 2 innate lymphoid cells. Nat Med 2017;23(8):938–44.

[22] Bernal-Alferes B, Gómez-Mosqueira R, Ortega-Tapia GT, Burgos-Vargas R, García-Latorre E, ML Domínguez-López, et al. The role of γδ T cells in the immunopathogenesis of inflammatory diseases: from basic biology to therapeutic targeting. J Leukoc Biol 2023;114(6):557–70.

[23] Gaur P, Misra R, Aggarwal A. Natural killer cell and gamma delta T cell alterations in enthesitis related arthritis category of juvenile idiopathic arthritis. Clin Immunol 2015;161(2):163–9.

[24] Stavre Z, Bridgewood C, Zhou Q, Maeda Y, Huang TT, Karman J, et al. A role for neutrophils in early enthesitis in spondyloarthritis. Arthritis Res Ther 2022;24(1):24.

[25] Watad A, Rowe H, Russell T, Zhou Q, Anderson LK, Khan A, et al. Normal human enthesis harbours conventional CD4+ and CD8+ T cells with

regulatory features and inducible IL-17A and TNF expression. Ann Rheum Dis 2020;79(8):1044–54.
[26] Milz S, Tischer T, Buettner A, Schieker M, Maier M, Redman S, et al. Molecular composition and pathology of entheses on the medial and lateral epicondyles of the humerus: a structural basis for epicondylitis. Ann Rheum Dis 2004;63(9):1015–21.
[27] Groves C, Chandramohan M, Chew NS, Aslam T, Helliwell PS. Clinical examination, ultrasound and MRI imaging of the painful elbow in psoriatic arthritis and rheumatoid arthritis: which is better, ultrasound or MR, for imaging enthesitis? Rheumatol Ther 2017;4(1):71–84.
[28] Kim HJ, Seo SJ, Kim JY, Kim YG, Lee Y. IL-17 promotes osteoblast differentiation, bone regeneration, and remodeling in mice. Biochem Biophys Res Commun 2020;524(4):1044–50.
[29] Jo S, Wang SE, Lee YL, Kang S, Lee B, Han J, et al. IL-17A induces osteoblast differentiation by activating JAK2/STAT3 in ankylosing spondylitis. Arthritis Res Ther 2018;20(1):115.
[30] Huang W, La Russa V, Alzoubi A, Schwarzenberger P. Interleukin-17A: a T-cell-derived growth factor for murine and human mesenchymal stem cells. Stem Cells 2006;24(6):1512–8.

Ann Rheum Dis 85 (2026) 297–307

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Axial spondyloarthritis

Development and validation of a Disease Activity index for PSoriatic Arthritis based on 44 joints (DAPSA44)

Dafne Capelusnik[1,2,*], Clementina Lopez-Medina[3,4], Désirée van der Heijde[5], Daniel Aletaha[6], Josef Smolen[6], Anna Molto[7,8], Sofia Ramiro[5,9]

[1] *Care and Public Health Research Institute, Maastricht University, Maastricht, The Netherlands*
[2] *Department of Rheumatology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel*
[3] *Department of Rheumatology, Reina Sofia University Hospital, Cordoba, Spain*
[4] *Maimonides Biomedical Research Institute of Cordoba, University of Cordoba, Cordoba, Spain*
[5] *Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands*
[6] *Department of Rheumatology, Medical University of Vienna, Wien, Austria*
[7] *Department of Rheumatology, Hospital Cochin, APHP, Paris, France*
[8] *INSERM U-1153, Center for Research in Epidemiology and Statistics, Université Paris Cité, Paris, France*
[9] *Department of Rheumatology, Zuyderland Medical Center, Heerlen, The Netherlands*

ARTICLE INFO

Article history:
Received 14 March 2025
Received in revised form 20 August 2025
Accepted 24 August 2025

ABSTRACT

Objectives: This study aims to develop a modified Disease Activity index for PSoriatic Arthritis (DAPSA) score using the 44-joint count instead of the 66 swollen/68 tender joint counts (SJC/TJC) in patients with spondyloarthritis (SpA) (axial SpA [axSpA], peripheral SpA [pSpA], and psoriatic arthritis [PsA]) and evaluate its construct validity.

Methods: Patients with axSpA, pSpA, and PsA included in the Assessment of SpondyloArthritis international Society-PerSpA (PERipheral involvement in SpondyloArthritis) study were randomly allocated 1:2 stratified for diagnosis and country into a derivation and validation cohort. For all patients, the 66SJC/68TJC were available, from which the SJC44/TJC44 were extracted. The derivation cohort was used to calculate the conversion factors for SJC66/TJC68 into SJC44/TJC44 to obtain the DAPSA44 using mixed-effects linear regression models. The validation cohort assessed the DAPSA44 construct validity through the agreement between continuous original-DAPSA/DAPSA44 (intraclass correlation coefficient [ICC]) and DAPSA disease activity states (weighted-kappa), Bland-Altman plot, Spearman correlations between original-DAPSA/DAPSA44 and external constructs, and discrimination between known groups (standardised mean differences [SMDs]) after stratifying patients into active/inactive based on a combination of patient global assessment (≥5/<5) and SJC (≥1/0 or ≥2/<2).

Results: A total of 4121 patients (65% axSpA, 10% pSpA, and 25% PsA) were included. Conversion factors from the derivation cohort (n = 1364) for TJC and SJC were 1.30 and 1.34, respectively. The validation cohort (n = 2757) analyses showed almost-perfect agreement between original-DAPSA and DAPSA44 (ICC 0.98, kappa 0.95). Spearman correlation coefficients between DAPSA44 and external constructs fell within the predefined 0.3-wide range of

*Correspondence to Dr Dafne Capelusnik, Care and Public Health Research Institute, Maastricht University, Maastricht, The Netherlands.
E-mail address: capelusnikdafne@gmail.com (D. Capelusnik).

Handling editor Josef S. Smolen

Anna Molto and Sofia Ramiro contributed equally.

https://doi.org/10.1016/j.ard.2025.08.027

corresponding original-DAPSA coefficients. DAPSA44 demonstrated excellent discriminatory ability (SMD > 0.8) across all scenarios of active/inactive disease.
Conclusions: These findings support the use of DAPSA44 as a comparable tool to the original DAPSA for assessing disease activity due to peripheral arthritis in SpA, especially in situations where only a reduced joint count is available.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Axial spondyloarthritis (SpA) clinical trials mostly include the 44-joint count, as it is the instrument recommended in the latest update of the Assessment of SpondyloArthritis international Society core domain set.
- The Disease Activity index for Psoriatic Arthritis (DAPSA), which uses the 66/68 joint count, has been validated in psoriatic arthritis. While recent findings suggest that DAPSA performs well in assessing disease activity due to peripheral arthritis in axial SpA, it is not possible to calculate it based on the 44-joint count.

WHAT THIS STUDY ADDS

- We present a modified version of the original DAPSA score that can be used when only a 44-joint count is available, calculated by applying a conversion factor to the 66-swollen and tender joint counts included in the original DAPSA.
- Construct validity analysis supports the use of DAPSA44 as a comparable tool to the original DAPSA for assessing disease activity, particularly in settings where a reduced joint count is the only option.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- By adapting the original-DAPSA to a 44-joint count, this version aims to capture disease activity related to peripheral arthritis in SpA while remaining practical for use in diverse research settings—and potentially in clinical practice—where only the 44-joint count is available.

INTRODUCTION

Spondyloarthritis (SpA) is a heterogeneous disease that can present with several phenotypes such as axial SpA (axSpA), peripheral SpA (pSpA), or psoriatic arthritis (PsA). Peripheral manifestations such as arthritis, dactylitis, and enthesitis may occur alongside axial disease or independently [1,2].

Recently, the Assessment of SpondyloArthritis international Society (ASAS) and Outcomes Measures in Rheumatology (OMERACT) updated the core domain set (COS) for axSpA, identifying peripheral manifestations as a mandatory domain for inclusion in clinical trials evaluating disease-modifying antirheumatic drugs [3]. During the subsequent instrument selection process, only 2 tools were evaluated for assessing peripheral arthritis: the swollen joint counts of 66 and 44 joints (SJC66 and SJC44), and among these, the SJC44 was selected as the preferred instrument for inclusion in the COS [4]. This means that no composite scores were tested for the measurement of disease activity due to peripheral arthritis.

The 2017 treat-to-target recommendations for SpA advocated for the use of the Axial Spondyloarthritis Disease Activity Score (ASDAS) [5] for axSpA and the Disease Activity index for PSoriatic Arthritis (DAPSA) [6] or minimal disease activity (MDA) [7] for PsA to define treatment targets [8]. While all recommended instruments are composite scores, the assessment for axSpA focused on the general assessment of disease activity without any recommendation for the assessment of disease activity specifically due to peripheral arthritis.

Recent studies have explored the performance of different outcome measurement instruments for peripheral arthritis in SpA. In an ancillary analysis of the ASAS-perSpA (PERipheral involvement in SpondyloArthritis) study, the DAPSA demonstrated a strong capacity to discriminate between active and inactive disease in axSpA, pSpA, and PsA [9]. Similarly, in the CRESPA trial (Clinical REmission in peripheral SPondyloArthritis), DAPSA showed superior longitudinal construct validity and trial discrimination compared to ASDAS in patients with pSpA [10]. These findings suggest that DAPSA could be a promising tool for assessing peripheral arthritis in SpA, though its psychometric properties require further evaluation, particularly in the population of axSpA.

Originally developed for reactive arthritis [11] and later validated for PsA [6], the DAPSA combines patient-reported outcomes (global and pain assessments), the SJC66, 68 tender joint count (TJC68), and C-reactive protein (CRP, mg/dL) in a simple sum calculation. Given the joint involvement typical in PsA (e.g. distal interphalangeal, ankle, and foot joints), the extensive 66/68 joint counts are preferred. Nevertheless, despite the lack of face validity [12], sometimes only the 28-joint counts are collected in databases and registries. To address this limitation, the DAPSA28, based on a 28-joint count, was previously developed and validated for PsA [13]. While it performed comparably to the original-DAPSA, it showed better agreement at lower disease activity levels and poorer psychometric performance in moderate-to-high activity states. Thus, the original DAPSA remains the preferred tool when feasible.

Expanding the use of the DAPSA to axSpA, the original 66-joint version can still be applied when the full joint data are available; however, the SCJ44 is the instrument recommended by the ASAS COS for the evaluation of peripheral arthritis, and thus more likely to be the 1 included in clinical trials. Therefore, a modified version of DAPSA may be necessary; while the DAPSA28 is 1 option, developing a DAPSA44, using the SJC44, should allow for the inclusion of data on more joints with better face validity.

The objective of this study was to develop a modified DAPSA score, incorporating the 44-joint count, in patients with SpA (including axSpA, pSpA, and PsA), and to evaluate its construct validity.

METHODS

Study design and population

Data from the ASAS-perSpA study were used. Briefly, the ASAS-perSpA study is an international, multicentre study with 23 participating countries, in which data of consecutive patients with SpA (based on the physician's diagnosis) were cross-sectionally collected between July 2018 and February 2020 [14,15]. Patients with a clinical diagnosis of axSpA, pSpA, or PsA and with complete data of all disease activity instruments

and external constructs as described below were included in the study.

Written informed consent was obtained from all patients before enrolment and Ethics Committees from the individual participating centres approved the study.

Joint counts

The 66/68-joint counts encompass a broad range of joints, including distal small joints (distal and proximal interphalangeal joints [DIP and PIP]), as well as large joints (hips) and intermediate joints (midtarsal, temporomandibular, sternoclavicular, and acromioclavicular joints). The 44-joint count includes a slightly more focused set compared to the 66/68 joint count, excluding the distal small joints of the hands (DIP) and feet (PIP) and midtarsal joint. In contrast, the 28-joint count is more limited, focusing primarily on the most commonly affected joints in rheumatoid arthritis, including the metacarpophalangeal joints, PIP joints, wrists, elbows, shoulders, and knees.

The details of the joints included in the 3 different joint counts (66/68 SJC/TJC, 44 SJC/TJC, and 28 SJC/TJC) are provided in Figure 1, highlighting the similarities and differences across the 3 measures.

Statistical analysis

The overall SpA population was randomly divided into 2: a derivation cohort and a validation cohort, in a 1:2 ratio. This division was performed using the *splitsample* command in STATA, with stratification by diagnosis (i.e. disease phenotype) and country to ensure a balanced representation. Descriptive statistics were used to summarise patients' demographic and clinical characteristics for the population, by SpA phenotypes, and for each randomised group.

Development of the DAPSA44 score

The derivation cohort was used for the development of the DAPSA44 score. Analyses were conducted first in the overall population and subsequently within each SpA phenotype (axSpA, pSpA, and PsA).

The DAPSA44 was calculated by modifying the joint count component of the original DAPSA formula, incorporating conversion factors to adjust the 66/68-joint count to 44-joint equivalents. Taking into account that the DAPSA is calculated by a simple sum of its individual components, with the conversion factors for SJC/TJC, the formula was as follows: DAPSA44 = (44TJC × conversion *factor1*) + (44SJC × conversion *factor2*) + patient global assessment (PGA, 0–10) + patient peripheral pain assessment (0–10) + CRP (mg/dL). For peripheral pain assessment, the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) question 3 ('How would you describe your overall level of pain/swelling in joints other than neck, back or hips') was used [16].

To determine the conversion factors, mixed-effects linear regression models were employed, with the 68TJC/66SJC as dependent variables (separate models) and 44TJC/44SJC as independent variables, respectively. The models included the country of residence as a random intercept to account for geographic reduced variability.

Construct validity assessment

Construct validity was evaluated using the validation cohort through the following assessments:

1. *Agreement between original DAPSA and DAPSA44 disease activity:* The agreement between original-DAPSA and DAPSA44 was assessed as a continuous score, with intraclass correlation coefficient (ICC) using a 2-way random effects model. The agreement between original DAPSA and DAPSA44 disease activity stated as a categorical variable was assessed using weighted-kappa coefficients. The scores were categorised according to the following established cut-offs: remission ≤ 4, low disease activity >4 and ≤14, moderate disease activity >14 and ≤28, and high disease activity >28 [17]. ICC values between 0.75 and 0.90 indicate 'good' reliability, and values greater than 0.90 indicate 'excellent' reliability [18]. A κ value between 0.81 and 0.90 indicates 'strong' agreement, whereas between 0.91 and 0.99 indicates 'almost perfect' agreement [19]. Prespecified desired levels of values of ICC and kappa (κ) were established above 0.80.
2. To visualise this agreement, a Bland-Altman plot with 95% limits of agreement (LoA) [20] comparing the original DAPSA and DAPSA44 was created, and systematic error (mean difference) and random error (scedasticity of the plot) were assessed.

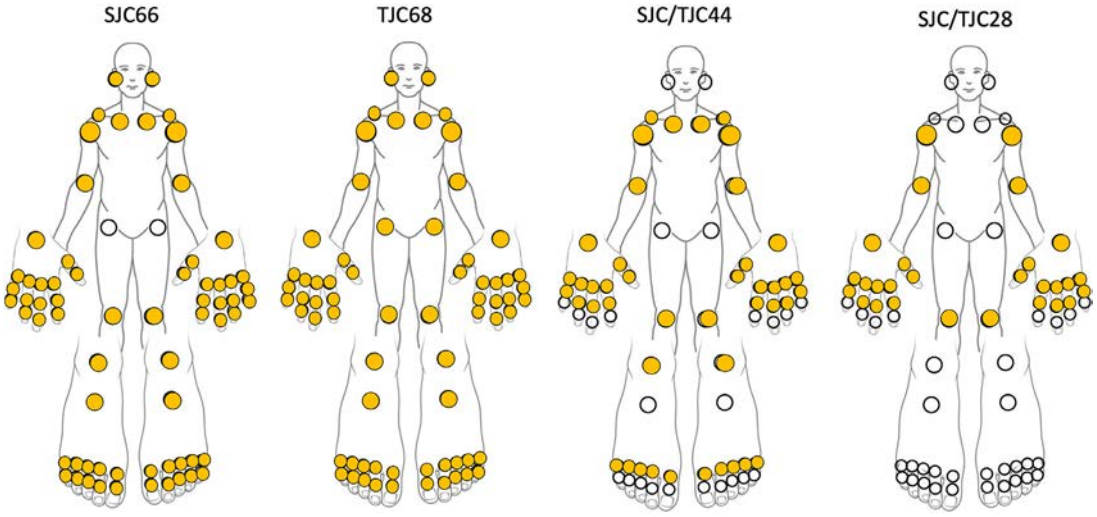


Figure 1. Different joint counts and the joints assessed according to each 1 of them. SJC, swollen joint count; TJC, tender joint count.

3. *Correlation with external constructs:* Spearman correlation coefficients between each of the original DAPSA and DAPSA44 against different external constructs were calculated. The external constructs included disease activity instruments BASDAI [16], ASDAS [5], DAS28/44 [21,22], CRP and other instruments, namely instruments assessing functional ability and overall functioning and health (Bath Ankylosing Spondylitis Functional Index [23], EQ-5D [24], ASAS Health Index [25]). It was predefined that correlation coefficients between the DAPSA44 and external constructs had to fall within a 0.3-wide band around the corresponding correlation coefficients between the original DAPSA and the external constructs.
4. *Discrimination between known groups for disease activity states:* Since no external gold standard is available to define disease activity states in patients with peripheral arthritis, we used different combined definitions based on PGA (as a patient reported outcome that includes the perception on both axial and peripheral involvement) and SJC (as an objective and exclusively peripheral outcome) to stratify the population into active/inactive disease. The stratification on PGA was based on the middle of the scale, so at a value of 5. Due to the high frequency of patients without arthritis, the stratification on SJC was performed in 2 ways: (i) no swollen joints vs any (≥1) swollen joint; (ii) SJC below the median SJC vs equal or above the median SJC. Combining both stratifications on PGA and SJC, patients were considered to be in 'low disease activity' in case they had a PGA < 5 and no swollen joints vs in 'high disease activity' in case they had a PGA ≥ 5 and at least 1 swollen joint.

Subsequently, comparisons were made across all possible subgroups combining the stratifications based on PGA and SJC: (i) PGA < 5 and SJC < median vs PGA < 5 and SJC ≥ median; (ii) PGA < 5 and SJC = 0 vs PGA < 5 and SJC ≥ 1; (iii) PGA ≥ 5 and SJC < median vs PGA ≥ 5 and SJC ≥ median; (iv) PGA > 5 and SJC = 0 vs PGA > 5 and SJC ≥ 1. Then, the standardised mean difference (SMD) was calculated both for the original DAPSA and DAPSA44 as the difference between the group means divided by the pooled SD. This unitless measure allows for comparison across different disease measures. A higher absolute SMD indicates better discriminatory capacity, and an SMD above 0.8 is considered 'good' [26–28].

Also, the same analyses were conducted in the different subpopulations (axSpA, pSpA, and PsA), as sensitivity analyses.

In order to make the calculation easier, an extra DAPSA44 calculation was conducted by simplifying the conversion factors to a more rounded number (a single decimal digit).

Additionally, to assess the robustness of the DAPSA44, we created 2 additional scores by using the lower and upper boundaries of the coefficients derived from the mixed-effects linear regression models as conversion factors. These scores were named DAPSA44min (using the lower coefficients obtained from both the SJC and TJC models) and DAPSA44max (using the higher coefficients from the SJC and TJC models).

All the analyses were performed using Stata SE V.17 (StataCorp).

RESULTS

From the 4185 patients included in the ASAS-perSpA study, 4121 could be included. Among them, 2679 (65%) had axSpA, 428 (10%) had pSpA, and 1014 (25%) had PsA based on the rheumatologist's diagnosis.

A history of peripheral arthritis was reported in 2291 (56%) patients, predominantly with oligoarticular (43%) or polyarticular involvement (45%). The prevalence of peripheral arthritis varied across disease phenotypes, being lower in axSpA (36%) compared to pSpA (95%) and PsA (91%). A total of 53 patients (0.6%) had at least 1 SJC66 ≥ 1 but an SJC44 = 0, and 319 patients (4%) had a TJC68 ≥ 1 but a TJC44 = 0. Sociodemographic and clinical characteristics of the overall SpA population and the different phenotypes can be found in Supplementary Table S1. Additionally, Supplementary Table S2 presents the

Table 1
Patient characteristics in the overall SpA population and after random allocation into the derivation and validation cohorts

	Derivation cohort (n = 1364)	Validation cohort (n = 2757)	*P* value
axSpA (%)	885 (64.9)	1794 (65.1)	.90
pSpA (%)	143 (10.5)	285 (10.3)	.88
PsA (%)	336 (24.6)	678 (24.6)	.98
Age (years)	45.13 (13.74)	44.32 (13.76)	.07
Male gender (%)	829 (61)	1692 (61)	.71
Symptom duration (years)	21.83 (19.01)	20.49 (17.91)	.03
Disease duration (years)	15.08 (11.85)	14.17 (11.09)	.02
BMI (kg/m^2)	26.52 (5.57)	26.41 (5.34)	.54
Axial involvement[a] (%)	1075 (79)	2140 (78)	.39
Peripheral arthritis[a] (%)	776 (57)	1515 (55)	.24
Proportion of patients with SJC66 ≥ 1 and SJC44 = 0	19 (1.4)	28 (1.0)	.28
History of			
Monoarticular[b] involvement	101 (8)	203 (9)	.94
Oligoarticular[b] involvement	110 (9)	244 (10)	.37
Polyarticular[b] involvement	61 (5)	135 (6)	.51
Dactylitis[a] (%)	209 (15)	423 (15)	.99
Enthesitis[a] (%)	600 (44)	1212 (44)	.99
CRP (mg/L)	12.48 (31.5)	11.65 (24.6)	.40
BASFI (0-10)	3.07 (2.67)	2.96 (2.65)	.24
ASAS HI (0-17)	6.59 (4.55)	6.59 (4.62)	.97
EuroQoL (0-1)	0.65 (0.24)	0.66 (0.24)	.55
Patient Global Assessment (0-10)	4.40 (2.66)	4.37 (2.72)	.68
Patient Assessment of Pain (BASDAI Q3)	3.36 (3.03)	3.35 (3.05)	.90
BASDAI (0-10)	3.91 (2.43)	3.85 (2.44)	.41
ASDAS	2.56 (1.23)	2.53 (1.15)	.52
Original-DAPSA	12.56 (12.52)	12.11 (11.14)	.26
DAS28	2.63 (1.06)	2.60 (1.07)	.30
DAS44	1.24 (0.81)	1.21 (0.78)	.28
SJC (0-66)	0.81 (3.63)/0 (0-0)	0.80 (2.79)/0 (0-0)	.95
TJC (0-68)	2.74 (6.83)/0 (0-3)	2.43 (5.72)/0 (0-2)	.14
SJC (0-44)	0.63 (2.64)/0 (0-0)	0.67 (2.29)/0 (0-0)	.61
TJC (0-44)	2.07 (5.10)/0 (0-2)	1.83 (4.36)/0 (0-2)	.13

ASAS HI, Assessment of SpondyloArthritis international Society Health Index; ASDAS, Axial Spondyloarthritis Disease Activity Score; axSpA, axial spondyloarthritis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Score; BASFI, Bath Ankylosing Spondylitis Functional Index; BMI, body mass index; CRP, C-reactive protein; DAPSA, Disease Activity in Psoriatic Arthritis; DAS, Disease Activity Score 28/44; EuroQoL, Euro Quality of life 5 Dimensions; PsA, psoriatic arthritis; pSpA, peripheral spondyloarthritis; SJC, swollen joint count; SpA, spondyloarthritis; TJC, tender joint count.
Results reflect mean (SD)/median (IQR) or n (%).
Disease phenotype was defined by the physician. Data were incomplete for: BMI (n = 15), ASAS HI (n = 2), and EuroQoL (n = 14).
[a] Manifestation ever present.
[b] History of type of involvement: monoarticular, 1 swollen joint; oligoarticular, 2-4 swollen joints; polyarticular, 5 or more swollen joints.

proportion of patients with joint involvement, at each joint level.

After random allocation, patient characteristics were similar in the derivation (n = 1364) and validation cohorts (n = 2757) (Table 1).

Development of the DAPSA44

The conversion factor obtained through the mixed-effect linear regression models was 1.30 for TJC and 1.34 for SJC, leading to the following formula:

DAPSA44 = (44TJC × 1.30) + (44SJC × 1.34) + PGA (0–10) + patient peripheral pain assessment (0–10) + CRP (mg/dL).

These conversion factors obtained from the overall SpA population were considered stable across disease phenotypes (Supplementary Table S3).

The DAPSA44 was similar in the derivation and validation cohorts, with a mean of 12.6 (SD 12.3) and 12.2 (12.4), respectively.

Construct validity

The agreement between original DAPSA and DAPSA44 was excellent as a continuous score in the overall SpA population (ICC 0.98 [95% CI 0.98-0.99]) and also in the 3 disease phenotypes (0.99 [0.99-0.99] in axSpA, 0.99 [0.98-0.99] in pSpA, and 0.97 [0.97-0.98] in PsA). Based on disease activity states, the agreement was also almost perfect, with a weighted κ of 0.95 in the overall SpA population, and between κ 0.92 to 0.96 for the disease phenotypes, with higher values corresponding to axSpA (Tables 2 and 3 and Supplementary Table S4).

As depicted in Figure 2a, the Bland-Altman plot showed a mean difference between the original DAPSA and DAPSA44 of −0.05 points with a 95% LoA of −3.91, 3.82, with a positive difference representing a higher DAPSA original. The plot showed minimal difference between the methods and the uniform scatter of points around the mean indicates consistent agreement across the range of measurements (homoscedasticity). The same was found for each disease phenotype (Supplementary Fig S1).

The correlation analysis revealed a correlation between original DAPSA and DAPSA44 of 0.99 (Fig 2b). All Spearman correlation coefficients between DAPSA44 and external constructs fell within the predefined 0.3-wide range of the corresponding coefficients for the original DAPSA. Some coefficients were even identical. In the overall SpA population, differences between the 2 scores and external constructs ranged from approximately 0.00 to 0.01. Similar patterns were observed across individual phenotypes, with differences

Table 2
Agreement between original-DAPSA and DAPSA44 disease activity states

	DAPSA44[a]				
Original-DAPSA[a]	Remission 1.98 (1.21)	Low disease activity 8.52 (2.85)	Moderate disease activity 19.45 (3.83)	High disease activity 41.58 (14.37)	Total
Remission 1.97 (1.20)	**588 (99%)/(97%)**	6 (1%)/(0.5%)	0 (0%)/(0%)	0 (0%)/(0%)	594
Low disease activity 8.56 (2.85)	20 (1%)/(3%)	**1252 (97%)/(97%)**	24 (2%)/(4%)	0 (0%)/(0%)	1296
Moderate disease activity 19.42 (3.71)	0 (0%)/(0%)	32 (5%)/(3%)	**604 (90%)/(93%)**	30 (5%)/(14%)	666
High disease activity 40.73 (14.83)	0 (0%)/(0%)	0 (0%)/(0%)	22 (11%)/(3%)	**179 (89%)/(86%)**	201
Total	608	1290	650	209	

Agreement 98.4%.
Weighted Kappa: 0.95
Bold values indicate the number (and percentage) of patients correctly clasified.
n (%) within original-DAPSA disease activity states/(%) within DAPSA44 disease activity states.
DAPSA, Disease Activity in PSoriatic Arthritis; DAPSA44, Disease Activity in PSoriatic Arthritis based on 44 joints.
[a] Below each disease activity state, mean overall (SD).

Table 3
Agreement between original-DAPSA and DAPSA44 (using the single 1-decimal conversion factor) disease activity states

	DAPSA44[a]				
Original-DAPSA[a]	Remission 1.98 (1.21)	Low disease activity 8.51 (2.85)	Moderate disease activity 19.41 (3.81)	High disease activity 41.38 (14.21)	Total
Remission 1.97 (1.20)	**589 (99%)/(97%)**	5 (1%)/(0.5%)	0 (0%)/(0%)	0 (0%)/(0%)	594
Low disease activity 8.56 (2.85)	20 (1%)/(3%)	**1252 (97%)/(97%)**	24 (2%)/(4%)	0 (0%)/(0%)	1296
Moderate disease activity 19.42 (3.71)	0 (0%)/(0%)	32 (5%)/(2.5%)	**608 (91%)/(93%)**	26 (4%)/(14%)	666
High disease activity 40.73 (14.83)	0 (0%)/(0%)	0 (0%)/(0%)	22 (11%)/(3%)	**179 (89%)/(87%)**	201
Total	609	1289	654	205	

Agreement 98.4%.
Weighted Kappa: 0.95
Bold values indicate the number (and percentage) of patients correctly clasified.
n (%) within original-DAPSA disease activity states/(%) within DAPSA44 disease activity states. For this table, DAPSA44 was calculated using the single 1-decimal conversion factor 1.3.
DAPSA, Disease Activity in PSoriatic Arthritis; DAPSA44, Disease Activity in PSoriatic Arthritic based on 44 joints.
[a] Below each disease activity state, mean overall (SD).

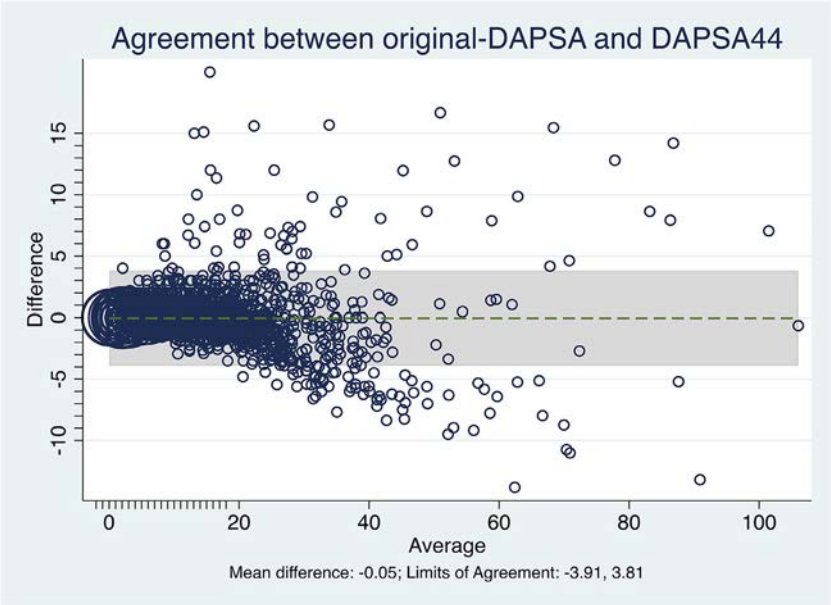


Figure 2. (a) Agreement between original DAPSA and DAPSA44. For the difference, DAPSA44 was subtracted from original DAPSA. A positive difference means a higher original DAPSA.

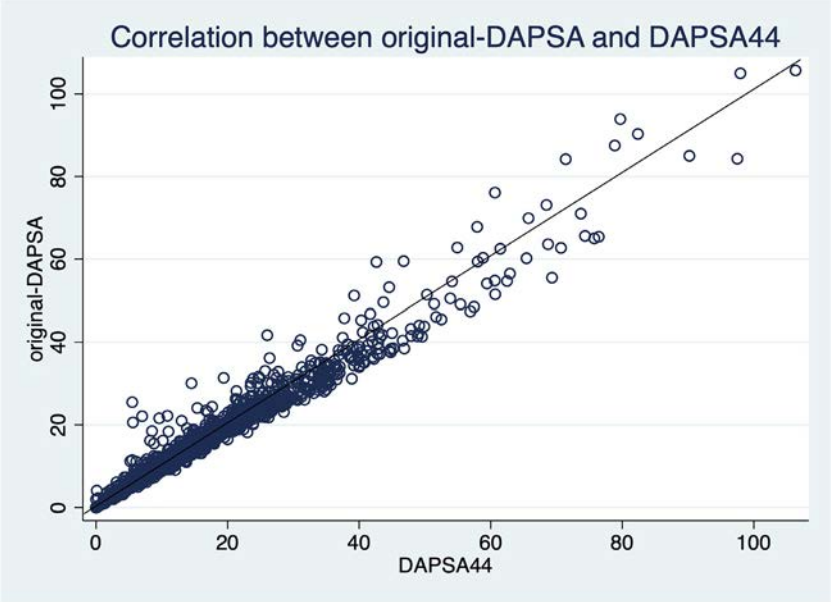


Figure 2. (b) Scatterplot showing the correlation between original DAPSA and DAPSA44 (rho = 0.991). DAPSA, Disease Activity index for PSoriatic Arthritis; DAPSA44, Disease Activity index for PSoriatic Arthritis based on 44 joints.

ranging from 0.001 to 0.01 in axSpA, 0.003 to 0.02 in pSpA, and 0.006 to 0.03 in PsA (Table 4).

As for the discrimination between known groups, DAPSA44 showed a good discriminatory capacity (SMD > 0.8) between active and inactive disease in all scenarios, in some of them (mainly in axSpA and pSpA) even slightly better than the original DAPSA (Tables 5 and 6).

Lastly, the sensitivity analyses showed no differences across the DAPSA44, the DAPSA44 version using the single 1-decimal conversion factor (1.3 for both SJC and TJC), DAPSA44, DAPSA44min (calculated using 1.31 and 1.28 as conversion factors for SJC and TJC, respectively) and DAPSA44max (calculated with 1.35 and 1.31 as conversion factors), adding robustness to the findings (Table 3 and Supplementary Tables S5-S12).

DISCUSSION

In this study, we present a modified version of the original DAPSA score. The development of the DAPSA44 provides a novel approach for assessing disease activity in patients with axSpA and pSpA, for which only a reduced joint count (SJC44) is often available, according to the ASAS axSpA COS [4]. By modifying the original DAPSA to focus on a 44-joint count, this version aims to capture the patterns of disease activity due to peripheral arthritis in SpA while remaining practical for use in diverse research and potentially also clinical settings. The single 1-decimal conversion factor of 1.3 for both SJC and TJC has been shown to perform well and is therefore recommended.

Our findings demonstrate that the DAPSA44 effectively reflects disease activity across a spectrum of axSpA and pSpA phenotypes, maintaining strong agreement with the original score and correlations with other established disease activity measures and patient-reported outcomes. Additionally, the DAPSA44 showed a good discriminatory capacity to distinguish between active and inactive disease, particularly in the axSpA and pSpA subgroups, even slightly larger than the original DAPSA. The sensitivity analyses applying both minimal and maximal conversion factors for the SJC and TJC offered a clear and informative means of capturing the potential range of variability in the measure. This design allowed a direct comparison of the performance between these 2 extreme scenarios and the selected conversion

Table 4
Correlation between disease activity measures and external constructs in the validation cohort (n = 2757) [29]

External constructs	Spearman correlation coefficient[a]							
	Overall SpA population (n = 2757)							
	BASDAI	ASDAS	DAS28	DAS44	CRP	BASFI	ASAS-HI	EuroQoL
Original-DAPSA	0.81	0.84	0.87	0.75	0.42	0.64	0.60	−0.62
DAPSA44	0.81	0.84	0.88	0.76	0.41	0.64	0.60	−0.62
axSpA (n = 1794)								
Original-DAPSA	0.83	0.88	0.86	0.69	0.44	0.69	0.61	−0.63
DAPSA44	0.83	0.88	0.86	0.69	0.43	0.69	0.61	−0.63
pSpA (n = 285)								
Original-DAPSA	0.78	0.84	0.87	0.81	0.47	0.61	0.59	−0.61
DAPSA44	0.77	0.83	0.89	0.83	0.47	0.59	0.58	−0.60
PsA (n = 678)								
Original-DAPSA	0.75	0.80	0.88	0.82	0.39	0.61	0.58	−0.60
DAPSA44	0.76	0.81	0.91	0.84	0.38	0.62	0.60	−0.61

ASAS HI, Assessment of SpondyloArthritis international Society Health Index; ASDAS, Axial Spondyloarthritis Disease Activity Score; axSpA, axial spondyloarthritis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Score; BASFI, Bath Ankylosing Spondylitis Functional Index; CRP, C-reactive protein; DAS, Disease Activity Score 28/44; DAPSA, Disease Activity in Psoriatic Arthritis; EuroQoL, Euro Quality of life 5 Dimensions; PsA, psoriatic arthritis; pSpA, peripheral spondyloarthritis; SpA, spondyloarthritis.
The correlation coefficients between the DAPSA44 and the different external constructs were within a 0.3-wide band around the corresponding correlation coefficients between the original DASPA and the external constructs.
[a] Weak < 0.30; moderate 0.30 to 0.69; strong ≥ 0.70.

Table 5
Discriminatory capacity

Assessment measure	Overall SpA population (axSpA, pSpA and PsA)			axSpA			pSpA			PsA		
	PGA < 5 and SJC = 0 (n = 1222)	PGA ≥ 5 and SJC ≥ 1 (n = 391)	SMD	PGA < 5 and SJC = 0 (n = 905)	PGA ≥ 5 and SJC ≥ 1 (n = 129)	SMD	PGA < 5 and SJC = 0 (n = 92)	PGA ≥ 5 and SJC ≥ 1 (n = 85)	SMD	PGA < 5 and SJC = 0 (n = 225)	PGA ≥ 5 and SJC ≥ 1 (n = 177)	SMD
Original-DAPSA	5.19 (4.84)	28.03 (14.20)	2.80	4.79 (4.36)	26.53 (12.07)	3.69	6.51 (6.09)	16.87 (14.50)	1.86	6.27 (5.76)	29.67 (15.34)	2.12
DAPSA44	5.09 (4.83)	28.97 (14.73)	2.85	4.70 (4.41)	27.56 (12.82)	3.74	6.36 (6.14)	28.27 (15.61)	1.88	6.14 (5.60)	30.34 (15.54)	2.17

axSpA, axial spondyloarthritis; DAPSA, Disease Activity in Psoriatic Arthritis; PGA, patient global assessment; PsA, psoriatic arthritis; pSpA, peripheral spondyloarthritis; SJC, swollen joint count; SMD, standardised mean difference; SpA, spondyloarthritis.
Analyses stratified by extreme groups according to PGA and SJC in the overall SpA population and the different phenotypes.
Values shown as mean (SD).
Large discrimination between known groups when SMD ≥ 0.8.

factors, thereby assessing whether relevant differences emerged across the validation analysis. The consistency of the results across these comparisons supports the robustness of the newly adapted score. Moreover, the simplified version, which applied a single 1-decimal conversion factor for SJC and TJC, resulted in nearly identical results, offering a more practical and thus more feasible calculation method.

As previously mentioned, this was not the first time the original DAPSA had been adapted into a version including a lower SJC; however, some differences between the 2 modified versions can be noticed. While both demonstrated good construct validity in general, DAPSA28 showed weaker agreement in moderate-to-high disease activity states than in remission or low disease activity states [13]. This outcome is not surprising, as a smaller joint count (TJC/SJC) increases the likelihood of missing affected joints, particularly in PsA, where joints of the feet are frequently affected. In contrast, DAPSA44 demonstrated strong agreement with original DAPSA across disease activity states, classifying patients as having the same disease activity state even in the presence of higher disease activity. This can also be observed in the Bland-Altman plots. Likely, this is due to the inclusion of a higher number of joints, particularly in the lower limbs, since arthritis of the lower limbs is more characteristic of SpA in general, as opposed to, for example, rheumatoid arthritis. For DAPSA44, agreement in the overall SpA population was nearly perfect in remission and low disease activity strata and very good in moderate and high disease activity states, with only a 3% difference in patient classification between the 2 scores. Lastly, no clear pattern was observed in the misclassified cases, with a maximum of misclassification of only 1 category. Additionally, using data from the ASAS-PerSpA cohort, which included the 3 disease phenotypes (axSpA, pSpA, and PsA), we demonstrated that the construct validity was robust across all phenotypes, with numerically better results observed in axSpA and pSpA compared to PsA. The correlation analysis between DAPSA44 and disease activity and nondisease activity constructs was closely aligned with the results of the original DAPSA. We deliberately selected disease activity scores for peripheral arthritis that are also used in other conditions such as axSpA and RA, in order to enhance comparability and contextualise our findings. Although a prespecified acceptable difference of 0.3 was allowed between the 2 scores, the correlations were practically equal in the overall SpA population and across the individual phenotypes. And lastly, the large capacity of DAPSA44 to

Table 6
Discrimination between known groups

	Patient global assessment < 5 (n = 1413)						Patient global assessment ≥ 5 (n = 1344)					
	Overall SpA population											
Assessment measure	SJC < 2 (n = 1947)	SJC ≥ 2 (n = 153)	SMD	SJC = 0 (n = 1222)	SJC ≥ 1 (n = 191)	SMD	SJC < 2 (n = 1067)	SJC ≥ 2 (n = 277)	SMD	SJC = 0 (n = 953)	SJC ≥ 1 (n = 391)	SMD
Original-DAPSA	5.48 (5.01)	18.56 (13.28)	2.18	5.19 (4.84)	14.35 (11.33)	1.49	14.79 (7.64)	30.80 (15.11)	1.66	14.01 (7.14)	28.03 (14.20)	1.44
DAPSA44	5.41 (5.03)	17.75 (11.87)	2.13	5.09 (4.84)	14.03 (10.27)	1.52	14.78 (7.79)	31.95 (15.77)	1.72	13.95 (7.31)	28.97 (14.73)	1.49
axSpA												
Assessment measure	SJC < 2 (n = 936)	SJC ≥ 2 (n = 24)	SMD	SJC = 0 (n = 905)	SJC ≥ 1 (n = 55)	SMD	SJC < 2 (n = 753)	SJC ≥ 2 (n = 81)	SMD	SJC = 0 (n = 705)	SJC ≥ 1 (n = 129)	SMD
Original-DAPSA	4.98 (4.53)	18.14 (14.45)	2.63	4.79 (4.36)	11.80 (11.05)	1.81	13.66 (9.72)	30.10 (8.96)	2.19	13.19 (6.39)	26.53 (12.07)	1.77
DAPSA44	4.90 (4.60)	18.43 (11.77)	2.76	4.70 (4.41)	14.13 (9.71)	1.94	13.60 (6.99)	31.50 (13.60)	2.27	13.10 (6.65)	27.56 (12.82)	1.82
pSpA												
Assessment measure	SJC < 2 (n = 114)	SJC ≥ 2 (n = 17)	SMD	SJC = 0 (n = 92)	SJC ≥ 1 (n = 39)	SMD	SJC < 2 (n = 97)	SJC ≥ 2 (n = 57)	SMD	SJC = 0 (n = 69)	SJC ≥ 1 (n = 85)	SMD
Original-DAPSA	7.33 (6.51)	14.30 (7.05)	1.06	6.51 (6.09)	12.31 (7.29)	0.90	16.81 (7.67)	29.54 (16.71)	1.08	14.94 (7.67)	26.87 (14.50)	1.00
DAPSA44	7.23 (6.47)	15.73 (7.84)	1.28	6.36 (6.14)	13.00 (7.52)	1.01	17.07 (8.62)	31.44 (17.95)	1.13	15.13 (8.54)	28.27 (15.61)	1.02
PsA												
Assessment measure	SJC < 2 (n = 261)	SJC ≥ 2 (n = 61)	SMD	SJC = 0 (n = 225)	SJC ≥ 1 (n = 97)	SMD	SJC < 2 (n = 217)	SJC ≥ 2 (n = 139)	SMD	SJC = 0 (n = 179)	SJC ≥ 1 (n = 177)	SMD
Original-DAPSA	6.50 (5.54)	19.90 (14.02)	1.70	6.27 (5.76)	15.48 (12.70)	1.09	17.81 (9.43)	31.72 (15.72)	1.13	16.88 (8.78)	29.67 (15.34)	1.02
DAPSA44	6.42 (5.45)	18.04 (12.89)	1.56	6.14 (5.60)	14.39 (11.52)	1.05	17.85 (9.05)	32.43 (16.09)	1.19	16.82 (8.44)	30.34 (15.54)	1.08

axSpA, axial spondyloarthritis; DAPSA, Disease Activity in Psoriatic Arthritis; PGA, patient global assessment; PsA, psoriatic arthritis; pSpA, peripheral spondyloarthritis; SJC, swollen joint count; SMD, standardised mean difference; SpA, spondyloarthritis.
Analyses stratified by both PGA and SJC in the overall SpA population (n = 2775). Population was stratified by PGA and SJC in different ways showing all the groups of such stratification allowing us to evaluate the performance of the indices in the complete population and according to the different levels of disease activity (including 2 different cut-off of SJC).
Values shown as mean (SD).
Large discrimination between known groups when SMD ≥ 0.8.

discriminate between active and inactive disease was almost identical to the original DAPSA, with even slightly better results for the newly developed version. Results were again consistent across different criteria used to classify active/inactive disease and across the 3 different phenotypes. These results confirm that the best is to use the original DAPSA, when only a 44-joint count is available then the DAPSA44 is preferable (also above DAPSA28) and when only a 28-joint count is available, then the DAPSA28 is the instrument to be used.

Although the original DAPSA is the recommended tool for PsA, a concise, equally valid alternative is valuable, especially for axSpA studies where the 66/68 joint count is not always available. It is important to emphasise that the primary purpose of DAPSA44 is to be applied in SpA conditions other than PsA or reactive arthritis, namely axSpA and pSpA. Nevertheless, while promising data support the use of composite scores like DAPSA for assessing peripheral arthritis in axSpA and pSpA [10,30,31], no official recommendation or endorsement currently exists for their use in these disease phenotypes, while the SJC44 is the recommended instrument according to the ASAS axSpA COS [4]. Further analyses are needed to evaluate all psychometric properties, as outlined by the OMERACT filter (truth, discrimination, and feasibility) [28], to support the endorsement of instruments for assessing this domain in these populations.

The main limitation of this study is the substitution of the patient assessment of peripheral pain by the BASDAI question 3. However, aside for excluding by the hip joints (that are specifically ignored in BASDAI question 3), the question is expected to capture a similar symptom. Another limitation is the cross-sectional nature of the dataset, which prevented the assessment of longitudinal construct validity (sensitivity to change) or trial discrimination. Nevertheless, since the original-DAPSA and DAPSA28 have been shown to be sensitive to change, it is highly likely that the DAPSA44, with demonstrated good validity and discrimination across known groups and used a joint count between the 1 from the original-DAPSA and the DAPSA28, will also be sensitive to change.

However, the strength of the study lies in the ASAS-perSpA international cohort, with data from diverse countries, varying socioeconomic status and cultural diversity resulting in a highly heterogeneous population [32]. Additionally, the inclusion of 3 disease phenotypes allowed us to evaluate the performance of DAPSA44 not only in the overall SpA population but also to confirm a good performance within each disease phenotype subgroup, adding robustness to the findings. Nevertheless, external validation of the DAPSA44 is warranted to further confirm its performance.

To summarise, when in existing databases only SJC44/TJC44 are available, the DASPA44 can be calculated by applying a conversion factor to the original DAPSA. Due to its easier calculation, with almost no difference in results, we recommend using the single 1-decimal conversion factor of 1.3 for both SJC and TJC. The construct validity analysis supports the use of DAPSA44 as a comparable tool to the original DAPSA for assessing disease activity, particularly in situations where only a reduced joint count is available.

Acknowledgements

We would like to thank all the collaborators who participated in the study: José Maldonado-Cocco (Buenos Aires University School of Medicine, Buenos Aires, Argentina), Hernán Maldonado Ficco (Hospital San Antonio de Padua, Rio Cuarto, Argentina), Rodolfo Pérez Alamino (Hospital Dr. Nicolás Avellaneda, Tucumán, Argentina), Emilio Buschiazzo (Hospital Señor del Milagro, Salta, Argentina), Romina Calvo (Hospital Provincial Dr. José M. Cullen, Santa Fé, Aregntina), Vanesa Duarte (Clínica Monte Grande, Buenos Aires, Argentina), Maria Victoria Martire (Instituto Médico Platense, La Plata, Argentina), Diego Baenas (Hospital Privado de Córdoba, Córdoba, Argentina), Dora

Pereira (Hospital Ricardo Gutiérrez, La Plata, Argentina), Adrian Salas (Consultorio Reumatológico, La Plata, Argentina), Juan Manuel Bande (Hospital General de Agudos Dr. E Tornú, Buenos Aires, Argentina), Alberto Berman (Centro Médico Privado de Tucumán, Tucumán, Argentina), Walter P Maksymowych (University of Alberta, Canada), Stephanie Belton (University of Alberta, Canada), Sebastián Ibáñez (Facultad de Medicina Clínica Alemana – Universdidad del Desarrollo, Santiago de Chile, Chile), María Paz Poblete (Facultad de Medicina Clínica Alemana – Universidad del Desarrollo, Santiago de Chile, Chile), Francisca Valenzuela (Facultad de Medicina Clínica Alemana – Universidad del Desarrollo, Santiago de Chile, Chile), Wilson Bautista-Molano (University Hospital Fundación Santa Fé de Bogotá, Bogotá, Colombia), Jieruo Gu (Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China), Min Xiao (Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China), CS Lau (Hong-Kong University, China), Ho Yin Chung (Hong-Kong University, China), Bassel Elzorkany (Cairo University, Cairo, Egypt), Sherif Gamal (Cairo University, Cairo, Egypt), Catherine Lebourlout (Cochin Hospital, Paris, France), Daniel Wendling (CHU Besançon, Besançon, France), Clément Prati (CHU Besançon, Besançon, France), Frank Verhoeven (CHU Besançon, Besançon, France), Martin Soubrier (CHU Clermont-Ferrand, Clermont-Ferrand, France), Carine Savel (CHU Clermont-Ferrand, Clermont-Ferrand, France), Trigui Alia (CHU Clermont-Ferrand, Clermont-Ferrand, France), Fan Angélique (CHU Clermont-Ferrand, Clermont-Ferrand, France), Pascal Claudepierre (Henri Mondor Hospital, Créteil, France), Valerie Farrenq (Henri Mondor Hospital, Créteil, France), Kamelia Faramarz (Henri Mondor Hospital, Créteil, France), Uta Kiltz (Rheumazentrum Ruhrgebiet, Herne, Germany), Isabella Sieber (Rheumazentrum Ruhrgebiet, Herne, Germany), Dories Morzeck (Rheumazentrum Ruhrgebiet, Herne, Germany), Fabian Proft (Charité University, Berlin, Germany), Pál Geher (Semmelweis University, Budapest, Hungary), Edit Toth (Flór Ferenc Hospital, Kistarcsa, Hungary), Katalin Nagy (Markhot Ferenc Hospital, Eger, Hungary), Attila Kovacs (MÁV Hospital, Szolnok, Hungary), Meghna Gavali (Nizam's Institute of Medical Sciences, Hyderabad, India), Liza Rajasekhar (Nizam's Institute of Medical Sciences, Hyderabad, India), Sapan Pandya (Smt NHL Medical College and Sardar Vallabhbhai Patel Hospital and Vedanta Institute of Medical Sciences, Ahmedabad, India), Bhowmik Meghnathi (Sri Sai Siri Hospital and Prathima Institue of Medical Sciences, Karimnagar, India), Carlomaurizio Montecucco (Fondazione IRCCS Policlinico San Matteo, Pavia, Italia), Sara Monti (Fondazione IRCCS Policlinico San Matteo, Pavia, Italia), Alessandro Biglia (Fondazione IRCCS Policlinico San Matteo, Pavia, Italia), Mitsumasa Kishimoto (Kyorin University School of Medicine, Tokyo, Japan), Akihiko Asahina (The Jikei University School of Medicine, Japan), Masato Okada (St Luke`s International University and Hospital, Japan), Tadashi Okano (Osaka City University, Japan), Yuko Kaneko (Keio University School of Medicine, Japan), Hideto Kameda (Toho University, Japan), Yoshinori Taniguchi (Kochi University, Japan), Naoto Tamura (Juntendo University School of Medicine, Japan), Shigeyoshi Tsuji (National Hospital Organization Osaka Minami Medical Center, Japan), Hiroaki Dobashi (Kagawa University Faculty of Medicine, Japan), Yoichiro Haji (Daido Hospital, Japan), Akimichi Morita (Nagoya City University, Japan), Nelly Ziade (Saint-Joseph University, Beirut, Lebanon), Nelly Salloum (Saint-Joseph University, Beirut, Lebanon), Rubén Burgos-Vargas (Hospital General de México Eduardo Liceaga, Mexico City, Mexico), Graciela Meza (CLIDITER), Julio Casasola-Vargas (Hospital General de Mexico, Mexico), César Pacheco-Tena (Hospital General Dr. Salvador Zubirán, Chihuahua, Mexico), Greta Reyes-Cordero (Hospital General Dr. Salvador Zubirán, Chihuahua, Mexico), César Ramos-Remus (Unidad de Investigación de Enfermedades Crónico Degenerativas, Jalisco, Mexico), J Dionisio Castillo (Unidad de Investigación de Enfermedades Crónico Degenerativas, Jalisco, Mexico), Laura González-López (Universidad de Guadalajara, Jalisco, Mexico), Iván Gámez-Nava (Unidad de Investigación Biomédica 02, Hospital de Especialidades, Centro Médico Nacional de Occidente, IMSS Guadalajara, Jalisco, Mexico), Najia Hajjaj-Hassouni (International University of Rabat (UIR), Rabat, Morocco), Fadoua Allali (University Mohammed V, CHU Ibn Sina, Rabat, Morocco), Hanan Rkain (University Mohammed V, CHU Ibn Sina, Rabat, Morocco), Lahcen Achemlal (University Mohammed V, CHU Ibn Sina, Rabat, Morocco), Taoufik Harzy (University Sidi Mohammed Benabdellah, CHU Hassan II, Fès, Morocco), Fernando M Pimentel-Santos (Universidade NOVA de Lisboa, Lisboa, Portugal), Santiago Rodrigues-Manica (Universidade NOVA de Lisboa, Portugal), Agna Neto (Universidade NOVA de Lisboa, Portugal), Jose Marona (Universidade NOVA de Lisboa, Portugal), Mª Joao Gonçalves (Universidade NOVA de Lisboa, Portugal), Ana Filipa Mourao (Universidade NOVA de Lisboa, Portugal), Rita Pinheiro Torres (Universidade NOVA de Lisboa, Portugal), Ruxandra Schiotis (Iuliu Hatieganu University of Medicine, Cluj-Napoca, Romania), Simona Rednic (Iuliu Hatieganu University of Medicine, Cluj-Napoca, Romania), Siao-Pin Simon (Iuliu Hatieganu University of Medicine, Cluj-Napoca, Romania), Laura Muntean (Iuliu Hatieganu University of Medicine, Cluj-Napoca, Romania), Ileana Filipescu (Iuliu Hatieganu University of Medicine, Cluj-Napoca, Romania), Maria Tamas (Iuliu Hatieganu University of Medicine, Cluj-Napoca, Romania), Laura Damian (Iuliu Hatieganu University of Medicine, Cluj-Napoca, Romania), Ioana Felea (Iuliu Hatieganu University of Medicine, Cluj-Napoca, Romania), Dana Fodor (Second Medical Clinic, Emergency Conty Hospital, Cluj-Napoca, Romania), Tae-Jong Kim (Chonnam National University Medical School and Hospital, South Korea), Hyun-Yi Kook (Chonnam National University Medical School and Hospital, South Korea), Hyun-Ju Jung (Chonnam National University Medical School and Hospital, South Korea), Tae-Hwan Kim (Hanyang University Hospital for Rheumatic Diseases, South Korea), Victoria Navarro-Compan (University Hospital La Paz, Madrid, Spain), Mireia Moreno (Hospital Parc Taulí, Barcelona, Spain), Eduardo Collantes-Estévez (Hospital Universitario Reina Sofía de Córdoba, Spain), M. Carmen Castro-Villegas (Hospital Universitario Reina Sofía, Córdoba, Spain), Cristina Fernández-Carballido (Hospital Universitario San Juan de Alicante, Alicante, Spain), Elizabeth Fernández (Hospital Universtario La Paz, Madrid, Spain), Marta Arévalo (Hospital Parc Taulí, Barcelona, Spain), Shue-Fen Luo (Chang Gung Memorial Hospital-Linkou, Taoyuan, Taiwan), Yeong-Jian Jan Wu (Chang Gung Memorial Hospital at Kee-Lung, Taiwan), Tian-Tsai Cheng (Chang Gung Memorial Hospital at Kao-Hsiung, Taiwan), Cheng-Chung Wei (Chung Sun Medical University, Taiwan), Tuncay Duruöz (Marmara University School of Medicine, Istanbul, Turkey), Servet Akar (Izmir Katip Çelebi University School of Medicine, Turkey), Ilhan Sezer (Akdeniz University School of Medicine), Umut Kalyoncu (Hacettepe University School of Medicine, Turkey), Sebnem Ataman (Ankara University School of Medicine, Turkey), Meltem Alkan Melikoglu (Erzurum Atatürk University School of Medicine, Turkey), Sami Hizmetli (Sivas Cumhuriyet University School of Medine, Turkey), Ozgur Akgul (Manisa Celal Bayar University School of Medicine, Turkey), Nilay Sahin (Balikesir University School of Medicine, Turkey), Erhan Capkin

(Karadeniz Teknik University School of Medicine, Turkey), Fatima Gluçin Ural (Ankara Yildirim Beyazit University School of Medicine, Turkey), Figen Yilmaz (Istanbul Sisli Etfal Training and Research Hospital), Ilknur Aktas (Istanbul Fatih Sultan Mehmet Training and Research Hospital, Turkey), Floris van Gaalen (Leiden University Medical Center, The Netherlands), Anne Boel (Leiden University Medical Center, The Netherlands), Mirian Starmans-Kool (Zuyderland Medical Center, The Netherlands), Femke Hoekstra-Drost (Zuyderland Medical Center, The Netherlands), Maha Abdelkadir (Maasstad Hospital in Rotterdam, The Netherlands), Angelique Weel (Maasstad Hospital in Rotterdam, The Netherlands), Pedro M. Machado (University College of London, London, UK), Marina Magrey (Cases Western Reserve University School of Medicine, Cleveland, Ohio, United States), Darerian Schueller (Cases Western Reserve University School of Medicine, Cleveland, Ohio, United States).

Contributors

DC, CL-M, AM, and SR designed the study and were involved in drafting the manuscript. DC and CL-M collected and/or prepared the data. DC analysed the data. All authors critically interpreted the results and revised the manuscript critically for important intellectual content and approved the final manuscript.

Funding

No funding was provided for the current analysis. The ASAS-perSpA study was funded by the ASAS with an unrestricted grant from AbbVie, Pfizer, Lilly, Novartis, UCB, Janssen, and Merck. The funders did not have any role in the design or conduct of the study; collection, management, analysis or interpretation of the data; preparation, review or approval of the manuscript; or decision to submit the manuscript for publication.

Competing interests

DC declares no conflict of interest. CL-M received consultant fees/speaker from UCB Novartis Janssen Lilly MSD Abbvie. DvdH received consultant fees from AbbVie, Argenx, BMS, Galapagos, Glaxo-Smith-Kline, Janssen, Lilly, Novartis, Pfizer, Takeda, and UCB Pharma. JS received grants from Abbvie, Astra-Zeneca, Eli Lilly and Company, Novartis, Galapagos, Roche, consulting fees or honoraria from AbbVie, Amgen, Ananda, Astro, BMS, Celltrion, Chugai, Eli Lilly and Company, Gilead, Immunovant, MSD, Novartis, Pfizer, Roche, R-Pharma, Samsung, Sanofi, UCB. DA received consulting fees, speakers' bureau fees, and grant/research support from AbbVie, Eli Lilly, Galapagos, Gilead, Janssen, Merck, Novartis, Pfizer, Sandoz and Sanofi. AM received grants from Biogen, Pfizer, and UCB and honoraria from AbbVie, UCB, Viatris, Amgen, BMS, Biogen, Galapagos, Janssen, Lilly and Novartis. SR received research grants from AbbVie, Galapagos, MSD, Novartis, Pfizer and UCB and honoraria or consultancy/speaker fees from AbbVie, Eli Lilly, Galapagos, Janssen, MSD, Novartis, Pfizer, UCB and Sanofi.

Patient consent for publication

Not applicable.

Ethics approval

The ethics committee from all participating hospitals have approved the study.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

No additional data are available.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.08.027.

Orcid

Dafne Capelusnik: http://orcid.org/0000-0001-9336-0416
Clementina Lopez-Medina: http://orcid.org/0000-0002-2309-5837
Désirée van der Heijde: http://orcid.org/0000-0002-5781-158X
Daniel Aletaha: http://orcid.org/0000-0003-2108-0030
Josef Smolen: http://orcid.org/0000-0002-4302-8877
Anna Molto: http://orcid.org/0000-0003-2246-1986
Sofia Ramiro: http://orcid.org/0000-0002-8899-9087

REFERENCES

[1] Rudwaleit M, van der Heijde D, Landewe R, Akkoc N, Brandt J, Chou CT, et al. The Assessment of SpondyloArthritis International Society classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general. Ann Rheum Dis 2011;70(1):25–31.
[2] Molto A, Sieper J. Peripheral spondyloarthritis: concept, diagnosis and treatment. Best Pract Res Clin Rheumatol 2018;32(3):357–68.
[3] Navarro-Compán V, Boel A, Boonen A, Mease P, Landewé R, Kiltz U, et al. The ASAS-OMERACT core domain set for axial spondyloarthritis. Semin Arthritis Rheum 2021;51(6):1342–9.
[4] Navarro-Compán V, Boel A, Boonen A, Mease PJ, Dougados M, Kiltz U, et al. Instrument selection for the ASAS core outcome set for axial spondyloarthritis. Ann Rheum Dis 2023;82(6):763–72.
[5] Lukas C, Landewé R, Sieper J, Dougados M, Davis J, Braun J, et al. Development of an ASAS-endorsed disease activity score (ASDAS) in patients with ankylosing spondylitis. Ann Rheum Dis 2009;68(1):18–24.
[6] Schoels M, Aletaha D, Funovits J, Kavanaugh A, Baker D, Smolen JS. Application of the DAREA/DAPSA score for assessment of disease activity in psoriatic arthritis. Ann Rheum Dis 2010;69(8):1441–7.
[7] Coates LC, Fransen J, Helliwell PS. Defining minimal disease activity in psoriatic arthritis: a proposed objective target for treatment. Ann Rheum Dis 2010;69(1):48–53.
[8] Smolen JS, Schöls M, Braun J, Dougados M, FitzGerald O, Gladman DD, et al. Treating axial spondyloarthritis and peripheral spondyloarthritis, especially psoriatic arthritis, to target: 2017 update of recommendations by an international task force. Ann Rheum Dis 2018;77(1):3–17.
[9] Capelusnik DMA, López Medina C, van der Heijde D, Landewé R, Dougados M, Sieper J, et al. Evaluation of instruments assessing peripheral arthritis in spondyloarthritis: an analysis of the ASAS-perSpA Study. Ann Rheum Dis 2024;84(8):1324–34.
[10] López-Medina C, Capelusnik D, Carron P, Webers C, Van den Bosch F, Boonen A, et al. Measurement properties of disease activity instruments in peripheral spondyloarthritis: a post-hoc analysis of the CRESPA trial. RMD Open 2025;11(3):e005525.
[11] Eberl G, Studnicka-Benke A, Hitzelhammer H, Gschnait F, Smolen JS. Development of a disease activity index for the assessment of reactive arthritis (DAREA). Rheumatology (Oxford) 2000;39(2):148–55.
[12] Duarte-García A, Leung YY, Coates LC, Beaton D, Christensen R, Craig ET, et al. Endorsement of the 66/68 joint count for the measurement of

musculoskeletal disease activity: OMERACT 2018 psoriatic arthritis workshop report. J Rheumatol 2019;46(8):996–1005.
[13] Michelsen B, Sexton J, Smolen JS, Aletaha D, Krogh NS, van der Heijde D, et al. Can disease activity in patients with psoriatic arthritis be adequately assessed by a modified Disease Activity index for PSoriatic Arthritis (DAPSA) based on 28 joints? Ann Rheum Dis 2018;77(12):1736–41.
[14] López-Medina C, Molto A, Sieper J, Duruöz T, Kiltz U, Elzorkany B, et al. Prevalence and distribution of peripheral musculoskeletal manifestations in spondyloarthritis including psoriatic arthritis: results of the worldwide, cross-sectional ASAS-PerSpA study. RMD Open 2021;7(1):e001450.
[15] López-Medina C, Chevret S, Molto A, Sieper J, Duruöz T, Kiltz U, et al. Identification of clinical phenotypes of peripheral involvement in patients with spondyloarthritis, including psoriatic arthritis: a cluster analysis in the worldwide ASAS-PerSpA study. RMD Open 2021;7(3):e001728.
[16] Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 1994;21(12):2286–91.
[17] Schoels MM, Aletaha D, Alasti F, Smolen JS. Disease activity in psoriatic arthritis (PsA): defining remission and treatment success using the DAPSA score. Ann Rheum Dis 2016;75(5):811–8.
[18] Portney LG, Watkins MP. Foundations of clinical research: applications to Practice. Pearson/Prentice Hall; 2015. p. 912.
[19] McHugh ML. Interrater reliability: the kappa statistic. Biochem Med (Zagreb) 2012;22(3):276–82.
[20] Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1(8476):307–10.
[21] Fuchs HA, Brooks RH, Callahan LF, Pincus T. A simplified twenty-eight-joint quantitative articular index in rheumatoid arthritis. Arthritis Rheum 1989;32(5):531–7.
[22] van der Heijde DM, van 't Hof MA, van Riel PL, Theunisse LA, Lubberts EW, van Leeuwen MA, et al. Judging disease activity in clinical practice in rheumatoid arthritis: first step in the development of a disease activity score. Ann Rheum Dis 1990;49(11):916–20.
[23] Calin A, Garrett S, Whitelock H, Kennedy LG, O'Hea J, Mallorie P, et al. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. J Rheumatol 1994;21(12):2281–5.
[24] EuroQol—a new facility for the measurement of health-related quality of life. Health Policy 1990;16(3):199–208.
[25] Kiltz U, van der Heijde D, Boonen A, Cieza A, Stucki G, Khan MA, et al. Development of a health index in patients with ankylosing spondylitis (ASAS HI): final result of a global initiative based on the ICF guided by ASAS. Ann Rheum Dis 2015;74(5):830–5.
[26] Cohen J. Statistical power analysis for the behavioral sciences. OMERACT; 1988 Associates neLE, editor:.
[27] Murad MH, Wang Z, Chu H, Lin L. When continuous outcomes are measured using different scales: guide for meta-analysis and interpretation. BMJ 2019;364:k4817.
[28] Maxwell LJ, Beaton DE, Boers M, D'Agostino MA, Conaghan PG, Grosskleg S, et al. The evolution of instrument selection for inclusion in core outcome sets at OMERACT: Filter 2.2. Semin Arthritis Rheum 2021;51(6):1320–30.
[29] Mukaka MM. Statistics corner: A guide to appropiate use of correlation coefficient in medical research. Malawa Med J 2012;24(3):69–71.
[30] Turina MC, Ramiro S, Baeten DL, Mease P, Paramarta JE, Song IH, et al. A psychometric analysis of outcome measures in peripheral spondyloarthritis. Ann Rheum Dis 2016;75(7):1302–7.
[31] Beckers E, Been M, Webers C, Boonen A, Ten Klooster PM, Vonkeman HE, et al. Performance of 3 composite measures for disease activity in peripheral spondyloarthritis. J Rheumatol 2022;49(3):256–64.
[32] Capelusnik D, Zhao SS, Boonen A, Ziade N, Medina CL, Dougados M, et al. Individual-level and country-level socio-economic factors and health outcomes in spondyloarthritis: analysis of the ASAS-perSpA study. Rheumatology (Oxford) 2022;61(5):2043–53.

Ann Rheum Dis 85 (2026) 308–318

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Axial spondyloarthritis

Final validation of the hierarchical framework for outcomes in axial spondyloarthritis: longitudinal determinants of health-related quality of life

Augusta Ortolan[1,2,6], Désirée van der Heijde[2], Laure Gossec[3,4], Sofia Ramiro[4,5,*]

[1] *Fondazione Policlinico Universitario Agostino Gemelli IRCSS, Department of Rheumatology, Rome, Italy*
[2] *Leiden University Medical Center, Department of Rheumatology, Leiden, The Netherlands*
[3] *INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, Sorbonne Universite, Paris, France*
[4] *Hopital Universitaire Pitie Salpetriere, APHP, Rheumatology Department, Paris, France*
[5] *Zuyderland Medical Center, Department of Rheumatology, Heerlen, The Netherlands*
[6] *Università Cattolica del Sacro Cuore, Rome, Italy*

ARTICLE INFO

Article history:
Received 21 August 2025
Received in revised form 13 October 2025
Accepted 14 October 2025

ABSTRACT

Objectives: This study aims to study the framework describing the longitudinal association between disease activity and function (independent variables) and health-related quality of life (HRQoL, outcome) in axial spondyloarthritis (axSpA).

Methods: Patients with AxSpA (symptoms <3 years) from the Devenir des Spondylarthropathies Indifférenciées Récentes cohort were followed up for 10 years. The association of disease activity (ASDAS) and function (BASFI) with HRQoL (physical component summary [PCS] and mental component summary [MCS] of 36-item short-form health survey, ankylosing spondylitis quality of life [ASQoL]) was assessed. Multivariable generalised estimating equations models were built with PCS, MCS, or ASQoL as outcomes (higher PCS/MCS = better HRQoL, higher ASQoL = worse), and ASDAS and BASFI as main predictors. Standard and autoregressive (i.e., corrected for prior HRQoL) models were used. The impact of ASDAS vs BASFI on HRQoL was compared through standardised coefficients. Mediation analysis assessed whether the effect of ASDAS on HRQoL was mediated by BASFI. Confounders/effect modifiers (eg, contextual factors) were considered in all models.

Results: A total of 663 patients with axSpA (46% males, mean age 33.5 [8.6] years) were included. In standard and autoregressive multivariable models, significant associations for ASDAS and BASFI with HRQoL were found (beta coefficient [95% CI] for autoregressive models: PCS −2.93 [−3.28, −2.58]; −2.13 [−2.31, −1.96]), MCS (−2.38 [−2.91, −1.86]; −1.10 [−1.36, −0.84]), ASQoL (1.18 [1.02, 1.36]; 1.08 [0.99, 1.17]). A larger influence of BASFI, compared to ASDAS, on HRQoL was noted. Mediation analysis showed the ASDAS effect on HRQoL was mostly (~75%) mediated by BASFI, but a direct effect (~25%) also existed.

Conclusions: A validated longitudinal framework has been established for interpreting HRQoL: even correcting for contextual factors, disease activity consistently impacts HRQoL—primarily through functional impairment, but also directly. While HRQoL is an overarching outcome in axSpA, targeting low disease activity is crucial to optimise HRQoL.

*Correspondence to Dr. Sofia Ramiro, Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands.
E-mail address: sofiaramiro@gmail.com (S. Ramiro).
Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.10.016

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Health-related quality of life (HRQoL) is a key outcome in axial spondyloarthritis (axSpA), known to depend on multiple factors, including disease activity and physical function. Previous cross-sectional studies in established radiographic axSpA suggested a hierarchical model where HRQoL is influenced by these domains.
- However, longitudinal confirmation of these relationships, especially across the full axSpA spectrum and in early disease, is still lacking. Furthermore, it is not known whether disease activity has a direct effect on HRQoL or mostly acts through alteration of physical function. Finally, the role of contextual factors has not been systematically explored.

WHAT THIS STUDY ADDS

- This study demonstrates that both disease activity and physical function are longitudinal determinants of HRQoL in patients with axSpA. It validates, over a 10-year follow-up, a hierarchical framework showing that HRQoL is influenced by disease activity both directly and indirectly through physical function.
- Additionally, functional impairment appears to exert a larger impact on HRQoL than disease activity itself. The longitudinal relationship among disease activity, function, and HRQoL, controlling for the relevant contextual factors (eg, gender, education, smoking), and extending to both radiographic and nonradiographic axSpA, is here demonstrated for the first time.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- This study confirms HRQoL as an overarching outcome in axSpA, depending longitudinally on various interrelated factors, with disease activity playing a key role.
- This study underscores the importance of persistently targeting low disease activity and preserving physical function throughout the disease course with the aim of optimising HRQoL.

INTRODUCTION

Health-related quality of life (HRQoL) in axial spondyloarthritis (axSpA) is a broad outcome influenced by multiple factors, including disease-related aspects, such as disease activity and physical function, as well as the patient's physical and social environment [1]. Previous studies in radiographic axSpA (r-axSpA, formerly ankylosing spondylitis) [2] have proposed a hierarchical model placing HRQoL at the top, as all other disease-related outcomes appear to contribute to it independently, to some extent (Supplementary Fig S1) [1].

This model was initially developed cross-sectionally in r-axSpA. More recently, longitudinal studies from the Devenir des Spondylarthropathies Indifférénciées Récentes (DESIR) cohort, including also nonradiographic axSpA (nr-axSpA), have confirmed several associations within this hierarchy. Specifically, a relationship over time between disease activity and spinal inflammation on one hand, and spinal mobility however, has been proven [3]. Moreover, disease activity over the axSpA course has been shown to be the main contributor of disability (i.e., loss of function), with spinal mobility becoming particularly relevant in advanced-stage disease [4]. What remains to be demonstrated is whether HRQoL truly depends on disease activity and physical function over the disease course (relationships with red lines in Supplementary Fig S1), and on their interaction with contextual factors. The importance of contextual factors has, in fact, been suggested by previous studies: maintaining low disease activity over time was associated with male sex, white-collar occupation, and higher education [5]. Cross-sectional analyses confirmed that disease activity and various non-disease-related factors contribute to HRQoL, though collectively accounting for only half of its variance [6]. Furthermore, it remains unclear whether these associations hold over time, in a longitudinal context.

In summary, the longitudinal relationship among disease activity, function, and HRQoL, across the whole spectrum of axSpA, particularly in earlier disease phases, and accounting for contextual factors, needs to be demonstrated. Thus, we set out to investigate these relationships in a representative axSpA population, including both r-axSpA and nr-axSpA.

Our primary aim was to investigate the longitudinal relationship between disease activity and physical function (independent variables) and HRQoL (dependent variable) over 10 years of follow-up. Our secondary aim was to determine which domain—disease activity or physical function—contributed more to HRQoL, and whether this contribution was direct or mediated, i.e., whether the impact of disease activity on HRQoL was direct or mediated through physical function.

METHODS

Patient population

Patients included in the DESIR cohort, diagnosed as axSpA by their treating rheumatologist, were included in the study. DESIR is an ongoing prospective observational and inception cohort involving 25 centres in France and enrolling patients with inflammatory back pain with a duration <3 years, and a high suspicion of axSpA (level of confidence ≥ 5 on a 0-10 scale), with a 10-year-long follow-up (NCT01648907) [7]. For the present study, only patients with a diagnosis of axSpA confirmed over the 10-year follow-up were considered. Patients' outcomes have been collected at baseline, every 6 months for the first 2 years, and yearly thereafter up to 10 years. The dataset used for this analysis was locked in November 2022. All patients provided signed informed consent before inclusion, and the study was approved by the French medical ethical committee.

Outcome: HRQoL

HRQoL was measured by different instruments, to verify consistency of the obtained results: the physical and mental component summary of the short form (36-item short-form health survey [SF-36]) (PCS, MCS) [8], a generic and multidimensional questionnaire, and the ankylosing spondylitis quality of life (ASQoL) questionnaire, more specific to axSpA [9]. SF-36 includes 36 questions assessing functional health and well-being, organised into 8 health domains, which contribute to PCS and MCS. Both are scored from 0 to 100, with higher scores indicating better health [10]. ASQoL is instead composed of 18 dichotomous items, with a total score ranging from 0 to 18, with higher scores indicating worse health [9].

Main independent variables: disease activity and function

The 2 main independent variables of interest, contributing to HRQoL (outcome), according to our hypothesis, were disease activity and function. Disease activity was primarily assessed by the Axial Spondyloarthritis Disease Activity Score (ASDAS),

which is the preferred measurement instrument according to the Assessment of SpondyloArthritis International Society (ASAS) core set, as it performs best in all the OMERACT filter 2.2 properties [11]. ASDAS is a weighted composite index including back pain (question #2 of the Bath Ankylosing Spondylitis Disease Activity Index [BASDAI]), peripheral pain (question #3 of the BASDAI), duration of morning stiffness (question #6 of the BASDAI), patient global assessment, and C-reactive protein (CRP). It gives a continuous score with higher values corresponding to worse disease activity, and has shown better validity and sensitivity to change than the other disease activity indices [11−13]. BASDAI and CRP were still taken into consideration as alternative measures of disease activity in a sensitivity analysis [14]. Physical function was assessed by Bath Ankylosing Spondylitis Functional Index (BASFI) [15], which ranges between 0 and 10, with higher scores indicating higher impairment in physical function.

Covariates: contextual factors

SpA features according to ASAS, personal and environmental factors, and therapy were considered as covariates (i.e., potential confounders or effect modifiers) in the relationship between ASDAS and BASFI, on one side, and HRQoL, on the other side. The following baseline covariates were used as time-fixed: sex, age (years), Human Leukocyte Antigen- B27 (HLA-B27) positivity, and positive baseline imaging (radiographic sacroiliitis according to modified New York Criteria or magnetic resonance imaging [MRI] sacroiliitis according to ASAS definition, according to central reading) [16,17]. Covariates that were collected at each follow-up point (time-varying covariates) were extra-musculoskeletal manifestations (psoriasis, inflammatory bowel disease [IBD], uveitis), peripheral manifestations (enthesitis, dactylitis, peripheral arthritis, enthesitis assessed by Maastricht Ankylosing Spondylitis Disease Activity score), body mass index (BMI, kg/m^2), smoking (current smoker vs nonsmoker), occupation (blue collar vs white collar), education (university education or equivalent yes/no), marital status (married/living together yes/no), parental status (number of children), use of nonsteroidal anti-inflammatory drugs (NSAIDs) (yes/no) or biological disease-modifying antirheumatic drugs (bDMARDs) (yes/no), comorbidities (simple comorbidity count, excluding depression due to its particular relationship with the outcome, especially MCS). Chronic comorbidities available in DESIR are lung disease, ischaemic heart disease, pericarditis, aortic insufficiency, other valvopathy, conduction system defects, cardiac insufficiency, stroke/transient ischaemic attack, cardiovascular accident, diabetes, upper gastrointestinal disease (ulcer, perforation, haemorrhage), and solid and lymphoproliferative neoplasm. To each of these comorbidities, a score of 1 was given if present (0-15).

Statistical analysis

The longitudinal association between disease activity, function, and HRQoL

Associations between the independent variables (ASDAS and BASFI) and the HRQoL outcomes over time were tested using generalised estimating equations (GEE) models: GEE is a technique suitable for modelling longitudinal data, as it makes use of all time points with information available, being relatively robust for missing data, and taking into account the intrasubject correlation across the various time points. The results are expressed in terms of the beta coefficient (which is an expression of the 'average' change in the units of outcome for a 1-unit increase of the independent variable) with its 95% CI. Among the GEE assumptions, it is required to specify a working correlation structure: in our case, the exchangeable correlation structure was selected, as it seemed to best fit the correlation structure between the outcomes at the various time points. First, univariable associations were investigated between ASDAS or BASFI as main independent variables and PCS, MCS, or ASQoL as outcome, each in a separate model. Since disease activity is known to influence function [18], we assessed whether there was an effect modification of the effect of BASFI on HRQoL by different levels of disease activity (ASDAS≥2.1 vs ASDAS < 2.1). Effect modification was considered to be potentially relevant if the interaction term was associated with the outcome with a $P <$ .15, in which case analyses would be stratified into different levels of the potential effect modifier, and clinical significance was judged, according to the different magnitude or direction of beta coefficients in the strata. In a further step, ASDAS and BASFI were considered together as main independent variables (so that each was adjusted for the other) in bivariable GEE models having PCS, MCS, or ASQoL as outcomes. Based on the literature and our hypotheses, the following covariates were tested as potential effect modifiers of the relationship between ASDAS or BASFI, on the one hand, and HRQoL, however: sex, HLA-B27, imaging at baseline (sacroiliitis modified New York, sacroiliitis MRI), occupation (blue collar vs white collar), smoking, NSAID-use, bDMARD-use, and comorbidity count [19−22].

The remaining contextual factors (age, psoriasis, IBD, uveitis, enthesitis, dactylitis, peripheral arthritis, BMI, smoking, education, marital status, parental status), as well as those shown not to be an effect modifier of the main relationships of interest, were tested as potential confounders. Confounders were considered to be relevant if the addition of the variable to the model led to a significant modification of the association between either ASDAS or BASFI and one of the HRQoL outcomes (variation of >10% of the coefficient). Furthermore, the univariable association between each of the covariates and each of the HRQoL outcomes was tested. These variables were included in the subsequent multivariable models if they were associated with the outcome with a P value <.1. Collinearity between covariates, as well as the opportunity to combine more variables into one (e.g., choosing between the variable grouping extramusculoskeletal manifestations or the single variables psoriasis, IBD, uveitis), were taken into consideration in the final covariate selection.

Multivariable models for the longitudinal relationship between disease activity, function, and HRQoL

Thereafter, three separate 'standard' GEE multivariable models were built, having PCS, MCS, or ASQoL as outcomes, ASDAS and BASFI as main independent variables, and all the covariates that had been found to be confounders, or significantly associated to the outcome in the univariable analysis, or that were considered to be important from a clinical point of view (sex, symptom duration, use of NSAIDS and bDMARDs were included *a priori*) [23−25]. To better identify the longitudinal effects, and to get more insight into the true longitudinal relationship between disease activity, function, and HRQoL, three 'autoregressive' GEE multivariable models GEE models were run: this means the models were adjusted for the outcome (ie, PCS, MCS, or ASQoL) at the previous time point (1 year earlier). For this analysis, visits at 6 and 18 months were dropped to ensure an equal lag between all timepoints. By comparing the autoregressive models with the standard models, we aimed to understand whether the associations were really present over

time, and not only cross-sectionally. Standard and autoregressive GEE multivariable models were also repeated with BASDAI and CRP instead of ASDAS as a sensitivity analysis.

Hypothesis about the magnitude and temporal structure of the influence of disease activity and function on HRQoL

To assess whether disease activity or function had a larger impact on HRQoL, we repeated the autoregressive GEE models using standardised coefficients, enabling comparison of effect sizes across variables with different scales. The computation applied to standardise each value of the independent variables of the models was standardised variable = (variable value − mean value)/SD. The obtained standardised variables were then used in the autoregressive models, thus obtaining standardised coefficients.

To explore whether disease activity influenced HRQoL through function or also independently, we conducted a mediation analysis. We hypothesised that ASDAS at a given time point affects BASFI (a), which in turn predicts HRQoL one year later (b), with a direct effect from ASDAS to HRQoL (c') also included for partial mediation (Fig 1A). The analysis used structural equation modelling, estimating both direct and indirect effects with maximum likelihood and 1000 bootstrap replications for robust SEs and 95% CIs. The indirect effect (a × b) was assessed via percentile-based bootstrap CI, preferred due to the non-normal distribution of indirect effects. The total effect (c) was the sum of direct (c') and indirect (a × b) effects (Fig 1A).

As a sensitivity analysis, we tested an alternative model where ASDAS at a given time point influenced BASFI 1 year later, which then predicted HRQoL at that same time.

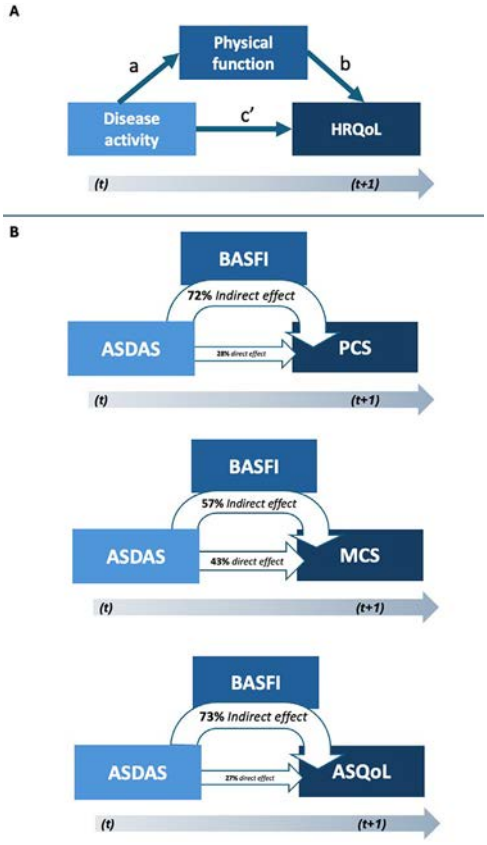


Figure 1. (A) Mediation hypothesis, general structure: the total effect (c) of disease activity (ASDAS) on HRQoL is the sum of a direct (c') and an indirect (a × b) effect, the latter mediated by BASFI. The timeline at the bottom of the figure indicates that a longitudinal relationship between BASDAI at a certain timepoint (t) and HRQoL at a following timepoint (t + 1) is assumed. (B) Mediation analysis, with actual estimation of direct and indirect effects. ASDAS, Axial Spondyloarthritis Disease Activity Score; ASQoL, Ankylosing Spondylitis Quality of Life questionnaire; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; HRQoL, health-related quality of life; MCS, mental component summary; PCS, physical component summary.

RESULTS

A total of 663 patients with axSpA were analysed, of whom 306 (46%) were males, 392 (59%) were HLA-B27 positive, and 141 (22%) were classified as r-axSpA at baseline. At inclusion in the cohort, mean (SD) age was 33.5 (8.6) years, and mean symptom duration was 1.5 (0.9) years (Table 1). Mean values of PCS, MCS, and ASQoL at baseline were 39.3 (9.5), 40.3 (11.2), and 9.3 (4.9), respectively, and had very little variation over time except a slight amelioration in HRQoL (increase in PCS/MCS and decrease in ASQoL) from baseline to 6 months (Supplementary Table S1).

Table 1
Baseline characteristics of the population

Variables	N = 663
Male sex	306 (46%)
Age (y)	33.5 (8.6)
Symptom duration (y)	1.5 (0.9)
HLA-B27 positive	392 (59%)
University education or equivalent	390 (59%)
Married/living maritally	413 (62%)
Parental status (n of children)	
No children	283 (44%)
One child	126 (19%)
Two or more children	254 (35%)
Employed	570 (86%)
Blue-collar job	98 (17%)
Active smoking	257 (37%)
Psoriasis (ever)	115 (17%)
IBD (ever)	35 (5%)
Uveitis (ever)	64 (10%)
Dactylitis (ever)	96 (14%)
Radiographic sacroiliitis (mNY)	141 (22%)
MRI sacroiliitis (ASAS definition)	233 (37%)
ASDAS	2.6 (0.9)
BASDAI (0-10)	4.4 (2.0)
BASFI (0-10)	3.0 (2.3)
MASES (0-13)	4.3 (5.9)
Swollen joint count (0-28)	0.2 (0.8)
Tender joint count (0-53)	4.2 (8.3)
Body mass index (kg/m^2)	23.9 (4.1)
NSAIDs use	614 (92%)
csDMARDs use	93 (13%)
bDMARDs use	0 (0%)
Comorbidity count (0-15)	
No comorbidities	336 (48%)
One comorbidity	311 (44%)
Two or more comorbidities	49 (8%)

ASAS, Assessment of SpondyloArthritis International Society; ASDAS, Axial Spondyloarthritis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; bDMARDs, biological disease-modifying antirheumatic drugs; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; HLA-B27, human leukocyte antigen B27; IBD, inflammatory bowel disease; MASES, Maastricht Ankylosing Spondylitis Disease Activity Score; MRI, magnetic resonance imaging; mNY, modified New York; NSAIDs, nonsteroidal anti-inflammatory drugs.

In the univariable analysis, ASDAS and BASFI were individually, strongly and significantly associated to PCS (beta −5.61 [95% CI: −5.80, −5.41] and −3.20 [−3.29, −3.11]), MCS (beta −3.99 [95% CI: −4.26, −3.71] and −2.06 [−2.20 −1.91], respectively), and ASQoL (beta 2.65 [2.55, 2.74] and 1.58 [1.53, 1.63]), with higher disease activity and higher functional disability associating to worse HRQoL scores. The interaction between disease activity and function was statistically significant, but when analysing function by disease activity strata, the effect of BASFI on the HRQoL outcomes was not substantially different between the strata, keeping effect sizes pointing in the same direction. Therefore, the interaction was not considered to be clinically relevant (Supplementary Table S2), so the models were not further stratified according to disease activity. In bivariable models, the association of ASDAS and BASFI, together as main independent variables, to PCS (−3.07 [95% CI: −3.32, −2.83] and −2.24 [−2.37, −2.11], respectively), MCS (beta −2.43 [95% CI: −2.84, −2.04] and 1.23 [−1.43, −1.03]), and ASQoL (1.35 [95% CI: 1.23, 1.48] and 1.16 [1.09, 1.22]) was confirmed.

When potential effect modifiers of the relationship between disease activity or function and HRQoL were considered, none of the contextual factors qualified as a clinically significant effect modifier. In other words, even when the interaction term had a $P < .15$, the beta coefficients for ASDAS or BASDAI in different strata of the covariate were not different enough to require separate models. Also, none of the remaining contextual factors qualified as confounders. Nonetheless, the univariable analysis between each contextual factor and the outcome was studied to select potentially relevant covariates to include in the multivariable models (Table 2).

Then, three different multivariable models having PCS, MCS, and AsQOL as outcome, and ASDAS and BASFI as main independent variables (across all models), along with the relevant covariates of Table 2 were then built, confirming the independent association between higher disease activity and higher functional impairment, on the one hand, and worse HRQoL however in the standard GEE model (Table 3).

For the autoregressive models, the univariable associations between each of the covariates and HRQoL were tested again, but this time correcting the models for the outcome at the previous time point (Supplementary Table S3). Then, the autoregressive multivariable models were built. The results of the models confirmed that indeed a higher disease activity and a worse function are major determinants for HRQoL outcomes, even when taking into consideration the strongest determinant of an outcome, which is the outcome in the previous time points, as well as taking other important covariates into account (Fig 2).

Results of the above models (standard and autoregressive models) were confirmed using BASDAI and CRP instead of ASDAS (Supplementary Tables S4 and S5). However, it has to be underlined that only BASDAI—and not CRP—was associated with PCS, MCS, and ASQoL. BASFI remained independently and strongly associated with all three HRQoL outcomes.

Moreover, when standardised variables were used in the autoregressive multivariable models, it was noted that standardised coefficients were consistently higher for BASFI than ASDAS, even though this was more evident for PCS and ASQoL rather than MCS (Table 4).

The results of the mediation analysis (Table 5) showed that, when PCS was considered as the key outcome, the total effect (direct and indirect) of ASDAS on PCS was −4.31, of which −1.22 being direct effect, and −3.09 indirect effect. Thus, the proportion of indirect (or mediated) effect was −3.09/−4.31, meaning that 72% of the effect of ASDAS on PCS was mediated by BASFI (Fig 1B). When MCS was considered as outcome, the

Table 2
Univariable associations with HRQoL outcomes

	PCS		MCS		ASQOL	
	Beta (95% CI)	*P* value	Beta (95% CI)	*P* value	Beta (95% CI)	*P* value
Male sex	4.17 (3.00, 5.35)	<.0001	2.55 (1.28, 3.82)	<.0001	−2.57 (−3.25, −1.90)	<.0001
Age (y)	−001 (−0.006, 0.003)	.67	−0.001 (−0.006, 0.005)	.80	0.0007 (−0.001, 0.003)	.54
HLA-B27 positive	3.61 (2.38, 4.83)	<.0001	2.59 (1.29, 3.88)	<.0001	−1.84 (−2.54, −1.13)	<.0001
Radiographic sacroiliitis (mNY)	1.70 (0.19, 3.20)	.027	0.59 (−0.97, 2.16)	.46	−0.59 (−1.46, 0.28)	.18
MRI sacroiliitis (ASAS definition)	2.31 (1.01, 3.61)	.001	1.15 (−0.20, 2.50)	.09	−1.15 (−1.90, −0.41)	.002
Psoriasis	0.84 (−0.04, 1.63)	.039	0.99 (0.04, 1.93)	.04	−0.99 (−1.40, −0.59)	<.001
IBD	−0.49 (−1.72, 0.75)	.44	−0.94 (−2.40, 0.53)	.21	−0.21 (−0.83, 0.41)	.51
Uveitis	−0.44 (−1.44, 0.56)	.39	1.69 (−0.51, 2.88)	.005	−0.58 (−1.08, −0.07)	.026
EMMs	0.11 (−0.55, 0.77)	.75	0.79 (−0.005, 1.59)	.048	−0.79 (−1.12, 0.45)	<.0001
MASES (0-13)	−0.09 (−0.12, −0.06)	<.0001	−0.06 (−0.09, −0.02)	.003	0.05 (0.03, 0.06)	<.0001
SJC (0-28)	−0.65 (−0.89, −0.40)	<.0001	−0.49 (−0.80, −0.19)	.002	0.25 (0.13, 0.36)	<.0001
Dactylitis	0.29 (−0.56, 1.15)	.50	1.38 (0.36, 2.40)	.008	−0.45 (−0.89, −0.01)	.045
Peripheral manifestations	−0.37 (−1.22, 0.48)	.39	−0.04 (−0.11, 0.10)	.92	−0.19 (−0.56, 0.17)	.30
BMI (kg/m^2)	−0.14 (−0.23, −0.05)	.002	−0.003 (−0.08, 0.14)	.95	0.02 (−0.02, 0.07)	.37
University education	3.84 (2.61, 5.07)	<.0001	3.57 (2.29, 4.86)	<.0001	−2.50 (−3.20, −1.81)	<.0001
Married/living maritally	−0.10 (−0.69, 0.50)	.75	0.53 (−0.19, 1.25)	.15	−0.001 (−0.30, 0.29)	.99
Parental status (n of children)	−0.04 (−0.36, 0.29)	.82	−0.12 (−0.50, 0.25)	.53	−0.13 (−0.30, 0.03)	.12
Blue-collar job	0.51 (−0.37, 1.37)	.25	−0.29 (−1.32, 0.73)	.57	0.04 (−0.39, 0.47)	.86
Active smoking	−0.55 (−1.13, 0.03)	.06	−1.45 (−2.16, −0.78)	<.0001	0.55 (0.26, 0.84)	<.0001
NSAIDs	−3.19 (−3.67, −2.72)	<.0001	−1.96 (−2.56, −1.37)	<.0001	1.72 (1.49, 1.95)	<.0001
bDMARDs	1.47 (0.92, 2.05)	<.0001	1.36 (0.67. 2.05)	<.0001	−1.24 (−1.52, −0.96)	<.0001
Comorbidity count (0-15)	−0.43 (−0.79, −0.08)	.016	0.28 (−0.15, 0.72)	.20	0.07 (−0.11, 0.25)	.44

ASAS, Assessment of SpondyloArthritis International Society; ASQoL, Ankylosing Spondylitis Quality of Life questionnaire; bDMARDs, biological disease-modifying antirheumatic drugs; BMI, body mass index; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; EMMs, extra musculoskeletal manifestations; HLA, human leukocyte antigen; HRQoL, health-related quality of life; IBD, inflammatory bowel disease; MASES, Maastricht Ankylosing Spondylitis Disease Activity Score; MCS, mental component summary; mNY, modified New York; MRI, magnetic resonance imaging; NSAIDs, nonsteroidal anti-inflammatory drugs; PCS, physical component summary; SJC, swollen joint count.

Table 3
'Standard' GEE multivariable models highlighting the associations between the main independent variables (disease activity and function) and the outcome (HRQoL) over time

	PCS N = 603 Beta (95% CI)	MCS N = 614 Beta (95% CI)	ASQOL N = 613 Beta (95% CI)
ASDAS	−3.01 (−3.27, −2.74)[a]	−2.74 (−3.16, −2.33)[a]	1.38 (1.25, 1.51)[a]
BASFI	−2.25 (−2.38, −2.12)[a]	−1.08 (−1.28, −0.87)[a]	1.10 (1.03, 1.16)[a]
Male sex	1.12 (0.40, 1.84)[a]	1.03 (−0.13, 2.21)	−1.23 (−1.65, −0.81)[a]
Age (y)	0.02 (−0.01, 0.05)	0.01 (−0.04, 0.06)	−0.02 (−0.04, −0.01)[a]
HLA-B27	0.67 (−0.07, 1.41)	0.78 (−0.41, 1.97)	−0.38 (−0.81, 0.05)
Radiographic sacroiliitis (mNY)	0.51 (−0.41, 1.44)	N/A	N/A
MRI sacroiliitis (ASAS definition)	0.74 (−0.04, 1.52)	0.59 (−0.60, 1.77)	−0.48 (−0.91, −0.05)[a]
Psoriasis	0.07 (−0.53, 0.68)	0.56 (−0.40, 1.53)	−0.22 (−0.54, 0.10)
IBD	N/A	N/A	N/A
Uveitis	N/A	0.85 (−0.38, 2.09)	−0.08 (−0.49, 0.33)
EMM	N/A	N/A	N/A
MASES (0-13)	−0.01 (−0.03, 0.01)	−0.01 (−0.03, 0.04)	0.01 (−0.002, 0.02)
SJC (0-28)	−0.01 (−0.23, 0.26)	−0.13 (−0.53, 0.26)	0.08 (−0.19, 0.03)
Dactylitis	N/A	0.10 (−0.97, 1.18)	0.18 (−0.18, 0.54)
Peripheral manifestations	N/A	N/A	N/A
BMI	0.08 (0.01, 0.15)[a]	N/A	N/A
University education	0.21 (−0.51, 0.95)	0.67 (−0.50, 1.85)	−0.62 (−1.04, −0.20)[a]
Married/living maritally	N/A	N/A	N/A
Parental status (n of children)	N/A	N/A	N/A
Blue-collar job	N/A	N/A	N/A
Active smoking	0.43 (−0.04, 0.91)	−1.04 (−1.79, −0.29)[a]	0.18 (−0.05, 0.43)
NSAIDs	−1.10 (−1.51, −0.70)[a]	−0.38 (−1.02, 0.26)	0.60 (0.40, 0.80)[a]
bDMARDs	−1.40 (−1.86, −0.95)[a]	−0.47 (−1.20, 0.25)	0.25 (0.02, 0.49)[a]
Comorbidity count (0-15)	−0.38 (−0.67, −0.09)[a]	N/A	N/A

ASAS, Assessment of SpondyloArthritis International Society; ASDAS, Axial Spondyloarthritis Disease Activity Score; ASQoL, Ankylosing Spondylitis Quality of Life questionnaire; BASFI, Bath Ankylosing Spondylitis Functional Index; bDMARDs, biological disease-modifying antirheumatic drugs; BMI, body mass index; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; EMMs, extramusculoskeletal manifestations; GEE, generalised estimating equation; HRQoL, health-related quality of life; HLA, human leukocyte antigen; IBD, inflammatory bowel disease; MASES, Maastricht Ankylosing Spondylitis Disease Activity Score; MCS, mental component summary; mNY, modified New York; MRI, magnetic resonance imaging; N/A, not included in the final multivariable model due to collinearity with other variables, or because $P > .1$ at univariable analysis; NSAIDs, nonsteroidal anti-inflammatory drugs; PCS, physical component summary; SJC, swollen joint count.
[a] Indicates significant results ($P < .05$).

total effect was −3.64, with −1.55 of direct effect and −2.09 indirect effect, meaning 57% of the ASDAS effect on MCS was mediated by BASFI (Fig 1B). When ASQoL was considered as outcome, the total effect was 2.17 (0.59 direct and 1.58 indirect effect), meaning that 73% of the ASDAS effect on ASQoL was mediated by BASFI (Fig 1B). The mediation analysis carried out with the second hypothesis (ASDAS influencing BASFI one year later, in turn influencing HRQoL at that same time point) showed very similar results (Supplementary Table S6 and Supplementary Fig S2).

Following all these analyses, and taking into account the previously presented parts of the framework showing a longitudinal relationship, we were able to complete the longitudinal validation of the hierarchical model for outcomes in axSpA, where HRQoL is the overarching outcome, and finalise it (Fig 3).

DISCUSSION

This work provided a final explanatory framework to describe the longitudinal relationship between disease activity and function, on the one hand, and HRQoL, on the other hand, correcting for the main contextual factors. Our results allow us to interpret HRQoL as an overarching outcome in axSpA: in other words, an outcome that can be seen as a consequence of other aspects of the disease. We confirmed the existence of a relationship between the outcomes of interest, not only at a cross-sectional level in r-axSpA [1], but also over time, and including nr-axSpA as well, therefore, in the whole spectrum of axSpA. We also went further by providing a relative 'weight' of the effect that disease activity and function, respectively, exert on HRQoL, and by analysing the mediation path that links disease activity and HRQoL through functional impairment. Because disease activity and function are closely linked, both were modelled as independent variables, allowing mutual adjustment and testing of mediation pathways to clarify their distinct effects on HRQoL. Therefore, we were able to demonstrate that targeting low disease activity is crucial to optimise HRQoL and hereby provide a full explanatory model of outcomes in axSpA (Fig 3).

The longitudinal association between disease activity, function, and HRQoL might seem intuitive, but it is not to be taken for granted, as many factors can influence HRQoL [6]; thus, the importance of disease activity in determining HRQoL could be questioned. Instead, we were able to complete the validation of the model originally proposed at a cross-sectional level by Machado et al. [1], and complement previous studies which had already elucidated other longitudinal relationships in the model [3,26].

The issue of what explains HRQoL is not an easy one to tackle, as it has been shown, as mentioned, that several nondisease related characteristics—such as age, smoking, socioeconomic background, and education—can influence HRQoL [21,22,27,28]. While smoking is a potentially modifiable factor,

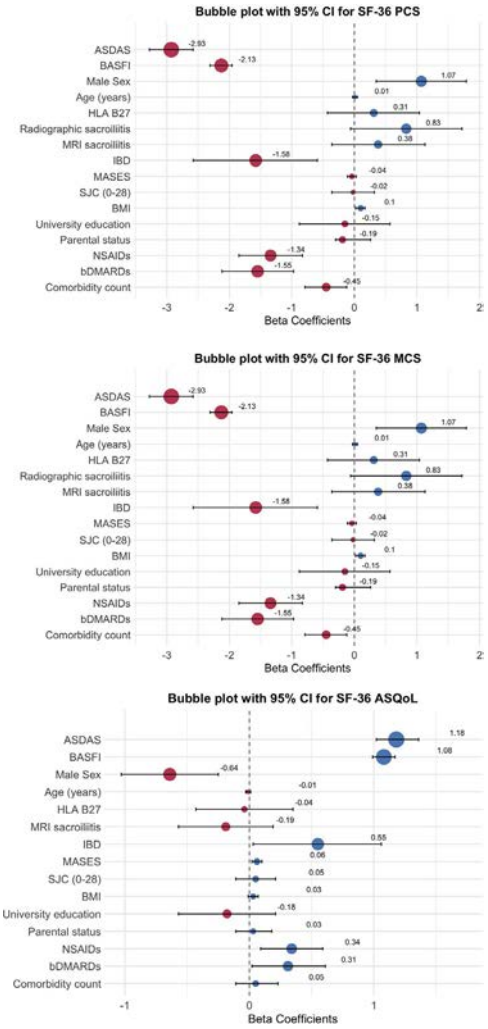


Figure 2. Longitudinal relationship between disease activity (ASDAS) and function (BASFI), on the one hand, and HRQoL outcomes (MCS, PCS, and ASQoL) on the other hand. Estimates reflect regression coefficients from autoregressive multivariable GEE models. Blue dots indicate positive regression coefficients, while red dots indicate negative regression coefficients. Independent variables ordered for comparison across models as main independent variables (ASDAS and BASFI), demographics, entry criteria of ASAS criteria, SpA features significant at univariable analysis, socioeconomic variables, therapy, comorbidity count. ASDAS, Axial Spondyloarthritis Disease Activity Score; ASQoL, Ankylosing Spondylitis Quality of Life questionnaire; BASFI, Bath Ankylosing Spondylitis Functional Index; bDMARDs, biological disease-modifying antirheumatic drugs; BMI, body mass index; GEE, generalised estimating equation; HLA, human leukocyte antigen; HRQoL, health-related quality of life; IBD, inflammatory bowel disease; MCS, mental component summary; MRI, magnetic resonance imaging; MASES, Maastricht Ankylosing Spondylitis Disease Activity Score; NSAIDs, nonsteroidal anti-inflammatory drugs; PCS, physical component summary; SF-36, 36-item short-form health survey; SJC, swollen joint count; SpA, spondyloarthritis.

the other factors are largely nonmodifiable or only minimally. This might lead to the perception that HRQoL is also scarcely modifiable, therefore potentially less of a priority for a therapeutic intervention. Our study considered disease-related and non-disease-related factors, both modifiable and non-modifiable, and came to the result that HRQoL is very largely and consistently influenced by disease activity and function in patients with axSpA, even when taking into account important confounders.

While acknowledging and confirming the role of non-disease-related factors such as sex, education, or comorbidities, our study highlighted how BASFI and ASDAS significantly impacted the physical and mental aspects of HRQoL. The fact that this relationship stands in a longitudinal way means that not only the current disease activity and function influence HRQoL [23,27–29], but that the entire course of the disease determines HRQoL over time. Translated into practice, this means we should not only aim at achieving remission or low disease activity at the beginning of the disease, but persistently over the axSpA course.

It is true, however, that most of the HRQoL improvement is achieved in the DESIR cohort over the first 6 months, with little variation over the following years [30]. It has also been previously reported that HRQoL seems to follow, over time, 2 types of trajectories: either the 'good HRQoL', with values of HRQoL close to the population norms, or the 'altered HRQoL', where, despite the initial improvement, HRQoL values seem to stay within the worst range [30]. It has been hypothesised that coping and personality traits (not collected in DESIR) might be at least partially responsible for the stable trajectory of HRQoL over time [30], especially considering that coping strategies have indeed been observed to be remarkably stable [31]. Rather than discourage intervention at later stages, though, this observation should prompt an investigation into how to better address HRQoL in the whole course of the disease of axSpA, to achieve the goal set from the 2022 ASAS-EULAR recommendations for axSpA management, which is maximising HRQoL [32]. It is reassuring to note how, compared to other causes of chronic back pain, patients with axSpA have, in fact, a higher likelihood of HRQoL improvement, once they are treated [33].

A further result of our study is that, between disease activity and function, the second seemed to have a higher impact on HRQoL: when standardised coefficients were compared, these were steadily higher for BASFI than for ASDAS. This was, however, less evident in the models with MCS as outcome, compared with models with PCS or ASQoL as outcomes, hinting at a more important association of functional ability with the physical aspects of HRQoL, rather than with the mental component. This outcome was—to some extent—expected, due to the fact that the definition of HRQoL refers to limitations and impairments that patients experience due to their disease [34,35]. Accordingly, in the mediation analysis, the majority of ASDAS's effect on HRQoL was mediated through BASFI. However, this does not necessarily imply that targeting function should take precedence over targeting disease activity. Physical function is largely influenced by disease activity, and functional impairment often results from previous periods of uncontrolled inflammation [36]. These considerations, together with the observation that ASDAS still exerts a meaningful direct effect on HRQoL, accounting for approximately one-quarter of the total effect (an even larger proportion in the case of MCS), suggest that effectively and persistently targeting disease activity remains the best strategy to prevent functional decline and to optimise HRQoL. Once substantial functional impairment has occurred, the potential for recovery is limited, which may contribute to the relative stability of HRQoL trajectories observed over time. Alongside disease activity, addressing functional aspects (eg, utilising specific measures such as physiotherapy) and psychological aspects can be very useful as a complementary measure [37].

It is worth underlining that the association between disease activity, function, and HRQoL was demonstrated to be robust by our results: it held consistently, with different HRQoL outcomes, over the entire axSpA spectrum, and it was confirmed by a sensitivity analysis with a different disease activity measure, BASDAI. This ensures the validity and the correct interpretation of our

Table 4
Autoregressive GEE multivariable models highlighting the associations between the main independent variables (disease activity and function) and the outcome (HRQoL) over time, with standardised beta coefficients

	PCS N = 509	MCS N = 551	ASQOL N = 510
	St. Beta (95%CI)	St. Beta (95%CI)	St.Beta (95%CI)
ASDAS	−2.81 (−3.15, −2.48)[a]	−2.29 (−2.80, −1.79)[a]	1.14 (0.98, 1.31)[a]
BASFI	−4.62 (−5.00, −4.24)[a]	−2.39 (−2.96, −1.82)[a]	2.34 (2.15, 2.53)[a]
Male sex	0.53 (0.17, 0.89)[a]	−0.18 (−0.70, 0.35)	−0.32 (−0.51, −0.13)[a]
Age	0.42 (−1.19, 2.02)	−0.73 (−3.08, 1.63)	−0.41 (−1.23, 0.43)
HLA B27	0.15 (−0.21, 0.51)	0.22 (−0.30, 0.74)	−0.02 (−0.21, 0.17)
Radiographic sacroiliitis (mNY)	0.34 (−0.03, 0.71)	N/A	N/A
MRI sacroiliitis (ASAS definition)	0.18 (−0.18, 0.55)	N/A	−0.09 (−0.27, 0.09)
Psoriasis	N/A	N/A	N/A
IBD	0.44 (−0.72, −0.16)[a]	−0,27 (−0.69, 0.15)	0.15 (0.01, 0.30)[a]
Uveitis	N/A	N/A	N/A
EMM	N/A	N/A	N/A
MASES (0-13)	−0.12 (−0.36, 0.12)	−0.15 (−0.50, 0.21)	0.20 (0.09, 0.32)[a]
SJC (0-28)	−0.01 (−0.26, 0.23)	N/A	0.03 (−0.08, 0.15)
Dactylitis	N/A	N/A	N/A
Peripheral manifestations	N/A	N/A	N/A
BMI	0.42 (0.09, 0.75)[a]	0.25 (−0.23, 0.73)	−0.12 (−0.29, 0.05)
University education	−0.07 (−0.45, 0.28)	−0.12 (−0.64, 0.40)	−0.09 (−0.28, 0.10)
Married/living maritally	N/A	0.71 (0.29, 1.14)[a]	
Parental status (n of children)	−0.02 (−0.37, 0.32)	−0.28 (−0.82, 0.27)	0,04 (−0.14, 0.22)
Blue-collar job	N/A	N/A	N/A
Active smoking	0.27 (0.01, 0.53)[a]	−0,30 (−0.69, 0.10)	N/A
NSAIDs	−0.60 (−0.83, −0.12)[a]	0,05 (−0.29, 0.39)	0.15 (0.04, 0.27)[a]
bDMARDs	−0.74 (−1.02, −0.46)[a]	−0,44 (−0.85, −0.04)[a]	0.15 (0.01, 0.29)[a]
Comorbidity count (0-15)	−0.48 (−0.83, −0.12)[a]	N/A	0.05 (−0.12, 0.23)

ASAS, Assessment of SpondyloArthritis international Society; ASDAS, Axial Spondyloarthritis Disease Activity Score; ASQoL, Ankylosing Spondylitis Quality of Life questionnaire; BASFI, Bath Ankylosing Spondylitis Functional Index; bDMARDs, biological disease-modifying antirheumatic drugs; BMI, body mass index; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; EMMs, extra musculoskeletal manifestations; GEE, generalised estimating equation; HRQoL, health-related quality of life; HLA, human leukocyte antigen; IBD, inflammatory bowel disease; MASES, Maastricht Ankylosing Spondylitis Disease Activity Score; MCS, mental component summary; mNY, modified New York; MRI, magnetic resonance imaging; N/A, not included in the final multivariable model due to collinearity with other variables, or because $P > .1$ at univariable analysis; NSAIDs, nonsteroidal anti-inflammatory drugs; PCS, physical component summary; SJC, swollen joint count.
[a] Indicates significant results ($P < .05$).

Table 5
Mediation analysis considering a direct and indirect (mediated by function, BASFI) effect of disease activity (ASDAS) on HRQoL

Analysed paths	Coefficients	95% CI
Models with PCS as outcome[a]		
ASDAS → BASFI	1.49	(1.43, 1.56)
BASFI → PCS	−2.07	(−2.26, −1.87)
ASDAS → PCS (direct effect)	−1.22	(−1.65, −0.80)
ASDAS → BASFI → PCS (indirect)	−3.09	(−3.38, −2.80)
Models with MCS as outcome[b]		
ASDAS → BASFI	1.58	(1.52, 1.64)
BASFI → MCS	−1.32	(−1.57, −1.07)
ASDAS → MCS (direct effect)	−1.55	(−2.11, −1.00)
ASDAS → BASFI → MCS (indirect effect)	−2.09	(−2.50, −1.71)
Models with ASQoL as outcome[c]		
ASDAS → BASFI	1.38	(1.31, 1.46)
BASFI → ASQoL	1.14	(1.03, 1.25)
ASDAS → ASQoL (direct effect)	0.59	(0.36, 0.82)
ASDAS → BASFI → ASQoL (indirect effect)	1.58	(1.41, 1.75)

ASDAS, Axial Spondyloarthritis Disease Activity Score; ASQoL, Ankylosing Spondylitis Quality of Life questionnaire; BASFI, Bath Ankylosing Spondylitis Functional Index; bDMARDs, biological disease-modifying antirheumatic drugs; BMI, body mass index; HRQoL, health-related quality of life; IBD, inflammatory bowel disease; MASES, Maastricht Ankylosing Spondylitis Disease Activity Score; MCS, mental component summary; NSAIDs, nonsteroidal anti-inflammatory drugs; PCS, physical component summary.
Models (all analysed paths) adjusted for significant covariates in the multivariable autoregressive model.
[a] Adjusted for sex, IBD, BMI, NSAID use, bDMARDs use, and comorbidity count.
[b] Adjusted for marital/social status and bDMARDs use.
[c] Adjusted for sex, IBD, MASES, NSAIDs use, and bDMARDs use.

findings. Notably, in the model where BASDAI and CRP replaced the ASDAS, CRP was not associated with HRQoL, but this should not be a reason for concern, nor be interpreted as a contrasting result. In fact, in the DESIR cohort (much like in axSpA in general), less than 30% of the subjects had an elevated CRP at baseline, and it is very well-known how CRP, albeit prognostically very relevant, is infrequently abnormal [38].

Limitations of our study include, first, the fact that DESIR is an observational cohort, having therefore missing data (up to 30% of baseline patients had missing data over 10 years, as expected in long-term follow-ups). However, we used GEE as a method of analysis because it can handle missing data, as its estimates are relatively robust [39]. Second, we could not analyse the impact of variables that are unavailable in DESIR, such as fibromyalgia/widespread pain, or socioeconomic deprivation, and that can influence HRQoL [27,28,40]. Using surrogates such as MCS ≤ 38/50 for depression [41] or a score ≥8 on 3 out of the first 5 BASDAI questions for fibromyalgia [42] was not feasible in our case due to the risk of circular reasoning: the same variables would have been used as both independent and outcome variables in the case of depression and MCS, or duplicated as independent variables in the case of extreme BASDAI values and fibromyalgia. Nevertheless, since the association between disease activity, function, and HRQoL was quite consistent, it is unlikely that the addition of these factors would fundamentally alter the framework. However, we acknowledge that these aspects are clinically relevant and could modulate the

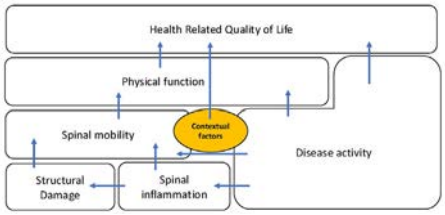


Figure 3. Hierarchical relationship between disease outcomes in axSpA (modified from Machado et al. Ann Rheum Dis 2011). Each domain is presented as a separate box. The final model includes HRQoL as an overarching outcome, depending longitudinally on disease activity and function (which, in turn, are also influenced by other outcomes). All confirmed associations are represented as solid arrows (both confirmed by previous studies and the present study). Contextual factors are positioned centrally, reflecting their potential role as confounders or effect modifiers across all these associations. AxSpA, axial spondyloarthritis; HRQoL, health-related quality of life.

magnitude of associations within the model. Third, a conceptual limitation is the use of patient reported outcomes (PROs) to define physical function and HRQoL: because it is well-known that PROs tend to correlate, the association could have a different magnitude if more 'objective' measurement instruments were used [43]. This is, however, purely hypothetical, as we used measurement instruments indicated as core outcomes in axSpA [44]. Strengths of the present study are the use of different outcome measures to corroborate the robustness of findings, the long follow-up time, and the considerable sample size.

In conclusion, our study establishes a validated framework clarifying how disease activity and function drive HRQoL in axSpA. HRQoL can thus be regarded as an overarching outcome, and achieving low disease activity is crucial to optimise it. This framework, both robust and generalisable, offers a valuable basis for interpreting future studies.

Competing interests

The authors declare the following financial interests/personal relationships, which may be considered as potential competing interests: DvdH reports a relationship with AbbVie Inc that includes consulting or advisory. DvdH reports a relationship with Alfasigma SpA that includes consulting or advisory. DvdH reports a relationship with Argenx BV that includes consulting or advisory. DvdH reports a relationship with Bristol-Myers Squibb Company that includes consulting or advisory. DvdH reports a relationship with Eli Lilly and Company that includes consulting or advisory. DvdH reports a relationship with Grey Wolf Therapeutics Limited that includes consulting or advisory. DvdH reports a relationship with Janssen Pharmaceuticals Inc that includes consulting or advisory. DvdH reports a relationship with Novartis that includes consulting or advisory. DvdH reports a relationship with Pfizer Inc that includes consulting or advisory. DvdH reports a relationship with Takeda Pharmaceuticals International AG that includes consulting or advisory. DvdH reports a relationship with UCB Pharma SA that includes consulting or advisory. SR reports a relationship with AbbVie Inc that includes consulting or advisory and funding grants. SR reports a relationship with Alfasigma SpA that includes consulting or advisory and funding grants. SR reports a relationship with Merck Sharp & Dohme Corp that includes consulting or advisory and funding grants. SR reports a relationship with Novartis Pharmaceuticals Corporation that includes funding grants and speaking, and lecture fees. SR reports a relationship with Pfizer Inc that includes consulting or advisory and funding grants. SR reports a relationship with UCB Pharma SA that includes funding grants and speaking, and lecture fees. SR reports a relationship with Eli Lilly and Company that includes consulting or advisory and speaking and lecture fees. SR reports a relationship with Janssen Pharmaceuticals Inc that includes consulting or advisory. LG reports a relationship with AbbVie Inc that includes consulting or advisory and funding grants. LG reports a relationship with Biogen Inc that includes: funding grants. LG reports a relationship with Amgen Inc that includes consulting or advisory. LG reports a relationship with Bristol-Myers Squibb Company that includes consulting or advisory. LG reports a relationship with Celltrion Pharm, Inc. that includes consulting or advisory. LG reports a relationship with Janssen Pharmaceuticals Inc that includes consulting or advisory. LG reports a relationship with Eli Lilly and Company that includes consulting or advisory and funding grants. LG reports a relationship with Merck Sharp & Dohme Corp that includes consulting or advisory. LG reports a relationship with Novartis that includes consulting or advisory and funding grants. LG reports a relationship with Pfizer Inc that includes consulting or advisory. LG reports a relationship with Sandoz Inc that includes consulting or advisory. LG reports a relationship with UCB Pharma SA that includes consulting or advisory. AO reports a relationship with AbbVie Inc that includes consulting or advisory. AO reports a relationship with Johnson & Johnson MedTech that includes consulting or advisory. AO reports a relationship with Eli Lilly and Company that includes consulting or advisory. AO reports a relationship with Novartis Pharmaceuticals Corporation that includes consulting or advisory. AO reports a relationship with UCB Pharma SA that includes consulting or advisory. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Augusta Ortolan: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Désirée van der Heijde:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Conceptualization. **Laure Gossec:** Writing – review & editing, Validation, Supervision, Methodology, Data curation. **Sofia Ramiro:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Acknowledgements

The DESIR cohort is conducted with Assistance Publique-Hopitaux de Paris (AP-HP, Paris, France) as the sponsor and under the umbrella of the French Society of Rheumatology. We thank the Clinical Research Unit Paris Centre (AP-HP, Paris, France), and the Institut national de la sante et de la recherche medicale (Inserm). We also thank the investigators: Pr Maxime Dougados, Dr Anna Molto (Paris-Cochin), Pr Philippe Dieudé (Paris-Bichat), Pr Laure Gossec (Paris-La Pitie-Salpétriere), Pr Francis Berenbaum (Paris-Saint-Antoine), Pr Pascal Claudepierre (Creteil), Pr Félicie Costantino (Boulogne-Billancourt), Dr Bernadette Saint-Marcoux (Aulnay-sous-Bois), Pr Philippe Goupille (Tours), Dr Emmanuelle Dernis et Dr Guillaume Direz (Le Mans), Pr Daniel Wendling, Pr Clément Prati (Besançon), Pr Bernard Combe, Pr Cédric Lukas (Montpellier), Pr Véronique Breuil (Nice), Pr Pascal Richette (Paris Lariboisière), Pr Thao Pham (Marseille), Dr Pauline Lasselin (Amiens), Pr Anne Tournadre

(Clermont-Ferrand), Dr Nadia Mehsen (Bordeaux), Pr Damien Loeuille (Nancy), Pr Rene-Marc Flipo (Lille), Pr Alain Saraux (Brest), Dr Stephan Pavy (Le Kremlin-Bicêtre), Pr Adeline Ruyssen-Witrand (Toulouse), Pr Olivier Vittecoq, Pr Thierry Lequerre (Rouen).

Funding

The DESIR cohort is run with the support of unrestricted grants from (in order of decreasing support) Pfizer France, AbbVie, Biogen, UCB, Novartis, Lilly, Galapagos, MSD, Celltrion HealthCare, and Fresenius.

Patient consent for publication

Written inform consent was signed by all patients prior to the study inclusion.

Ethics approval

This study was approved by Comité de protection des personnes CCP Ile de France III, Number 2457, EUDRACT number 2007- A00608-45.

Provenance and peer review

Not commissioned; externally peer reviewed.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.10.016.

Orcid

Augusta Ortolan: http://orcid.org/0000-0002-3131-0939
Désirée van der Heijde: http://orcid.org/0000-0002-5781-158X
Laure Gossec: http://orcid.org/0000-0002-4528-310X
Sofia Ramiro: http://orcid.org/0000-0002-8899-9087

REFERENCES

[1] Machado P, Landewé R, Braun J, Hermann KG, Baraliakos X, Baker D, et al. A stratified model for health outcomes in ankylosing spondylitis. Ann Rheum Dis 2011;70:1758–64.
[2] Boel A, Molto A, van der Heijde D, Ciurea A, Dougados M, Gensler LS, et al. Do patients with axial spondyloarthritis with radiographic sacroiliitis fulfil both the modified New York criteria and the ASAS axial spondyloarthritis criteria? Results from eight cohorts. Ann Rheum Dis 2019;78:1545–9.
[3] Carvalho PD, Ruyssen-Witrand A, Fonseca J, Marreiros A, Machado PM. Determining factors related to impaired spinal and hip mobility in patients with axial spondyloarthritis: longitudinal results from the DESIR cohort. RMD Open 2020;6:e001356.
[4] Carvalho PD, Ruyssen-Witrand A, Marreiros A, Machado PM. Long-term association between disease activity and disability in early axial spondyloarthritis: results from a prospective observational study of inflammatory back pain. Arthritis Care Res (Hoboken) 2022;74:768–75.
[5] Portier E, Benattar L, Resche-Rigon M, Dougados M, Gossec L, Molto A, et al. Different disease activity trajectories in early axial spondyloarthritis lead to significantly different long-term outcomes: a trajectory-based analysis of the DESIR cohort over 10 years. RMD Open 2024;10:e004910.
[6] Drouet J, López-Medina C, Granger B, Fautrel B, Landewe RBM, Molto A, et al. Disease activity and widespread pain are main contributors to patient-reported global health in axial spondyloarthritis: an analysis of 6064 patients. Rheumatol Int 2024;44:1455–68.
[7] Dougados M, Etcheto A, Molto A, Alonso S, Bouvet S, Daurès JP, et al. Clinical presentation of patients suffering from recent onset chronic inflammatory back pain suggestive of spondyloarthritis: The DESIR cohort. Joint Bone Spine 2015;82:345–51.
[8] Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473–83.
[9] Doward LC, Spoorenberg A, Cook SA, Whalley D, Helliwell PS, Kay LJ, et al. Development of the ASQoL: a quality of life instrument specific to ankylosing spondylitis. Ann Rheum Dis 2003;62:20–6.
[10] Ware JE, Kosinski M, Dewey JE. How to score version 2 of the SF-36 health survey: (standard & acute forms); [SF-36v2]. 3rd ed. Lincoln, RI: QualityMetric; 2001.
[11] Machado P, Landewé R, Lie E, Kvien TK, Braun J, Baker D, et al. Ankylosing Spondylitis Disease Activity Score (ASDAS): defining cut-off values for disease activity states and improvement scores. Ann Rheum Dis 2011;70:47–53.
[12] Lukas C, Landewé R, Sieper J, Dougados M, Davis J, Braun J, et al. Development of an ASAS-endorsed disease activity score (ASDAS) in patients with ankylosing spondylitis. Ann Rheum Dis 2009;68:18–24.
[13] van der Heijde D, Lie E, Kvien TK, Sieper J, Van den Bosch F, Listing J, et al. ASDAS, a highly discriminatory ASAS-endorsed disease activity score in patients with ankylosing spondylitis. Ann Rheum Dis 2009;68:1811–8.
[14] Calin A, Garrett S, Whitelock H, Kennedy LG, O'Hea J, Mallorie P, et al. A new approach to defining functional ability in ankylosing spondylitis: the development of the Bath Ankylosing Spondylitis Functional Index. J Rheumatol 1994;21:2281–5.
[15] Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the bath ankylosing spondylitis disease activity index. J Rheumatol 1994;21:2286–91.
[16] van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984;27:361–8.
[17] Lambert RG, Bakker PA, van der Heijde D, Weber U, Rudwaleit M, Hermann KG, et al. Defining active sacroiliitis on MRI for classification of axial spondyloarthritis: update by the ASAS MRI working group. Ann Rheum Dis 2016;75:1958–63.
[18] Landewé R, Dougados M, Mielants H, van der Tempel H, van der Heijde D. Physical function in ankylosing spondylitis is independently determined by both disease activity and radiographic damage of the spine. Ann Rheum Dis 2009;68:863–7.
[19] Nikiphorou E, Ramiro S, van der Heijde D, Norton S, Moltó A, Dougados M, et al. Association of comorbidities in spondyloarthritis with poor function, work disability, and quality of life: results from the assessment of spondyloarthritis international society comorbidities in spondyloarthritis study. Arthritis Care Res (Hoboken) 2018;70:1257–62.
[20] Ulas ST, Proft F, Diekhoff T, Rios V, Rademacher J, Protopopov M, et al. Sex-specific diagnostic efficacy of MRI in axial spondyloarthritis: challenging the "One Size Fits All" notion. RMD Open 2023;9:e003252.
[21] Nikiphorou E, Ramiro S, Sepriano A, Ruyssen-Witrand A, Landewé RBM, van der Heijde D. Do smoking and socioeconomic factors influence imaging outcomes in axial spondyloarthritis? Five-year data from the DESIR cohort. Arthritis Rheumatol 2020;72:1855–62.
[22] Chung HY, Machado P, van der Heijde D, D'Agostino MA, Dougados M. Smokers in early axial spondyloarthritis have earlier disease onset, more disease activity, inflammation and damage, and poorer function and health-related quality of life: results from the DESIR cohort. Ann Rheum Dis 2012;71:809–16.
[23] Marzo-Ortega H, Navarro-Compán V, Akar S, Kiltz U, Clark Z, Nikiphorou E. The impact of gender and sex on diagnosis, treatment outcomes and health-related quality of life in patients with axial spondyloarthritis. Clin Rheumatol 2022;41:3573–81.
[24] van Lunteren M, Ez-Zaitouni Z, de Koning A, Dagfinrud H, Ramonda R, Jacobsson L, et al. In early axial spondyloarthritis, increasing disease activity is associated with worsening of health-related quality of life over time. J Rheumatol 2018;45:779–84.
[25] Capelusnik D, Benavent D, van der Heijde D, Landewé R, Poddubnyy D, van Tubergen A, et al. Treating spondyloarthritis early: does it matter? Results from a systematic literature review. Rheumatology 2023;62:1398–409.
[26] Machado P, Landewé RB, Braun J, Baraliakos X, Hermann KG, Hsu B, et al. MRI inflammation and its relation with measures of clinical disease activity and different treatment responses in patients with ankylosing spondylitis treated with a tumour necrosis factor inhibitor. Ann Rheum Dis 2012;71:2002–5.
[27] Macfarlane GJ, Rotariu O, Jones GT, Pathan E, Dean LE. Determining factors related to poor quality of life in patients with axial spondyloarthritis: results from the British Society for Rheumatology Biologics Register (BSRBR-AS). Ann Rheum Dis 2020;79:202–8.
[28] Dean LE, Macfarlane GJ, Jones GT. Five potentially modifiable factors predict poor quality of life in ankylosing spondylitis: results from the Scotland registry for ankylosing spondylitis. J Rheumatol 2018;45:62–9.

[29] López-Medina C, Garrido-Castro JL, Castro-Jiménez J, González-Navas C, Calvo-Gutiérrez J, Castro-Villegas MC, et al. Evaluation of quality of life in patients with axial spondyloarthritis and its association with disease activity, functionality, mobility, and structural damage. Clin Rheumatol 2018; 37:1581–8.
[30] Puyraimond-Zemmour D, Granger B, Molto A, Gaujoux-Viala C, Guillemin F, Ruyssen-Witrand A, et al. Similar alteration for mental and physical aspects in health-related quality of life over 5 to 8 years in 1347 patients with early arthritis and early inflammatory back pain. Arthritis Res Ther 2019;21:63.
[31] van Lunteren M, Landewé R, Fongen C, Ramonda R, van der Heijde D, van Gaalen FA. Do Illness perceptions and coping strategies change over time in patients recently diagnosed with axial spondyloarthritis? J Rheumatol 2020;47:1752–9.
[32] Ramiro S, Nikiphorou E, Sepriano A, Ortolan A, Webers C, Baraliakos X, et al. ASAS-EULAR recommendations for the management of axial spondyloarthritis: 2022 update. Ann Rheum Dis 2023;82:19–34.
[33] Bento da Silva A, Ramiro S, Boel A, van Lunteren M, Marques ML, van de Sande M, et al. Do quality of life and work productivity change in early axial spondyloarthritis and non-axial spondyloarthritis patients after 2 years? Rheumatology (Oxford) 2025;64(4):1826–34.
[34] Kiltz U, Kiefer D, Boonen A. Health-Related) quality of le as an outcome in studies of axial spondyloarthritis. Rheum Dis Clin North Am 2020;46:379–93.
[35] Kiltz U, van der Heijde D, Boonen A, Akkoc N, Bautista-Molano W, Burgos-Vargas R, et al. Measurement properties of the ASAS Health Index: results of a global study in patients with axial and peripheral spondyloarthritis. Ann Rheum Dis 2018;77:1311–7.
[36] Capelusnik D, Ramiro S, Schneeberger EE, Citera G. Peripheral arthritis and higher disease activity lead to more functional impairment in axial spondyloarthritis: longitudinal analysis from ESPAXIA. Semin Arthritis Rheum 2021;51:553–8.
[37] Ortolan A, Webers C, Sepriano A, Falzon L, Baraliakos X, Landewé RB, et al. Efficacy and safety of non-pharmacological and non-biological interventions: a systematic literature review informing the 2022 update of the ASAS/EULAR recommendations for the management of axial spondyloarthritis. Ann Rheum Dis 2023;82:142–52.
[38] Maksymowych WP. Biomarkers for diagnosis of axial spondyloarthritis, disease activity, prognosis, and prediction of response to therapy. Front Immunol 2019;10:305.
[39] Salazar A, Ojeda B, Dueñas M, Fernández F, Failde I. Simple generalized estimating equations (GEEs) and weighted generalized estimating equations (WGEEs) in longitudinal studies with dropouts: guidelines and implementation in R. Stat Med 2016;35:3424–48.
[40] Hollick RJ, Stelfox K, Dean LE, Shim J, Walker-Bone K, Macfarlane GJ. Outcomes and treatment responses, including work productivity, among people with axial spondyloarthritis living in urban and rural areas: a mixed-methods study within a national register. Ann Rheum Dis 2020;79:1055–62.
[41] Nikiphorou E, Carvalho PD, Boonen A, Fautrel B, Richette P, Machado PM, et al. Sick leave in early axial spondyloarthritis: the role of clinical and socioeconomic factors. Five-year data from the DESIR cohort. RMD Open 2021;7: e001685.
[42] Santos-Faria D, Dougados M, Gossec L, Perrot S, Moltó A. Evaluation of the performance of extreme patient-reported outcomes as surrogate markers for fibromyalgia in axial spondyloarthritis. Rheumatol Int 2019;39:141–6.
[43] Gossec L, Dougados M, Dixon W. Patient-reported outcomes as end points in clinical trials in rheumatoid arthritis. RMD Open 2015;1:e000019.
[44] Navarro-Compán V, Boel A, Boonen A, Mease P, Landewé R, Kiltz U, et al. The ASAS-OMERACT core domain set for axial spondyloarthritis. Semin Arthritis Rheum 2021;51:1342–9.

Ann Rheum Dis 85 (2026) 319–327

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Axial spondyloarthritis

Evaluation of pregnancy outcomes in patients with spondyloarthritis compared to the general population: results from a French national prospective and matched study

Sabrina Hamroun[1], Grégoire Martin de Frémont[2], Nathalie Costedoat-Chalumeau[3,4], Marion Couderc[5,6], René-Marc Flipo[7], Jérémie Sellam[8], Christophe Richez[9], Rakiba Belkhir[2], Laure Gossec[10,11], Hubert Marotte[12], Emmanuel Dernis[13], Aline Frazier-Mironer[14], Elisabeth Gervais[15], Cédric Lukas[16], Valérie Devauchelle-Pensec[17], Alban Deroux[18], Véronique Le Guern[4], Gaëlle Guettrot-Imbert[4], Nathalie Lelong[19], Emmanuelle Pannier[20], Loic Sentilhes[21], Camille Le Ray[22,23], Raphaèle Seror[23], Anna Molto[3,24,25,*], on behalf of the GR2 and the ENP2021 Study Groups

[1] *Rheumatology Department, NOVO Hospital, Pontoise, France*
[2] *Rheumatology Department, Kremlin-Bicêtre University Hospital, Le Kremlin-Bicêtre, France*
[3] *Université Paris Cité and Université Sorbonne Paris Nord, INSERM, INRAE, Centre for Research in Epidemiology and Statistics (CRESS), Paris, France*
[4] *Internal Medicine Department, Referral Center for Rare Autoimmune and Systemic Diseases, Cochin Hospital, Paris, France*
[5] *Rheumatology Department, Gabriel-Montpied Hospital, Clermont-Ferrand University Hospital, Clermont-Ferrand, France*
[6] *IMOST team–INSERM (1240), Clermont Auvergne University, Clermont-Ferrand, France*
[7] *Rheumatology Department, Salengro Hospital, Lille University Hospital, Lille, France*
[8] *Rheumatology Department, Sorbonne University, CRSA INSERM UMRS8938, Saint-Antoine University Hospital, AP–HP Centre, Paris, France*
[9] *Rheumatology Department, Bordeaux University Hospital, Bordeaux, France*
[10] *Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, Team Pepites, Paris, France*
[11] *AP-HP, Pitié Salpêtrière Hospital, Rheumatology Department, Paris, France*
[12] *Université Jean Monnet, Service de Rhumatologie, CHU Saint-Etienne, INSERM, Mines Saint Etienne, SAINBIOSE U1059, Saint Etienne, France*
[13] *Rheumatology Department, Le Mans Hospital, Le Mans, France*
[14] *Rheumatology Department, Lariboisière University Hospital, Paris, France*
[15] *Rheumatology Department, University Hospital and LITEC UR15560 Poitiers, France*
[16] *Rheumatology Department, Montpellier University Hospital, Montpellier, France*

*Correspondence to Pr Anna Molto, Université Paris Cité, Rheumatology Department Bichat Hospital, Paris, France.
E-mail address: anna.molto@aphp.fr (A. Molto).

ENP2021 Study Group: Camille Le Ray, Nathalie Lelong, Hélène Cinelli, Béatrice Blondel, Nolwenn Regnault, Virginie Demiguel, Elodie Lebreton, Benoit Salanave, Jeanne Fresson, Annick Vilain, Thomas Deroyon, Philippe Raynaud, Sylvie Rey, Khadoudja Chemlal, Nathalie Rabier-Thoreau, Frédérique Collombet-Migeon.

SH and GMDF have contributed equally.

RS and AM have contributed equally.

Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.10.024

[17] Rheumatology Department, Centre National de Référence des Maladies Auto-Immunes Rares du Nord-Ouest (CERAINOM), Brest University Hospital, Brest, France
[18] Internal Medicine Department, Grenoble University Hospital, Grenoble, France
[19] University of Paris, INSERM Obstetrical Perinatal and Pediatric Epidemiology Research Team, Paris, France
[20] Port-Royal Maternity Unit, Assistance Publique-Hôpitaux de Paris, Hôpital Cochin, FHU Préma, Paris, France
[21] Service de Gynécologie Obstétrique, Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France
[22] Université Paris Cité, Institut Santé des femmes, U1153, Centre of Research In Epidemiology and Statistics (CRESS), Obstetrical, Perinatal and Paediatric Epidemiology Research Team (EPOpé), INSERM, INRAE, Paris, France
[23] Université Paris-Saclay, UVSQ, INSERM CESP U1018, Gustave Roussy, Villejuif, France
[24] Université Paris Cité, UFR Médecine, Paris, France
[25] Rheumatology Department, Bichat Hospital, AP-HP Nord, Paris, France

ARTICLE INFO

Article history:
Received 22 July 2025
Received in revised form 22 October 2025
Accepted 23 October 2025

ABSTRACT

Objectives: The aim of the study was to determine the frequency of adverse pregnancy outcomes in women with spondyloarthritis (SpA) in comparison to controls from the French general population, and to identify factors associated with these adverse pregnancy outcomes.
Methods: This French prospective multicentre cohort study included pregnant women with SpA (both axial and peripheral) according to their treating rheumatologist between December 2015 and June 2021. Maternal characteristics, disease activity, treatments, and pregnancy outcomes were analysed. Outcomes of pregnancies in women with SpA were compared to that of matched (1:4) general population women from the 2016 and 2021 French National Perinatal Surveys, including pregnancy, neonatal, and maternal outcomes. For adverse pregnancy outcomes that are significantly more frequent in patients with SpA, logistic regression analysis was performed to identify potential risk factors.
Results: A total of 135 SpA pregnancies in 124 women were analysed: they have a mean age of 32.1 years, a mean disease duration of 6.3 years, and 50.4% were nulliparous. Small for gestational age (SGA) was the most common adverse pregnancy outcome and occurred more frequently in women with SpA compared to controls (17.4% vs 9.8%, odds ratios = 1.94, 95% CI 1.09-3.39). Other adverse pregnancy outcomes rates, including preterm birth and caesarean delivery, were comparable to the general population. No predictor of SGA was identified in women with SpA.
Conclusions: In this contemporary cohort, compared to the general population, SpA was associated with a higher risk of SGA, but no other adverse pregnancy outcomes, providing reassurance for most pregnant women with SpA.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- An increased risk of adverse pregnancy outcomes has been reported in women with spondyloarthritis (SpA).

WHAT THIS STUDY ADDS

- In this prospective study, the most frequent adverse pregnancy outcome in women with SpA was small for gestational age (SGA) births, occurring in 17.4% of pregnancies.
- Women with SpA had a 2-fold increased risk of SGA compared to the general population.
- The rate of caesarean section delivery and other adverse pregnancy outcomes was comparable to that of the general population.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Our results suggest that pregnancies in women with SpA have a good prognosis, with no increased risk of adverse pregnancy outcomes compared to the general population, except for a nearly 2-fold higher risk of SGA.

INTRODUCTION

Spondyloarthritis (SpA), encompassing both axial and peripheral forms, is recognised as one of the most prevalent chronic rheumatic inflammatory diseases, particularly affecting women of childbearing age [1,2]. Nevertheless, there remains a paucity of knowledge regarding the influence of disease activity and therapeutic strategies on pregnancy outcomes. This lack of comprehensive data is further compounded by the increasing prevalence of SpA among women, particularly following the recognition of nonradiographic forms of axial SpA [3,4], and, as a result, the growing number of pregnancies in this population. A systematic review of the literature and a meta-analysis regarding reproductive health in women with SpA [5] have identified an association between SpA and an elevated risk of adverse pregnancy outcomes, including preterm birth, pre-eclampsia, being small for gestational age (SGA), and the necessity for caesarean section [5]. This heightened risk has also been documented in cases of rheumatoid arthritis and juvenile arthritis [6–9]. Despite certain limitations present in the studies included in the meta-analysis on SpA, such as a retrospective design, the absence of a control group, and insufficient data concerning potential confounders, these studies demonstrated limited heterogeneity. Additionally, sensitivity analyses consistently indicated an increased risk of adverse pregnancy outcomes. Consequently, the underlying reasons for this additional risk remain to be elucidated.

The absence of a clear causal explanation for the adverse pregnancy outcomes observed in SpA is partly influenced by the nature of published studies [10]. Existing population-based studies indicate a risk of adverse pregnancy outcomes due to

their large sample sizes. However, the claims databases utilised in these studies often lack important adjustment factors, such as disease activity, severity, or past reproductive history, which limits the exploration of the association between adverse pregnancy outcomes and other variables. Conversely, pregnancy registries like the Groupe de Recherche sur la Grossesse et les maladies Rares (GR2) study collect various prospective variables that could affect reproductive health in women with chronic rheumatic inflammatory diseases, making them more appropriate for investigating the causes of adverse pregnancy outcomes. In addition, the availability of the National Perinatal Surveys (Enquêtes Nationales Périnatales [ENPs] [French perinatal survey]) offers a distinctive chance to compare pregnancies from the GR2 study with a contemporary French control group.

Based on these remarks, we conducted this study to determine the prevalence of adverse pregnancy outcomes in women with SpA from the GR2 study, compare their prevalence to matched pregnancies from the general population, and identify predictors of adverse pregnancy outcomes in women with SpA.

METHODS

Study design and populations

- Patients with SpA: GR2 cohort

The GR2 study is a French multicentre prospective observational study of women planning to conceive or already pregnant (included in the first trimester of pregnancy) with rare and/or rheumatological diseases. It has been conducted since October 2014 across 76 centres, and since December 2015 for rheumatic diseases. Women are included by their treating specialists (rheumatologists or internal medicine doctors) and are followed for up to 12 months postpartum [11,12]. Treatment decisions are left to the discretion of the treating physicians. The GR2 study is part of the European network of pregnancy registers in rheumatology [13] and follows the European Alliance of Associations for Rheumatology (EULAR) recommendations regarding core data sets for pregnancy registers in rheumatology [14].

In the present study, patients were eligible if they were included in the GR2 study before June 28, 2021, and were diagnosed with SpA (axial or peripheral). The SpA diagnosis was defined by a rheumatologist, and all items necessary for the Assessment in Spondyloarthritis International Society classification criteria calculation were collected [15]. If a patient presented with multiple pregnancies that were prospectively included in the GR2 study throughout the follow-up, all pregnancies were documented. To avoid reporting bias, only women with pregnancies included before 12 weeks of gestation (WG) were considered in the present analysis.

- Control population: ENP study

Starting in 1995, the French ENP has had a standard design and aims to assess the health status of mothers and children in France [16,17]. Approximately every 5 years, data on all births ≥22 WG or newborns weighing ≥500 g are collected for 1 week in all public and private maternity units, including midwife units. There is a core set of information common to all ENPs since inception, to which specific demands are added (or retrieved) for each survey. The data sources include medical records and face-to-face interviews performed by trained midwives 2 to 3 days after birth to collect information. Women are orally informed and consent to participate. Full details can be found in the reports. Since births before 22 WG and/or weighing <500 g are excluded from the ENP, only GR2 women with SpA giving birth ≥22 WG and newborns weighing ≥500 g were included in the matched controlled study. Thus, we distinguished the total SpA population (all pregnancies in the GR2 cohort) from the SpA subpopulation matched to the general population (ENP), excluding pregnancies that did not meet ENP inclusion criteria.

For the current comparison of the GR2 study to the general population, we used the ENPs 2016 and 2021 [16,18].

Data collection in the GR2 study

At inclusion, maternal demographics, SpA characteristics and activity, SpA history of treatment, obstetric history, including live births, abortions (ie, the right of women in France to end a pregnancy up to 14 weeks of pregnancy, medical termination of pregnancy, miscarriages, and intrauterine deaths), use of assisted reproductive techniques (ARTs), and comorbidities, were collected. SpA disease activity, treatments, and therapeutic changes were collected at each follow-up visit.

Variables and definitions

- Women's characteristics: age; body mass index (BMI); use of tobacco and/or alcohol during pregnancy (yes/no), comorbidities as chronic hypertension; and pregestational diabetes.
- SpA-related characteristics: history of corticosteroids and/or conventional disease-modifying antirheumatic drugs (csDMARDs) and/or tumour necrosis factor inhibitor (TNFi) use (any use of these treatment categories since diagnosis, before study inclusion), SpA disease activity during pregnancy defined as a Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) ≥4 (0–10 scale) at least once during pregnancy [19]. Axial Spondyloarthritis Disease Activity Score (ASDAS) was not used due to the high number of missing C-reactive protein (CRP) values.
- Obstetric history: parity; history of preterm birth (birth <37 WG); history of SGA (a birthweight below the 10th percentile if not otherwise specified); ART use (including ovarian stimulation, egg donation, artificial insemination, and *in vitro* fertilisation).
- Pregnancy outcomes: either foetal loss before 22 WG, foetal loss after 22 WG or live birth; foetal growth restriction (FGR) diagnosed by the obstetrician; SGA; low birth weight (birthweight <2500 g); macrosomia (birthweight >4000 g); preterm birth; delivery by caesarean section; induction of labour; neonatal transfer to an intensive care unit (ICU) or neonatology department; congenital malformations.
- Maternal outcomes: gestational diabetes; hypertensive disorders of pregnancy defined as gestational hypertension (any arterial hypertension starting during pregnancy unless an onset before 20 WG was specified) and/or pre-eclampsia (including eclampsia, Haemolysis, Elevated Liver enzymes, Low Platelets syndrome and placental abruption); premature rupture of the membranes; severe postpartum haemorrhage defined as a maternal bleeding ≥1000 mL, need for embolisation or surgery, or for blood transfusion; maternal transfer to an ICU.

Statistical analyses

Continuous variables were expressed by their means ± SD and qualitative variables as number and percentages (%).

First, we described the characteristics of the GR2-SpA population and the frequency of each pregnancy outcome.

Second, we compared the pregnancy outcome frequency between patients with SpA-GR2 and the general population (ENPs). This analysis was restricted to GR2 pregnancies meeting the ENP inclusion criteria (ie, birth at ≥22 WG or birthweight ≥500 g). Each GR2 pregnancy was matched to 4 ENP pregnancies by age group (≤29, 30-34, 35-39, or ≥40 years), parity (nulliparity vs multiparity), residential area (French region), and singleton vs multiple pregnancies. These matching variables were chosen because they are associated with the risk of adverse pregnancy outcomes and are therefore potential confounders. Furthermore, depending on the year of delivery, women with SpA-GR2 were matched either to the 2016 (ie, GR2 deliveries between 2016 and 2018) or 2021 (ie, GR2 deliveries between 2019 and 2021) ENP women, respectively. Odds ratios (ORs) and their 95% CI were calculated for each adverse pregnancy outcome.

Finally, for adverse pregnancy outcomes identified as significantly more frequent in SpA compared to the general population of women, a multilevel logistic regression was performed to identify factors independently associated with each adverse pregnancy outcome. We applied mixed-effects logistic regression. A random intercept at the centre level accounted for heterogeneity in clinical practice across sites. A patient-level random intercept accounted for multiple pregnancies within the same woman. This multilevel structure appropriately modelled within-centre and within-patient correlation [20]. Univariate and multivariate analyses were performed. We used multiple imputation (using multiple imputaion by chained equations) [21] to address missing data among the explanatory variables.

All analyses were conducted with R. Studio R v. 4.0.3 (2020-10-10). The *MatchIt* package was used to match participants between the GR2 and the ENP.

Ethics

According to the French regulation, women received information on the study from the investigators and had to orally express that they did not oppose the use of their data for this study. The local Ethics Committee (Committee for the Protection of Persons [CPP] Île-de-France VI) approved the GR2 study (29/08/2012). The ENP 2016 was approved by the National Council on Statistical Information (Comité du Label no. 2016 × 703SA), the French Data-Protection Authority (Commission Nationale de l'Informatique et des Libertés [CNIL] no. 915197), and the INSERM Ethics Committee (IRB00003888 no. 14-191). The ENP 2021 was approved by the Label Committee (Label of general interest and statistical quality, Visa n°2021 × 701SA, order of November 23, 2020), CPP (July 7, 2020), Committee of Ethics and Scientists for Research, Studies and Evaluations (June 12, 2020) and CNIL (DR-2020-391 of December 31, 2020 and DR-2022-179 of July 28, 2022). The studies were conducted following the Declaration of Helsinki.

Patient and public involvement

No patient representatives were involved in the project.

RESULTS

Patient characteristics

From December 2015 to June 2021, 172 pregnancies in 155 women with SpA were enrolled in the GR2 study. Among them, 135 pregnancies in 124 women, all singletons, were included in this study (Fig). The mean age at pregnancy was 32.1 (±4.3) years, the mean BMI was 23.8 (±4.7) kg/m^2, and 14 (10.4%) women were active smokers during pregnancy. SpA was mainly axial (51.1%), with a mean disease duration of 6.3 (±5.9) years (Table 1). Before pregnancy, 100 (74.1%) women had been treated with at least 1 TNFi and almost 10% by at least 3 biologics. Reproductive history revealed that 68 (50.4%) were nulliparous, 14 (10.4%) had a history of abortion, and 17 (12.6%) had

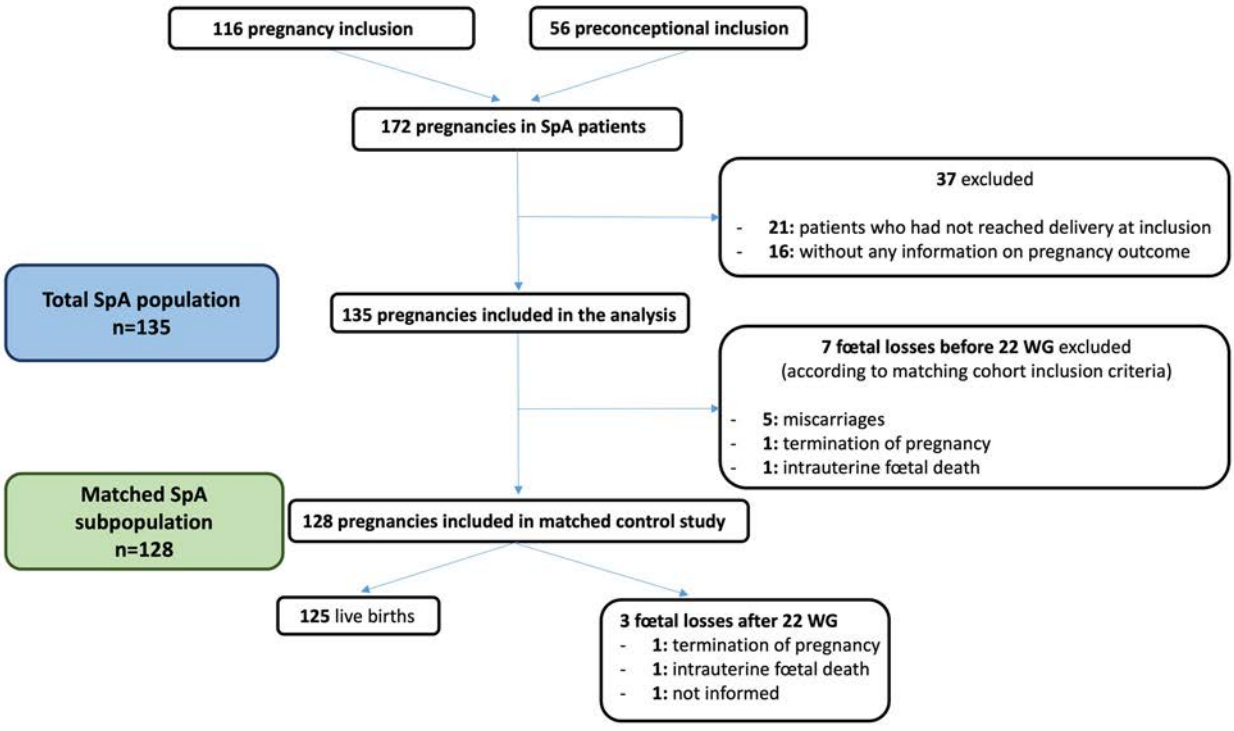


Figure. Flow chart of patients with SpA with pregnancy from the GR2 cohort*. GR2, Groupe de Recherche sur la Grossesse et les maladies Rares; SpA, spondyloarthritis; WG, weeks of gestation. *French multicentre prospective observational cohort study of women planning to conceive or already pregnant with rare and/or rheumatological diseases.

Table 1
Baseline characteristics of pregnant women with SpA

Baseline characteristics	Total SpA population (N = 135)
Women's characteristics[a]	
Age, y	32.1 (4.3)
Use of tobacco during pregnancy	14/130 (10.8)
Use of alcohol during pregnancy	3/125 (2.4)
BMI, kg/m^2	23.8 (4.7) for n = 131
Comorbidities	
Chronic hypertension	2 (1.5)
Pregestational diabetes	1 (0.7)
Obstetrical history	
Nulliparity	68/129 (52.7)
History of caesarean delivery	10 (7.4)
History of abortion	15 (11.1)
History of foetal loss before 22 WG (excl. abortion)	17 (12.6)
Miscarriage	16
Medical termination of pregnancy	1
History of foetal loss after 22 WG	5 (3.7)
Intrauterine foetal death	4
Medical termination of pregnancy	1
SpA-related characteristics	
Disease duration	6.3 (5.9) for n = 132
History of corticosteroid use	48 (35.6)
History of csDMARDs use	48 (35.6)
Sulfasalazine	26 (19.3)
Methotrexate	32 (23.7)
History of NSAIDs use	91 (67.4)
History of TNFi use	100 (74.1)
1 TNFi	61 (45.2)
2 TNFi	26 (19.3)
≥3 TNFi	11 (8.2)
SpA phenotype, n = 130	
Axial	69 (53.1)
Peripheral	30(23.1)
Both	31 (23.8)
Radiographic-axSpA	35 (26.0)
History of coxitis	4 (3.0)
History of total hip replacement	1 (0.8)
Syndesmophytes	2 (1.5)
Active disease (defined as BASDAI score ≥4/10 at least once during pregnancy)	51 (37.8) for n = 104
Treatment exposure during pregnancy	
Corticosteroids (yes/no)	31 (23.0)
Methylprednisolone pulses	3 (2.2)
csDMARDs	8 (5.9)
NSAIDs	15 (11.1)
Biologics	80 (59.3)

axSpA, axial SpA; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BMI, body mass index; csDMARD, conventional disease-modifying antirheumatic drug; NSAID, nonsteroidal anti-inflammatory drug; SpA, spondyloarthritis; TNFi, TNF inhibitor; WG, weeks of gestation.
[a] Numeric variables are presented as mean (and SD), while dichotomous variables are presented in number and percentage of the total available.

a history of miscarriage or medical termination of pregnancy before 22 WG (Table 1).

During pregnancy, 15 (11.1%), 31 (23.0%), and 80 (59.3%) women were exposed at least once to nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and biologics, respectively (Table 2). Of these, 71 (52.6%), 24 (17.8%), and 5 (3.7%) had received 1, 2, or 3 of these treatments during pregnancy, respectively. An active disease (BASDAI ≥4/10) at least once during pregnancy was found in 51 (37.8%) pregnancies (Table 2).

Pregnancy outcomes in the population with SpA

Of the 135 pregnancies (total SpA population), 125 (92.6%) resulted in a live birth, including 119 (88.1%) full-term births.

Table 2
Pregnancy and maternal adverse outcomes in women with SpA

Pregnancy and maternal adverse outcomes	Total SpA population (N = 135)
Pregnancy outcomes[a]	
Live birth	125 (92.6)
Foetal loss before 22 WG	7 (5.2)
Foetal loss after 22 WG	3 (2.2)
Preterm live birth <37 WG	6 (4.4)
SGA <10th percentile	21/121 (17.4)
SGA <3rd percentile	8/121 (6.6)
Foetal growth restriction	5 (3.7)
Congenital malformations	6 (4.4)
Maternal outcomes	
Assisted reproductive techniques use	16 (11.9)
Gestational diabetes	14 (10.4)
Pre-eclampsia, eclampsia, HELLP	5 (3.7)
Hypertensive disorders of pregnancy	7 (5.5)
Any haemorrhagic complication	6 (4.4)
Premature rupture of membrane	3 (2.2)
Caesarean section	21/124 (16.9)
Induction of labour	27/98 (27.6)

HELLP, Haemolysis, Elevated Liver enzymes, Low Platelets syndrome; SGA, small for gestational age below 10th percentile; SpA, spondyloarthritis; WG, weeks of gestation.
[a] Numeric variables are presented as mean (and SD), while dichotomous variables are presented in number and percentage of the total available.

The most frequent adverse pregnancy outcome was SGA, observed in 21 (15.6%) pregnancies, followed by preterm birth observed in 6 (4.4%) pregnancies. Other adverse pregnancy outcomes included foetal losses before 22 WG for 7 (5.2%) and foetal losses after 22 WG for 3 (2.2%) (Table 2).

Six (4.4%) newborns had a congenital malformation (Table 2): 2 had a major malformation (agenesis of the corpus callosum and unilateral renal agenesis), 3 a minor malformation (hydronephrosis with a pelvic dilation <10 mm, persistent foramen ovale and dacryocystocele), and 1 had a rare genetic syndrome (CHARGE syndrome, manifesting as short size, psychomotor retardation, facial palsy, facial dysmorphia, and vestibular syndrome in the child).

Comparison of pregnancy outcomes between patients with SpA and women from the general population

Of the 135 SpA pregnancies (total SpA population), 128 met ENP inclusion criteria (matched SpA subpopulation) and were matched to 512 control pregnancies (ie, 248 from the ENP 2016 and 264 from the ENP 2021). Comparison between SpA and the general population of women revealed that SGA was more frequent in newborns from mothers with SpA, both for SGA ≤10th (17.4% vs 9.8%, OR 1.94, 95% CI 1.09-3.39) and ≤3rd percentiles (6.6% vs 2.9%, OR 2.35, 95% CI 1.02-5.49). The frequency of other adverse pregnancy outcomes did not differ between women with SpA and the general population. Importantly, the rate of delivery by caesarean section was comparable between women with SpA and their controls (17.4% vs 18.1%, respectively) (Table 3).

Predictors of adverse pregnancy outcomes in SpA pregnancies

As only SGA was significantly more frequent among patients with SpA compared to controls, a logistic regression analysis was performed to identify factors potentially contributing to this

Table 3
Pregnancy and maternal outcomes of the GR2 pregnancies and matched control pregnancies from the 2016 and 2021 ENPs

	Matched SpA subpopulation (GR2) N = 128	French general population (ENP) N = 512	Crude OR 95% CI
Maternal characteristics			
Maternal age by category, y, n (%):			
≤29	33 (25.8)	132 (25.8)	–
30-34	53 (41.4)	212 (41.4)	
35-39	33 (25.8)	132 (25.8)	
≥40	9 (7.0)	36 (7.0)	
Maternal age, y, mean (SD)	32.8 (4.1)	32.2 (5.2)	–
Nulliparity, n (%)	68 (53.1)	272 (53.1)	–
BMI >30 kg/m^2, n (%)	14/116 (12.1)	63/496 (12.7)	–
BMI, kg/m^2, mean (SD)	23.7 (4.8)	24.0 (5.2)	–
Use of tobacco during pregnancy, n (%)	14/124 (11.3)	74/482 (15.4)	–
History of arterial hypertension (chronic or gestational), n (%)	3 (2.3)	12/511 (2.3)	–
History of diabetes (chronic or gestational), n (%)	3 (2.3)	8/510 (1.6)	–
History of preterm birth, n (%)	6 (4.7)	16/511 (3.1)	–
History of SGA, n (%)	4 (3.1)	25/511 (4.9)	–
Pregnancy outcomes			
Preterm live birth, n (%)	6 (4.7)	36 (7.0)	0.65 (0.29-1.55)
SGA <10th percentile, n (%)	21/121 (17.4)	50 (9.8)	**1.94 (1.09-3.39)**
SGA <3rd percentile, n (%)	8/121 (6.6)	15 (2.9)	**2.35 (1.02-5.49)**
Low birth weight, n (%)	6/121 (5.0)	30 (5.9)	0.84 (0.36-2.07)
Macrosomia, n (%)	4/121 (3.3)	32 (6.3)	0.51 (0.19-1.43)
Neonatal transfer to an ICU or neonatology department, n (%)	7 (5.5)	38/508 (7.5)	0.72 (0.30-1.58)
Congenital malformations, n (%)	6 (4.7)	15/506 (3.0)	1.61 (0.63-4.20)
Maternal outcomes			
Assisted reproductive techniques use, n (%)	15 (11.7)	39 (7.6)	1.61 (0.83-2.97)
Gestational diabetes, n (%)	14 (10.9)	70 (13.7)	0.78 (0.43-1.39)
Hypertensive disorders of pregnancy, n (%)	7 (5.5)	17[a] (3.3)	1.68 (0.64-3.93)
Caesarean section, n (%)	21/121 (17.4)	89/493 (18.1)	0.95 (0.56-1.60)
Induction of labour, n (%)	27/98 (27.6)	118 (23.0)	1.27 (0.79-2.08)
Severe postpartum haemorrhage, n (%)	2 (1.6)	11/503 (2.2)	0.71 (0.16-2.94)
Maternal transfer to an ICU, n (%)	2 (1.6)	4/497 (0.8)	1.96 (0.37-8.46)

BMI, body mass index; ENP, Enquête Nationale Périnatale (French perinatal survey); GR2, Groupe de Recherche sur la Grossesse et les maladies Rares; ICU, intensive care unit; OR, odds ratio; SGA, small for gestational age; SpA, spondyloarthritis; WG, weeks of gestation.
[a] The date of diagnosis of the hypertensive disorder was not available for 4 of the 17 cases. Significant results are presented in **bold**.

increased risk. Variables tested in the model as potential predictors of SGA were maternal age, disease duration, SpA form, parity, disease activity during pregnancy, smoking during pregnancy, BMI, and exposure to corticosteroids, NSAIDs, csDMARDs, and TNFi during pregnancy. No significant association was found in univariate and multivariate analyses between SGA and these variables (Table 4). When assessed individually at each visit, trimester-specific disease activity (measured by BASDAI score)—including that observed in the third trimester—was not associated with an increased risk of SGA (OR 0.99, 95% CI 0.54-1.81). Furthermore, the rate of SGA was similar between radiographic and nonradiographic forms of SpA (20% vs 14%, OR 0.63, 95% CI 0.23-1.75), indicating no significant difference between the 2 groups. We also assessed the impact of TNFi withdrawal during pregnancy on the risk of SGA. For this analysis, we distinguished the impact of early withdrawal on the risk of SGA. SGA rates did not differ significantly between groups: 10 (18.2%) in pregnancies without TNFi, 5 (13.5%) in

Table 4
Factors associated with small for gestational age: results from logistic regression model

	Univariate analyses		Multivariate analyses	
	Crude OR 95% CI	*P* value	Adjusted OR 95% CI	*P* value
Age	1.03 (0.91-1.16)	.75	1.04 (0.83-1.29)	.77
BMI	0.99 (0.89-1.10)	.97	0.99 (0.82-1.20)	.74
Nulliparity	2.11 (0.93-4.48)	.09	2.62 (0.90-9.16)	.07
Smoking	0.81 (0.28-3.43)	.58	0.83 (0.25-3.29)	.81
BASDAI score ≥4[a]	0.98 (0.40-2.43)	.96	1.15 (0.43-3.07)	.78
TNFi[a]	0.64 (0.29-1.69)	.37	0.67 (0.22-2.01)	.45
NSAIDs[a]	0.71 (0.15-2.39)	.19	0.68 (0.18-4.56)	.57
Corticosteroids[a]	1.09 (0.45-2.65)	.88	1.15 (0.51-2.71)	.90

BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BMI, body mass index; NSAID, nonsteroidal anti-inflammatory drug; OR, odds ratio; TNFi, tumour necrosis factor inhibitor.
[a] At least once during pregnancy.

those exposed during the first trimester only, and 6 (14.6%) in those exposed during the second and/or third trimester (P = .57 and .61, Supplementary Table).

When stratifying the cohort by calendar period (corresponding to the periods used for matching with the general population [2016-2018 vs 2019-2021]), the rate of SGA was similar between groups (15.4% vs 15.7%, P = .99), despite a significantly higher use of TNFi during pregnancy in the later period (46.2% vs 71.4%, P = .01).

DISCUSSION

In our study, SGA was the only adverse pregnancy outcome more frequent in newborns from mothers with SpA in comparison to women from the general population.

Retrospective data from the past decades indicated an increase in the risks of preterm birth, SGA, low birth weight, pre-eclampsia, and caesarean section in women with SpA. In contrast, our study found that, apart from the risk of SGA, adverse pregnancy outcomes rates were comparable to those observed in the general population. Along with other recent prospective findings [22], our results offer reassurance to women with SpA. Interestingly, recent data from a Swedish cohort reported a gradual decrease in the risk of adverse pregnancy outcomes from 2007 to 2020, coinciding with an increase in TNFi use during pregnancy [23]. The pregnancies included in our study, which reflect the current clinical management of SpA, with more than half of the women treated with biologics, support the fact that adverse pregnancy outcomes rates are now close to those of the general population, except SGA. For this outcome, the underlying cause remains unclear and does not appear to be fully explained by disease activity, as SGA was not associated with BASDAI scores at any trimester, including T3, and the risk remained stable between calendar periods despite a significantly higher use of TNFi in the later period. One of the major results is that the rate of delivery by caesarean section did not differ in our study between women with SpA and controls, in contrast with previous reports from earlier studies. Our results could be partly explained by the inclusion of patients mostly followed in tertiary centres, with a strong collaboration between rheumatologists and obstetricians to optimise maternal and pregnancy management. However, it could also be explained by a better therapeutic management of SpA during pregnancy and global awareness of the different healthcare professionals involved in the management of these pregnancies, as this decline has also been observed in other countries where the rate of caesarean section is approaching that of the general population [23].

Among the studies that have explored factors potentially associated with adverse pregnancy outcomes in women with SpA, some have found an association with NSAIDs and corticosteroids exposure during pregnancy, independently of disease activity [24,25], an association that we do not observe. Some factors could explain these discrepancies: in the study by Smith et al [25], pregnancies were conducted in earlier years (from 2004 to 2018), women were more exposed to corticosteroids (38% vs 23% in ours), and almost half of the women had psoriatic arthritis. Similarly, we did not find an association with disease activity, potentially due to the low disease activity overall of our patients, largely treated with TNFi.

The rates of exposure to different treatments in our cohort are also in line with the trends observed in the use of treatments during pregnancy in women with chronic inflammatory rheumatic disorders. Indeed, the increased frequency of pregnancies in this population and the better knowledge of the safety profile of biologics during pregnancy are accompanied by a shift in the use of treatments in pregnancy management of these patients. In a study, which involved 2645 pregnancies including 13.7% in women with SpA, Desai et al [26] looked at treatment prescription. They observed a decline in corticosteroid use over the years, even if it remained strong in 2012, in contrast to the use of biologics during pregnancy that increased 3-fold over 10 years. However, rates of discontinuation of preconceptional therapies such as biologics were high throughout the pregnancy, with 61% of the women included in the study having discontinued treatment during pregnancy [26].

Our study has several limitations. The statistical power was limited by the relatively limited number of patients, which did not allow for subgroup analyses on the different forms of SpA. Another limitation may have been a selection bias, as despite the national and multicentric nature of the cohort, patients were primarily recruited in tertiary rheumatology centres, potentially enriching our study population with more severe forms of SpA with an increased risk of adverse pregnancy outcomes. Nevertheless, it also reinforces the reassuring message of this study, as no major increase in the risk of adverse pregnancy outcomes was found, even among the most severe forms of SpA.

Our study also has some strengths worth mentioning. This is one of the few existing prospective studies worldwide aiming to describe pregnancy outcomes in patients with SpA, limiting recall bias and with an extensive data collection on disease characteristics of patients and their disease activity, while the available literature is essentially based on retrospective cohorts or administrative databases. Furthermore, disease activity was assessed by a validated tool, while a review of the literature highlighted the major heterogeneity between the tools used in clinical trials for disease activity assessment during pregnancy in women with rheumatic diseases [27]. Finally, the matched analysis allowed us to establish robust and contemporary comparisons to the general population.

To summarise, in our prospective study, only a moderate 2-fold increased risk of SGA was found in SpA pregnancies when compared to the general population. These results provide very reassuring data for patients and physicians and should help the counselling and management of pregnancy in women with SpA.

CRediT authorship contribution statement

Sabrina Hamroun: Writing – original draft, Methodology, Formal analysis, Data curation. **Grégoire Martin de Frémont:** Writing – original draft, Formal analysis, Data curation. **Nathalie Costedoat-Chalumeau:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Marion Couderc:** Writing – review & editing, Investigation, Conceptualization. **René-Marc Flipo:** Writing – review & editing, Investigation. **Jérémie Sellam:** Writing – review & editing, Investigation. **Christophe Richez:** Writing – review & editing, Investigation. **Rakiba Belkhir:** Writing – review & editing, Investigation. **Laure Gossec:** Writing – review & editing, Investigation. **Hubert Marotte:** Writing – review & editing, Investigation. **Emmanuel Dernis:** Writing – review & editing, Investigation. **Aline Frazier-Mironer:** Writing – review & editing, Investigation. **Elisabeth Gervais:** Writing – review & editing, Investigation. **Cédric Lukas:** Writing – review & editing. **Valérie Devauchelle-Pensec:** Writing – review & editing, Investigation. **Alban Deroux:** Writing – review & editing, Investigation. **Véronique Le Guern:** Writing – review & editing, Resources, Project administration, Methodology,

Investigation, Funding acquisition, Data curation, Conceptualization. **Gaëlle Guettrot-Imbert:** Writing – review & editing, Visualization, Validation, Project administration, Data curation. **Nathalie Lelong:** Writing – review & editing, Resources, Project administration, Methodology. **Emmanuelle Pannier:** Writing – review & editing, Investigation. **Loic Sentilhes:** Writing – review & editing. **Camille Le Ray:** Writing – review & editing, Supervision. **Raphaèle Seror:** Writing – review & editing, Supervision, Methodology, Formal analysis. **Anna Molto:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Acknowledgements

The authors thank Ada Clarke for her assistance in managing the e-case report form and collecting data. The authors thank the patients for agreeing to participate in the study and patient associations for their strong support. The authors would also like to thank the French Society of Internal Medicine (SNFMI), the French Society of Rheumatology (SFR), and the filière de santé des maladies auto-immunes et auto-inflammatoires rares (FAI^2R) for their scientific and technical support.

Funding

The GR2 study has received grants from patient associations (Lupus France; association des Sclérodermiques de France, association Gougerot Sjögren, Association Francophone contre la Polychondrite chronique atrophiante), from the AFM-Telethon, the SNFMI, the SFR, the CMEL commission for Research and Innovation of Cochin Hospital, the Ministère de la Santé (the Clinical Research Contract—Database CRCBDD17003), Foundation for Research in Rheumatology, ORRICK society (Prix Véronique ROUALET), and an unrestricted grant from UCB (the company had no role in the initiation, planning, conduct, data assembly, analysis, or interpretation of the study). The Direction de la Recherche Clinique et du Développement provided logistic and administrative support.

Competing interests

SH: none; GMdF: none, NC-C: grant from UCB and Roche to her institution and honoraria for symposium from Bristol Myers Squibb; MC: none; R-MF: none; JS: none; CR: none; RB: none; LG: disclosures outside the submitted work: research grants: AbbVie, Lilly, Novartis, and UCB; consulting fees: AbbVie, Alfa-Sigma, Amgen, BMS, Celltrion, J&J, Lilly, Moonlake, Novartis, Pfizer, Stada, and UCB; nonfinancial support: AbbVie, Amgen, Biogen, Celltrion, Janssen, Pfizer, and UCB; HM: none; ED: none; AF-M: none; EG: none; CL: none; VD-P: none; AD: none; VLG: honoraria for presentations or speaker bureaus from Bristol Myers Squibb, GSK, AstraZeneca-MedImmune, and Novartis; consulting fees from Novartis; and was supported by AstraZeneca-MedImmune and Novartis for attending meetings and travel; GG-I: none; NL: none; EP: none; LS: none; CLR: none; RS: consulting fee/honorarias from GlaxoSmithKline, Boehringer, Kiniska, Janssen, Astra ZENECA, alpha Sigma, Bristol Myers Squibb, and Novartis; grant support: Roche and Novartis; and support for attending meeting from GlaxoSmithKline, Novartis, and Amgen—none is related to this study; AM: consulting fees from AbbVie, Amgen, BMS, Galapagos, Gilead, Janssen, Lilly, Novartis, Pfizer, UCB Pharma, and Viatris; research grants from AbbVie and Biogen.

Patient consent for publication

According to the French regulation, women received information on the study from the investigators and had to orally express that they did not oppose the use of their data for this study and futher publication of the results.

Ethics approval

The local Ethics Committee (CPP Île-de-France VI) approved GR2 study (29/08/2012). The ENP 2016 was approved by the National Council on Statistical Information (Comité du Label no. 2016X703SA), the French Data-Protection Authority (CNIL no. 915197) and the INSERM Ethics Committee (IRB00003888 no. 14-191). The ENP 2021 was approved by the Label Committee (Label of general interest and statistical quality, Visa n°2021 × 701SA, order of November 23, 2020), Committee for the Protection of Persons (CPP) (July 7, 2020), Committee of Ethics and Scientists for Research, Studies and Evaluations (CESREES) (June 12, 2020) and the Commission Nationale de l'Informatique et des Libertés (CNIL) (DR-2020-391 of December 31, 2020 and DR-2022-179 of July 28, 2022).

Provenance and peer review

Not commissioned; externally peer reviewed.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.10.024.

Orcid

Laure Gossec: http://orcid.org/0000-0002-4528-310X
Anna Molto: http://orcid.org/0000-0003-2246-1986

REFERENCES

[1] Carmona L, Cross M, Williams B, Lassere M, arthritis March LRheumatoid. Best Pract Res Clin Rheumatol 2010;24:733–45. doi: 10.1016/j.berh.2010.10.001.
[2] Stolwijk C, Boonen A, van Tubergen A, Reveille JD. Epidemiology of spondyloarthritis. Rheum Dis Clin North Am 2012;38:441–76. doi: 10.1016/j.rdc.2012.09.003.
[3] Sieper J, Poddubnyy D. Axial spondyloarthritis. Lancet 2017;390:73–84. doi: 10.1016/S0140-6736(16)31591-4.
[4] Zeidler H, Amor B. The Assessment in Spondyloarthritis International Society (ASAS) classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general: the spondyloarthritis concept in progress. Ann Rheum Dis 2011;70:1–3. doi: 10.1136/ard.2010.135889.
[5] Hamroun S, Hamroun A, Bigna JJ, Allado E, Förger F, Molto A. Fertility and pregnancy outcomes in women with spondyloarthritis: a systematic review and meta-analysis. Rheumatology (Oxford) 2022;61:1314–27. doi: 10.1093/rheumatology/keab589.
[6] de Man YA, Hazes JM, van der Heide H, Willemsen SP, de Groot CJ, Steegers EA, et al. Association of higher rheumatoid arthritis disease activity during pregnancy with lower birth weight: results of a national prospective study. Arthritis Rheum 2009;60:3196–206. doi: 10.1002/art.24914.
[7] Nørgaard M, Larsson H, Pedersen L, Granath F, Askling J, Kieler H, et al. Rheumatoid arthritis and birth outcomes: a Danish and Swedish nationwide prevalence study. J Intern Med 2010;268:329–37. doi: 10.1111/j.1365-2796.2010.02239.x.
[8] Wallenius M, Salvesen KÅ, Daltveit AK. Skomsvoll JF. Rheumatoid arthritis and outcomes in first and subsequent births based on data from a national

birth registry. Acta Obstet Gynecol Scand 2014;93:302–7. doi: 10.1111/aogs.12324.
[9] Remaeus K, Johansson K, Askling J, Stephansson O. Juvenile onset arthritis and pregnancy outcome: a population-based cohort study. Ann Rheum Dis 2017;76:1809–14. doi: 10.1136/annrheumdis-2016-210879.
[10] Polachek A, Eder L. Pregnancy outcome in inflammatory arthropathies - are population database studies the right tool? J Rheumatol 2020;47:161–3. doi: 10.3899/jrheum.190631.
[11] Murarasu A, Guettrot-Imbert G, Le Guern V, Yelnik C, Queyrel V, Schleinitz N, et al. Characterisation of a high-risk profile for maternal thrombotic and severe haemorrhagic complications in pregnant women with antiphospholipid syndrome in France (GR2): a multicentre, prospective, observational study. Lancet Rheumatol 2022;4:e842–52. doi: 10.1016/S2665-9913(22)00308-3.
[12] de Frémont GM, Costedoat-Chalumeau N, Lazaro E, Belkhir R, Guettrot-Imbert G, Morel N, et al. Pregnancy outcomes in women with primary Sjögren's syndrome: an analysis of data from the multicentre, prospective, GR2 study. Lancet Rheumatol 2023;5:e330–40. doi: 10.1016/S2665-9913(23)00099-1.
[13] Meissner Y, Strangfeld A, Costedoat-Chalumeau N, Förger F, Goll D, Molto A, et al. European Network of Pregnancy Registers in Rheumatology (EuNeP)-an overview of procedures and data collection. Arthritis Res Ther 2019;21:241. doi: 10.1186/s13075-019-2019-3.
[14] Meissner Y, Fischer-Betz R, Andreoli L, Costedoat-Chalumeau N, De Cock D, Dolhain RJEM, et al. EULAR recommendations for a core data set for pregnancy registries in rheumatology. Ann Rheum Dis 2021;80:49–56. doi: 10.1136/annrheumdis-2020-218356.
[15] Rudwaleit M, Landewé R, van der Heijde D, Listing J, Brandt J, Braun J, et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part I): classification of paper patients by expert opinion including uncertainty appraisal. Ann Rheum Dis 2009;68:770–6. doi: 10.1136/ard.2009.108217.
[16] Le Ray C, Lelong N, Cinelli H, Blondel B. Collaborators - Members of the ENP2021 Study Group. Results of the 2021 French National Perinatal Survey and trends in perinatal health in metropolitan France since 1995. J Gynecol Obstet Hum Reprod 2022;51:102509. doi: 10.1016/j.jogoh.2022.102509.
[17] Jacques M, Chantry AA, Evrard A, Lelong N, Le Ray C. ENP2021 Study Group. Consent for interventions during childbirth: a national population-based study. Int J Gynaecol Obstet 2025;168:333–42. doi: 10.1002/ijgo.15830.
[18] Enquête nationale périnatale 2016. Les naissances et les établissements, situation et évolution depuis 2010 | Direction de la recherche, des études, de l'évaluation et des statistiques. [cited 2025 Jun 30]. Available from: https://drees.solidarites-sante.gouv.fr/publications/rapports/enquete-nationale-perinatale-2016-les-naissances-et-les-etablissements
[19] Sieper J, Rudwaleit M, Baraliakos X, Brandt J, Braun J, Burgos-Vargas R, et al. The Assessment of SpondyloArthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis. Ann Rheum Dis 2009;68(suppl 2) ii1-44. doi: 10.1136/ard.2008.104018.
[20] Hogan JW, Blazar AS. Hierarchical logistic regression models for clustered binary outcomes in studies of IVF-ET. Fertil Steril 2000;73:575–81. doi: 10.1016/s0015-0282(99)00577-4.
[21] Blazek K, van Zwieten A, Saglimbene V, Teixeira-Pinto A. A practical guide to multiple imputation of missing data in nephrology. Kidney Int 2021;99:68–74. doi: 10.1016/j.kint.2020.07.035.
[22] Meissner Y, Strangfeld A, Molto A, Forger F, Wallenius M, Costedoat-Chalumeau N, et al. Pregnancy and neonatal outcomes in women with axial spondyloarthritis: pooled data analysis from the European Network of Pregnancy Registries in Rheumatology (EuNeP). Ann Rheum Dis 2022;81:1524–33. doi: 10.1136/ard-2022-222641.
[23] Morin M, Frisell T, Stephansson O, Hellgren K. Temporal trends in adverse pregnancy outcomes in axial spondyloarthritis in Sweden: a cohort study. Lancet Rheumatol 2023;5:e121–9. doi: 10.1016/S2665-9913(23)00001-2.
[24] Pons M, Dougados M, Costedoat-Chalumeau N, Briot K, Goupille P, Roux C, et al. Pregnancy rates and outcomes in early axial spondyloarthritis: an analysis of the DESIR cohort. Joint Bone Spine 2021;88:105075. doi: 10.1016/j.jbspin.2020.09.007.
[25] Smith CJF, Bandoli G, Kavanaugh A, Chambers CD. Birth outcomes and disease activity during pregnancy in a prospective cohort of women with psoriatic arthritis and ankylosing spondylitis. Arthritis Care Res (Hoboken) 2020;72:1029–37. doi: 10.1002/acr.23924.
[26] Desai RJ, Huybrechts KF, Bateman BT, Hernandez-Diaz S, Mogun H, Gopalakrishnan C, et al. Brief report: patterns and secular trends in use of immunomodulatory agents during pregnancy in women with rheumatic conditions. Arthritis Rheumatol 2016;68:1183–9. doi: 10.1002/art.39521.
[27] Andreoli L, Gerardi MC, Fernandes M, Bortoluzzi A, Bellando-Randone S, Brucato A, et al. Disease activity assessment of rheumatic diseases during pregnancy: a comprehensive review of indices used in clinical studies. Autoimmun Rev 2019;18:164–76. doi: 10.1016/j.autrev.2018.08.008.

Ann Rheum Dis 85 (2026) 328–338

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Systemic lupus erythematosus

IL-2 signalling sustains pathogenic age-associated B cell homeostasis in lupus

Xiaxia Han[1], Yang Jiang[1], Shuangshuang Gu[1], Yiwei Shen[1], Huihua Ding[1], Sheng Chen[1], Qiong Fu[1], John B. Harley[3,4], Dai Dai[1,2,*], Nan Shen[1,2,3,4,*]

[1] *Shanghai Institute of Rheumatology, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*
[2] *State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*
[3] *Cincinnati Education and Research for Veterans Foundation, Cincinnati, OH, USA*
[4] *US Department of Veterans Affairs Medical Center, Research Service, Cincinnati, OH, USA*

ARTICLE INFO

Article history:
Received 17 December 2024
Received in revised form 10 June 2025
Accepted 9 August 2025

ABSTRACT

Objectives: Despite expanding regulatory T (Treg) cells, low-dose interleukin-2 (IL-2) therapy shows variable clinical efficacy in systemic lupus erythematosus (SLE). Here, we investigated whether previously unrecognised effects of IL-2 on age-associated B cells (ABCs) explain this therapeutic heterogeneity.

Methods: Integrated transcriptomic analysis was used to identify the prevalent signalling pathways associated with lupus pathogenic ABCs. The effects of IL-2 on ABCs were examined. TLR7-driven lupus-prone mice were administered to assess the efficacy of IL-2 therapy. IL-2 responsiveness of ABC was further evaluated in patients with SLE through bioinformatic analysis.

Results: Transcriptomic and single-cell analyses revealed elevated IL-2 signalling in ABCs, with a more pronounced IL-2 signature in patients with SLE than in healthy controls. IL2RB, a subunit of the IL-2 receptor, was significantly enriched in ABCs and showed increased chromatin accessibility at its promoter. IL-2 promoted ABC survival and differentiation in a stage-dependent manner. Prior IL-2 administration to recipient mice promoted the persistence of adoptively transferred ABCs. In lupus-prone mice, IL-2 administration increased both ABC and Treg populations without ameliorating disease manifestations with ABC's promotion being more dependent on the disease condition. Additionally, ABCs showed elevated expression of IL-2 receptor correlating with ABC frequency in our cohorts, and activation of IL-2 signalling was further observed in B cells and ABCs from patients with SLE.

Conclusions: This study identified ABCs as a novel target of IL-2, expanding the understanding of IL-2's role in SLE beyond the traditional Treg-centric view and offering insights into the variable therapeutic efficacy of IL-2 in this context.

*Correspondence to Dr. Nan Shen, Prof. Dai Dai, Shanghai Institute of Rheumatology, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, 145 Shan Dong Middle Road, Shanghai 200001, China.

E-mail addresses: daidaisibs@163.com (D. Dai), nanshensibs@gmail.com (N. Shen).

XH, YJ, SG, and YS contributed equally to this work.

Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.08.012

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Age-associated B cells (ABCs), the crucial pathogenic B cell subset, are greatly expanded in systemic lupus erythematosus (SLE) and involved in disease progression.
- Low-dose interleukin (IL)-2 therapy has been shown to be safe and effective in inducing Treg expansion and restoring immune tolerance in SLE, yet it displays therapeutic heterogeneity in clinical trials.
- Previous studies have found that IL-2 promotes effector B cell response, but these effects on pathogenic B cell subsets in autoimmune disease and the links with therapeutic outcomes are unknown.

WHAT THIS STUDY ADDS

- Based on the integrated transcriptomic analysis, this study demonstrated that ABCs shared a responsive IL-2 signalling pathway across multiple disease states.
- IL-2 was found to promote ABC survival and development, as evidenced by both experimental observations and transcriptome analysis.
- The dual effects of low-dose IL-2 therapy on ABCs and Tregs were observed in 2 TLR7-driven lupus models, with limited improvement in serological symptoms.
- The IL-2 signature was elevated in SLE ABCs, and surface IL-2 receptor expression of ABCs correlated with their accumulation in patients with SLE.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- This study identifies IL-2 as a novel cytokine niche for ABC development, expanding our understanding of ABC physiology in lupus pathogenesis.
- This study provides new insights into the stratification of low-dose IL-2 treatment in patients with SLE. The balance between ABCs and Tregs may serve as a predictive biomarker for IL-2 therapy, underscoring the need to refine strategies that enhance Treg benefits while limiting ABC-related effects.

INTRODUCTION

Interleukin (IL)-2 is a pleiotropic cytokine that modulates multiple immune cell subsets and signalling networks, exerting wide-ranging effects on immune regulation [1,2]. Beyond its classical functions in promoting Treg differentiation and maintenance, IL-2 exhibits diverse effects including augmentation of natural killer cell cytolytic activity and regulation of effector T cell responses, as well as B cell maturation [3–7]. This multifaceted nature creates intricate downstream effects that influence both immune homeostasis and pathological conditions, particularly in the context of autoimmune diseases where immune regulation is fundamentally disrupted.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterised by disrupted immune balance, resulting in dysregulated production of autoantibodies and proinflammatory cytokines, which lead to systemic inflammation and progressive organ damage [8]. Previous studies have long demonstrated that IL-2 production is defective in SLE, contributing to immune imbalance in patients [9–12]. Accordingly, low-dose IL-2 therapy has been explored as a novel immunomodulatory strategy in SLE [13–15]. However, 2 randomised controlled trials (RCTs) of low-dose IL-2 in SLE failed to meet their primary endpoints [16,17]. Nonetheless, both trials demonstrated positive outcomes based on secondary endpoints or post hoc analyses, underscoring the heterogeneity in therapeutic response. In addition, clinical observations have reported inconsistent patterns of IL-2 dysregulation across various autoimmune diseases [18–20], including SLE, further highlighting the complex role of IL-2 in SLE pathogenesis and treatment. These inconsistencies likely stem from SLE's complex aetiopathogenesis and our incomplete understanding of IL-2's diverse effects across immune cell populations.

Recent findings indicate that IL-2 can directly regulate B cells, which are key drivers of SLE pathogenesis, by guiding naïve B cells towards plasma cell differentiation and promoting extrafollicular B cell maturation [7,21]. Age-associated B cells, also termed as $CD11c^+$ B cells, $CD11c^{hi}T\text{-}bet^+$ B cells, double negative 2 B cells or atypical memory B cells, are a distinct effector B cell subset characterised by unique surface markers, transcriptome signature, and development pathway [22–28]. Age-associated B cells (ABCs) have been shown to expand in multiple autoimmune diseases and are considered major producers of autoantibodies [23,24,26,29]. ABCs are also found in affected tissues, where they contribute to tissue damage [28,30,31]. The formation of ABCs depends on both extrinsic signals and intrinsic regulators. Toll-like receptor (TLR) signalling, B-cell receptor (BCR) engagement, and T cell help collectively orchestrate ABC formation [22,23,32–36]. Intrinsic transcriptional regulators in B cells, including ZEB2, T-bet, and IRF5, play essential roles in ABC differentiation and function [30,35,37,38]. Notably, ZEB2 directs the extrafollicular development of ABCs by repressing germinal centre (GC) confinement, enabling ABCs to form at the T-B cell border, where they receive T cell help [30,39–41]. The well-established classical T cell signals for ABC development include IL-21 and interferon γ (IFN-γ) [22,42]. However, T cell-derived IL-2 is also implicated in B cell maturation, particularly in the extrafollicular effector B cell response [7]. Whether IL-2 directly influences the generation or maintenance of ABCs in autoimmune settings is unknown, representing a critical gap in our understanding of ABC regulation. Addressing this question is important, as IL-2's effects on pathogenic B cells could help explain the heterogeneity of clinical responses to IL-2 therapy in SLE.

Thus, we hypothesised that IL-2 may play a role in regulating ABCs in lupus, contributing to disease pathogenesis and potentially underlying the variable efficacy of IL-2 treatment. In this study, we employed a comprehensive research strategy to investigate the regulatory role of IL-2 on ABCs during lupus pathogenesis. Beginning with transcriptomic analysis of patients with SLE, we identified unexpected IL-2 signalling activation in pathogenic ABCs. We validated this observation through multiple approaches, including epigenetic profiling and single-cell RNA sequencing across various disease conditions, establishing IL-2 responsiveness as a conserved feature of ABCs. Functional studies including both *in vitro* culture and *in vivo* transfer experiments revealed that IL-2 promotes ABC survival and maintenance. Using 2 distinct lupus mouse models, we demonstrated that IL-2 administration simultaneously expands both protective Tregs and pathogenic ABCs, potentially explaining the variable therapeutic outcomes observed in clinical settings. Finally, we established clinical relevance by showing correlations between IL-2 signalling, ABC accumulation, and disease severity in lupus patients. Together, these findings reveal a previously unrecognised mechanism by which IL-2 influences lupus pathogenesis through direct effects on ABCs, suggesting that the balance between regulatory and pathogenic cell responses may determine therapeutic efficacy. This work provides new insights into the complex role of IL-2 in autoimmunity and offers potential strategies for improving cytokine-based therapies in lupus treatment.

METHODS

See supplementary materials.

RESULTS

ABCs exhibit activated IL-2 signalling

To identify key regulators of ABC biology, we first analysed transcriptomic profiles from the GSE118254 dataset, which includes ABCs, switched memory B cells (SWM), and naïve B cells sorted from patients with SLE and healthy controls [27]. Differential expression analysis comparing ABCs with both SWM and naïve B cells confirmed the classical ABC signature, including *ITGAX, TBX21,* and *ZEB2* (Supplementary Fig S1A). In addition to these canonical markers, pathway enrichment analysis of upregulated genes revealed a prominent enrichment of the IL-2 signalling pathway and antigen-activated B cell receptor generation of second messengers in ABCs, greater than in SWM or naïve B cells (Fig 1A). To validate these findings, we further examined 2 additional independent transcriptome dataset from patients with SLE (GSE92387 and GSE110999), focusing on

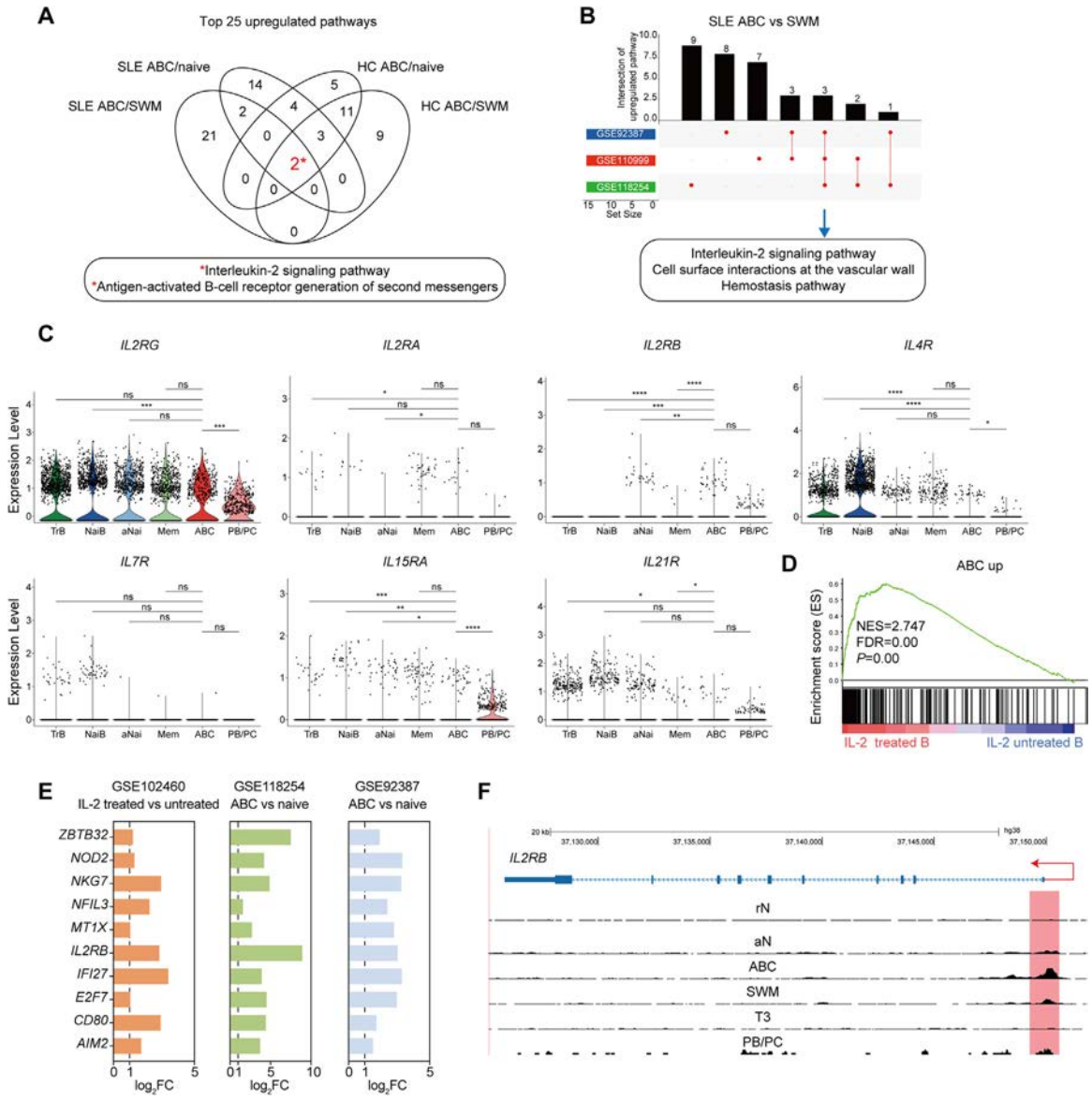


Figure 1. Transcriptional activation of interleukin (IL)-2 signalling in ABC. (A) Significantly upregulated genes ($P < .01$, $\log_2FC > 1$) were identified from ABC vs naïve, and ABC vs SWM B cell comparisons in patients with systemic lupus erythematosus (SLE) and healthy controls from public dataset GSE118254. BioPlanet pathway enrichment analysis of genes upregulated among these gene sets, performed using the Enrichr tool. The Venn diagram shows the overlap among the top 25 enriched pathways. (B) BioPlanet pathway enrichment analysis of genes upregulated in ABCs vs SWM from public dataset GSE118254, GSE92387, and GSE110999, performed using the Enrichr tool. UpSet plot depicting the intersection of top 15 enriched pathways among the indicated datasets. (C) Violin plots showing the expression levels of IL-2 family receptors (*IL2RG, IL2RA, IL2RB, IL4R, IL7R, IL15RA,* and *IL21R*) in B cell subsets from a patient with newly onset SLE, with log-normalised expression values labelled. (D) Gene set enrichment analysis (GSEA) enrichment plot for ABC-up gene set in IL-2 primed B cells and unprimed B cells (GSE102460). (E) Barplots depicting the $\log_2FC$ level of the selected IL-2 inducible genes in IL-2-treated B cells vs untreated B cells (GSE102460), ABCs vs resting naïve B cells from patients with SLE (GSE118254), and ABCs vs naïve B cells from patients with SLE (GSE92387). (F) The chromatin accessibility around *IL2RB* locus in B cell subsets from patients with SLE ATAC data (GSE118256), visualised with the UCSC genome browser (https://genome.ucsc.edu). *$P < .05$, **$P < .01$, ***$P < .001$; ****$P < .0001$; ns, not significant. Wilcoxon rank-sum test for comparing ABCs to other B cell subsets (C). ABC, age associated B cells; aN, activated naïve B cells; aNai, activated naïve B cells; FC, fold change; FDR, false discovery rate; HC, healthy controls; Mem, memory B cells; NaiB, naïve B cells; NES, normalised enrichment score; PB/PC, plasmablasts/plasma cells; rN, resting naïve B cells; SWM, switched memory B cells; T3, transitional 3 B cells; TrB, transitional B cells; ATAC, Assay for Transposase-Accessible Chomatin.

comparisons between ABCs and SWM (Supplementary Fig S1B) [25,26]. Consistently, IL-2 signalling pathway was again significantly enriched in ABCs across all 3 datasets, reinforcing the potential relevance of IL-2 signalling in ABC biology (Fig 1B). Given the known role of IL-2 family cytokines in shaping immune responses, we focused on the expression patterns of IL-2 receptor components. The IL-2 family—which includes IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21—shares the common gamma chain (γ_c) as a critical receptor subunit [43]. We observed that this γ_c was broadly expressed across multiple B cell subsets (Fig 1C), consistent with its fundamental role in immune regulation. For IL-2 signalling specifically, the γ_c pairs with the IL-2 receptor β chain (*IL2RB*, CD122) to form a dimeric receptor of moderate affinity. Incorporation of the IL-2 receptor α chain (*IL2RA*, CD25) generates a high-affinity trimeric receptor complex, and both the dimeric and trimeric forms are capable of initiating downstream signalling [2]. In our datasets, the IL-2 receptor β chain, which is critical for both intermediate and high-affinity IL-2 signalling, was specifically enriched in activated B cells known as ABC progenitors and mature ABCs (Fig 1C). To determine whether this transcriptional pattern corresponded to functional responsiveness, we performed gene set enrichment analysis (GSEA) and found that ABC-enriched genes overlapped significantly with IL-2-induced transcriptional signatures, including key immune regulators such as *NKG7, ZBTB32, CD80*, and *NFIL3* (Fig 1D,E). Notably, these IL-2-responsive genes were also upregulated in murine ABCs, indicating that this regulatory axis is conserved across species (Supplementary Fig S2A,B). Thus, ABCs appeared poised to respond robustly for IL-2 signalling, suggesting that IL-2 may influence ABC differentiation, survival, or effector functions.

To further corroborate this finding at the epigenetic level, we analysed ATAC-seq data from B cells of patient with SLE. ABCs showed increased chromatin accessibility at the *IL2RB* transcription start site, a feature mirrored in murine ABCs (Fig 1F and Supplementary Fig S2C). While increased accessibility does not confirm active signalling, this epigenetic evidence indicates that ABCs are permissively primed to engage IL-2 pathways. Collectively, these integrative analyses of transcriptional and epigenetic profiles provide compelling evidence that IL-2 signalling is functionally activated in ABCs and may serve as a critical regulator of ABC homeostasis. The conservation of these molecular features between human and mouse ABCs further suggests that IL-2 signalling represents a fundamental mechanism in ABC biology.

Conserved IL-2 signalling is enriched in ABCs across disease conditions

ABCs have been implicated in autoimmune processes and appear in diverse pathogenic contexts, including infectious diseases, genetic disorder, and cancers [30,31,44–46]. These cells often share broadly similar transcriptional signatures [44,47]. To further characterise their common features, we integrated single-cell RNA sequencing (scRNA-seq) datasets from multiple disease settings: peripheral blood from healthy controls [48], patients with SLE and rheumatoid arthritis [30,49], kidney tissue from patients with lupus nephritis (LN) [48,50], and blood from malaria-infected individuals [44] (Fig 2A). This integrated analysis confirmed a conserved ABC signature, marked by uniform expression of canonical ABC genes such as *ITGAX, ZEB2, ITGB2*, and *FCRL5* (Supplementary Fig S3). To assess whether IL-2 signalling is consistently active in ABCs across these conditions, we first conducted pathway enrichment analyses of the upregulated genes in ABC ($\log_2$FC > 0.25 and adjusted $P < .01$). This revealed that IL-2 signalling was significantly and consistently enriched in ABCs (Fig 2B).

Cytokines from the same family often utilise overlapping signalling pathways, and pleiotropic cytokines can exert distinct regulatory effects across different immune cell types. These characteristics may confound conventional pathway enrichment analyses and obscure cytokine-specific effects within B cell subsets. To better evaluate IL-2 responsiveness, we analysed transcription changes in human primary B cells following IL-2 stimulation. We defined the upregulated differentially expressed genes (DEGs) as the 'IL-2 module' and applied this module to each dataset, revealing consistently robust IL-2 signalling activity in ABCs (Fig 2C and Supplementary Fig S4A). Interestingly, plasma cells also exhibited elevated IL-2 module scores, consistent with previous studies highlighting the critical role of IL-2 in plasma cell differentiation [7,21,51].

To further investigate whether the IL-2 responsiveness is enhanced under pathological conditions, we compared IL-2 module scores between healthy controls and patients with SLE (Supplementary Fig S5A,B). Although ABCs from all conditions exhibited higher IL-2 module scores than naïve B cells (Fig 2D), both ABCs and naïve B cells from patients with SLE showed higher IL-2 signalling activity compared to their counterparts from healthy controls (Fig 2E and Supplementary Fig S5C). In contrast, plasma cells did not show significant differences between disease states, suggesting a more general role of IL-2 in terminal plasma cell differentiation (Supplementary Fig S5C). Notably, ABCs isolated from inflamed kidney tissue displayed the highest IL-2 module scores, indicating a potential association between elevated IL-2 signalling and the pathogenic activity of tissue-resident ABCs. Furthermore, we found that tissue-resident ABCs (CB0) within LN kidney samples expressed high levels of key IL-2 signalling components (*IL2RA, IL2RB,* and *STAT5A*), reaching levels comparable to those in regulatory T cells (CT3a) (Supplementary Fig S6A) [50]. Given the dominance of ABCs in the B cell compartment within affected tissues during autoimmune disease [30,31,50], these findings suggest that IL-2 signalling could be critical for ABC homeostasis or function in disease settings and affected tissues.

Taken together, these results establish that ABCs share a conserved and active IL-2 signalling feature across multiple disease states and tissue niches. The further enhancement of IL-2 responsiveness under disease conditions highlights the potential involvement of ABCs in the pathogenesis of autoimmune diseases.

IL-2 signalling ensures ABC maintenance and survival

The cytokine microenvironment that shapes ABC biology is well characterised, with IFN-γ and IL-21 exerting distinct, stage-specific roles in ABC development [25,32,42,51]. Given the enhanced IL-2 imprint observed in the ABC transcriptome, we next asked whether IL-2 also contributes to ABC development. We first confirmed that stimulation with TLR7—a key signal for ABC induction—markedly upregulated CD25 and CD122 on B cells, indicating that ABC-polarising stimuli significantly enhanced their sensitivity to IL-2 signalling (Supplementary Fig S7A).

We then investigated the regulatory effects of IL-2 on ABC biology and found that IL-2 supplementation moderately promoted the *in vitro* induction of both human and mouse ABCs during the early stage of differentiation (Fig 3A and Supplementary Fig S7B). However, this effect was less potent than

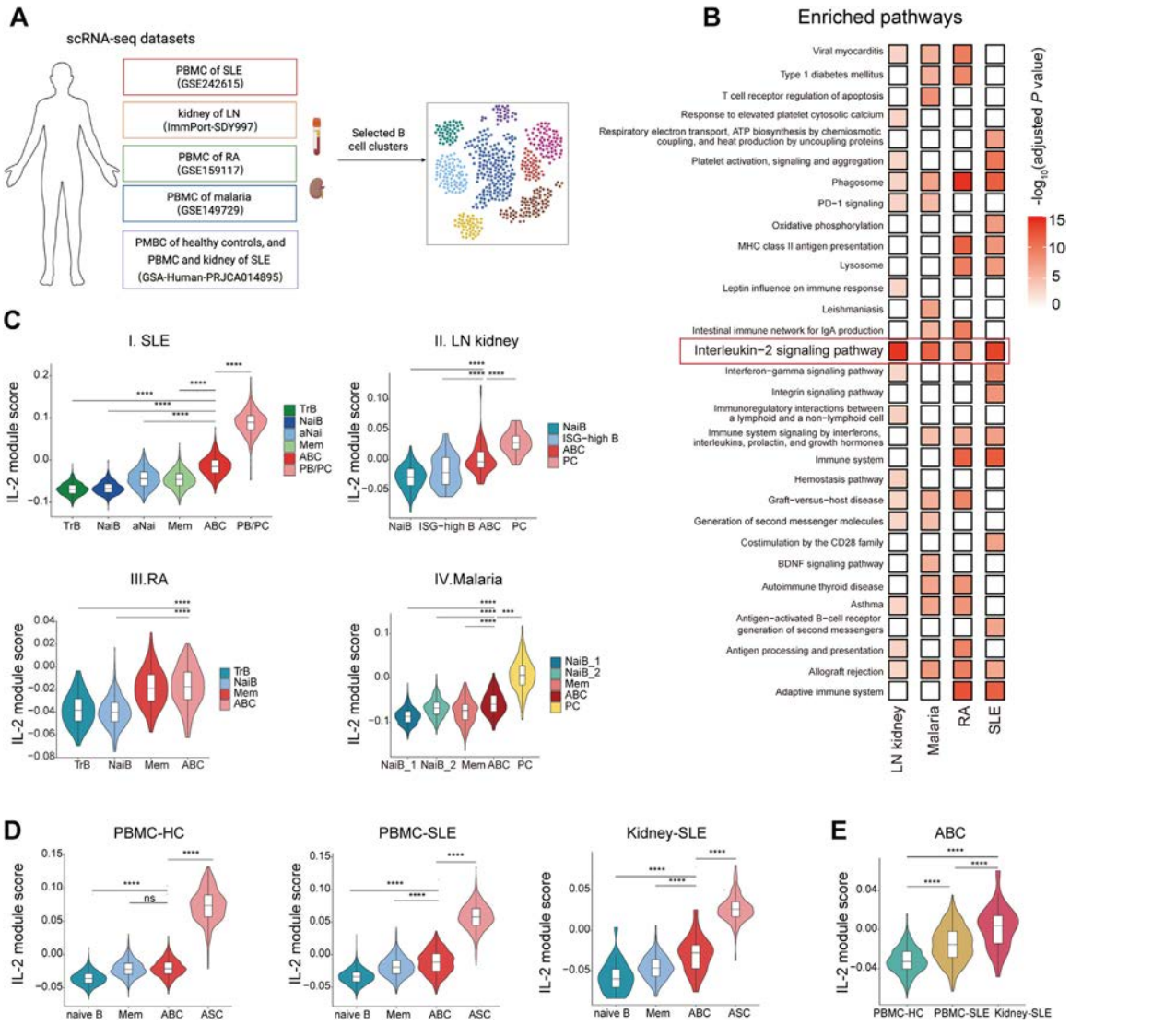


Figure 2. ABCs share upregulated interleukin (IL)-2 signalling across different disease states. (A) Schematic diagram illustrating the selected B clusters from single-cell RNA-seq datasets, including samples from the peripheral blood of healthy controls (HC), patients with SLE, patients with RA, and patients with malaria, as well as kidney tissue from patients with LN. (B) The top 15 significantly enriched pathways for the upregulated genes of ABCs of each indicated dataset, determined by the Enrichr tool. Adjusted *P* values were log-normalised and scaled by colour. Pathways not ranked within the top 15 for a given dataset were displayed as blank. (C) Violin plot comparing the IL-2 module scores across B cell clusters from the PBMC of patients with SLE, RA, and malaria, as well as kidney tissue from patients with LN, respectively. (D) Violin plot comparing the IL-2 module scores across B cell clusters from the PBMC of healthy controls, and the PBMC and kidney tissue of patients with SLE, respectively. (E) Violin plot comparing the IL-2 module scores of ABCs across indicated samples. IL-2 module gene set was constructed based on IL-2 inducible genes in activated B cells ($log_2FC > 1$, $P < .01$). *$P < .05$, **$P < .01$, ***$P < .001$; ****$P < .0001$; ns, not significant. Wilcoxon rank-sum test comparing ABCs to other B cell subsets (C and D) and Kruskal-Wallis test with Dunn's multiple comparisons test for (E). ABC, age-associated B cells; aNai, activated naïve B cells; ISG, interferon-stimulated genes; LN, lupus nephritis; Mem, memory B cells; NaiB, naïve B cells; PB/PC, plasmablasts/plasma cells; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TrB, transitional B cells; PBMC, peripheral blood mononuclear cell.

that of IL-21 (Supplementary Fig S7C). Notably, IL-2 enhanced cell survival during the induction phase, especially when survival signals like CD40 costimulation were limited (Supplementary Fig S7D). We next examined whether IL-2 contributes to the subsequent maintenance of ABCs and observed that IL-2 significantly enhanced the survival of both *in vitro*- and *in vivo*-derived ABCs (Fig 3B and Supplementary Fig S8A), whereas IL-21 impaired the survival of *ex vivo*-sorted ABCs (Supplementary Fig S8B). Moreover, IL-2, like IL-21, also facilitated the differentiation of ABCs into plasma cells, consistent with previous studies highlighting its role in supporting plasma cell generation (Fig 3C and Supplementary Fig S8C) [7,21,51]. Collectively, these findings underscore that although IL-2 and IL-21 both belong to the common γ-chain cytokine family, they exert distinct and nonredundant, stage-dependent functions in ABC biology, with IL-2 playing a particularly supportive role in the maintenance of ABCs (Supplementary Fig S8D).

We next evaluated the regulatory effects of IL-2 on ABC maintenance *in vivo*. To this end, we transferred CD45.1$^+$ ABCs sorted from lupus-prone mice into recipient mice pretreated with IL-2, and assessed the proportions of surviving ABCs among the transferred cells. IL-2 administration significantly enhanced the survival of transferred ABCs, resulting in approximately a twofold increase ($P = .0217$) (Fig 3D).

Taken together, these findings demonstrate that, in addition to the well-established roles of IL-21 and IFN-γ, IL-2 also contributes critically to ABC development and maintenance.

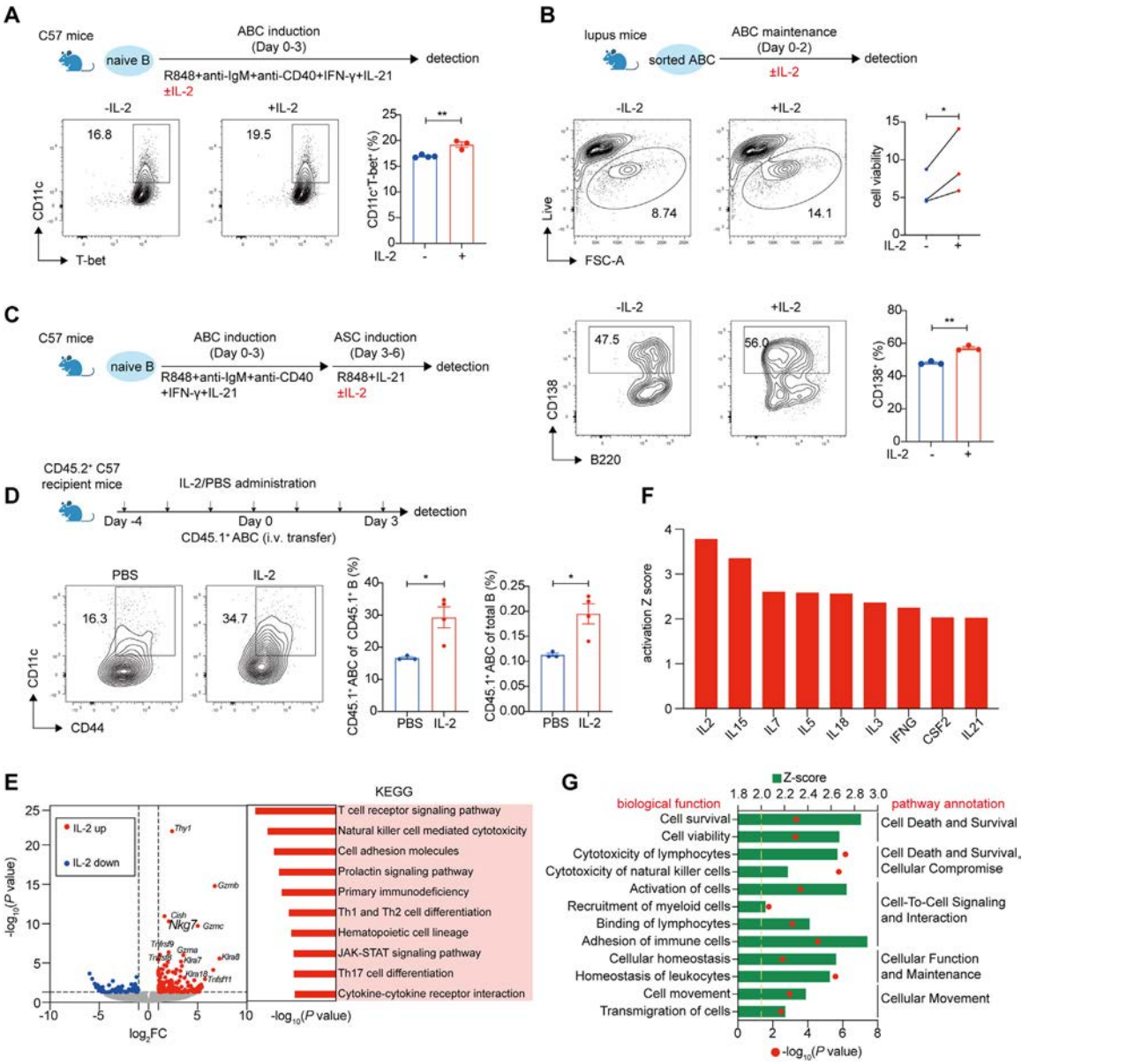


Figure 3. IL-2 contributes to ABC differentiation and survival. (A) Schematic diagram showing mouse naïve B cells were activated with ABC induction cocktail along with (n = 3) or without (n = 4) 10 ng/mL IL-2 stimulation for 3 d. Representative flow cytometry plots and quantitative analysis of in vitro induced ABC proportion. (B) Schematic diagram showing the ABCs sorted from lupus mice were cultured with or without 20 ng/mL IL-2 for 2 d (upper panel). Representative flow cytometry plots and quantitative analysis (lower panel) of the cultured ABC viability (n = 3 for each). (C) Schematic diagram showing that the naïve B cells were cultured with ABC induction cocktail for 3 d. Cells were then washed and recultured for an additional 3 d for further ASC differentiation along with or without 10 ng/mL IL-2 stimulation (n = 3 for each). Representative flow cytometry plots and quantitative analysis of in vitro induced ASC proportion. (D) ABCs sorted from IMQ-induced CD45.1$^+$ donor mice were transferred to recipient mice, which were pretreated with IL-2 (2000 IU/g/d) or PBS daily for 4 d and followed with IL-2 or PBS administration for another 3 d (upper panel). Representative flow cytometry plots and quantitative analysis (lower panel) of the transferred CD45.1$^+$ ABC (CD19$^+$CD11c$^+$CD44$^+$) in CD45.1$^+$ B cells and total B cells (PBS, n = 3; IL-2, n = 4). (E) ABC were sorted from lupus mice and cultured with or without 20 ng/mL IL-2 for 1 d before RNA sequencing. Volcano plots (left) of DEGs between IL-2-treated ABC and untreated ABC. Blue and red represent genes with downregulation and upregulation in IL-2-treated ABCs, respectively. KEGG enrichment analysis (right) of genes upregulated in IL-2-treated ABC. (F) Barplot of the activation *Z*-score for ingenuity pathway analysis (IPA)-predicted upstream regulators in IL-2-treated ABC. (G) Biological functions predicted to be activated in IL-2-treated ABCs compared to untreated ABCs are marked by a *Z*-score ≥ 2 (yellow dotted line). The significance of these predictions is depicted by -log$_{10}$(*P* value) (red dots). Data are shown as mean ± SEM. **P* < .05, ***P* < .01, ****P* < .001; ns, not significant. unpaired Student's t test for (A, C, and D) and paired Student's t test for (B). ABC, age-associated B cells; aNai, activated naïve B cells; DEGs, differentially expressed genes; IFN, interferon; IL, interleukin; IMQ, imiquimod; ASC, antibody-secreting cell; KEGG, Kyoto Encyclopedia of Genes and Genomes; PBS, phosphate buffered saline.

IL-2 programmes ABC cellular features and effector functions

To investigate the molecular mechanisms underlying the regulatory effects of IL-2 on ABC maintenance, we performed transcriptional profiling of IL-2-treated ABCs. RNA-seq analysis revealed 179 upregulated genes (log$_2$FC ≥ 1, *P* < .05) and 75 downregulated genes (log2FC ≤ −1, *P* < .05) including *Nkg7*, an ABC signature gene which was consistently upregulated in IL-2 primed human B cells (Fig 1E). Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis demonstrated that IL-2 stimulation predominantly induced expression of genes related to immune response and cytokine-receptor signalling (Fig 3E). To systematically identify the upstream regulators orchestrating these transcriptional changes, we performed ingenuity pathway

analysis (IPA), a causal analytics tool that predicts upstream molecular regulators based on observed gene expression changes. As expected, this analysis predicted activation of IL-2 family cytokines (IL-2, IL-15, IL-7, and IL-21) (Fig 3F). Moreover, integration of these activated regulators with their downstream DEGs revealed a coordinated transcriptional network governing ABC viability and cellular homeostasis (Supplementary Fig S8E). Biological function analysis further confirmed the activation of specific biological processes related to cell survival in IL-2-treated ABCs (Fig 3G). The data presented herein suggest that IL-2 signalling may promote ABC survival through modulation of fundamental cellular programmes, consistent with the observations from both *ex vivo* culture and *in vivo* transfer experiments.

Dual effects of IL-2 therapy limit efficacy in ABC-associated lupus models

Previous studies have demonstrated that low-dose IL-2 treatment (≤ 50,000 IU) effectively induces Treg expansion and alleviates inflammatory symptoms in NZB/W F1 and MRL/lpr mice [52,53]. However, the impact of IL-2 on ABCs and its role in ABC-mediated autoimmunity have not been evaluated in lupus models.

Considering the pivotal role of TLR7 in ABC development and lupus pathogenesis [36], we sought to comprehensively evaluate the dual effects of IL-2, which promotes both protective Tregs and pathogenic ABCs, in TLR7-relevant models. To this end, we administered low-dose recombinant human IL-2 (rIL-2) to B6.*Sle1yaa* and imiquimod (IMQ)-induced mice, 2 TLR7-driven lupus models characterised by spontaneous ABC accumulation and lupus-like symptoms. Flow cytometric analysis revealed that IL-2 treatment expanded the Foxp3$^+$ Treg population by approximately 30% compared to phosphate buffer saline (PBS)-treated controls (Fig 4A-C). However, concurrent analysis of B cell populations showed 3-fold increase in both ABC frequency and absolute numbers (Fig 4D,E and Supplementary Fig S9A-C), demonstrating simultaneous expansion of these opposing populations. Importantly, this effect was selective, as GC responses, including GC B cells, T follicular helper (Tfh) cells and plasma cells, remained unchanged (Supplementary Fig S9D-F). Interestingly, low-dose IL-2 treatment did not induce ABC accumulation in wild-type C57BL/6 mice (Supplementary Fig S10A-C), although splenic Tregs increased (Supplementary Fig S10D,E), suggesting that IL-2-mediated ABC expansion is more context-dependent and requires underlying inflammatory or autoimmune cues.

Despite the robust expansion of Tregs, IL-2 treatment led to an increase in ABC-associated anti-chromatin antibodies, while anti-dsDNA antibody levels showed no improvement (Fig 4F). Consistently, low-dose IL-2 treatment significantly promoted Treg expansion (P = .0190) in IMQ-treated mice (Fig 4G-I). We also found IL-2-treated IMQ-induced lupus mice showed elevated frequencies of ABCs (Fig 4J,K and Supplementary Fig S11A-C). The GC compartment remained stable, with comparable frequencies of GC B cells, plasma cells, and Tfh cells between treatment groups (Supplementary Fig S11D-I). Anti-dsDNA antibody titres showed no significant reduction (Supplementary Fig S11J), align with our observations in the B6.*Sle1yaa* mice.

Together, these results demonstrated that IL-2 treatment consistently induces parallel accumulation of both Tregs and ABCs in lupus models. In autoimmune conditions dominated by ABCs, the IL-2-induced enhancement of pathogenic ABCs appears to counteract the beneficial effects of Treg expansion. This balance between opposing cellular responses may explain the variable therapeutic outcomes observed in patients with lupus treated with IL-2. Our findings suggest that successful therapeutic strategies may require approaches that can preserve IL-2's beneficial effects on Tregs while selectively inhibiting ABC expansion.

Elevated IL-2 signalling in ABCs correlates with their accumulation in patients with SLE

Next, we investigated whether IL-2 signalling in ABCs was associated with ABC expansion and related disease symptoms in patients with SLE. To this end, we applied gene set variation analysis to a public large-scale SLE cohort and found that IL-2 signalling was upregulated in multiple B cell subsets, including double negative (DN) cells (enriched with ABCs), naïve B cells, and other B cell subsets in SLE. When comparing across B cell subsets, the most prominent enrichment of IL-2 signalling was observed in DN cells and plasma cells (Fig 5A). These findings further corroborated our single-cell results and reinforced the conclusion that IL-2-mediated effects on ABCs are prominently enriched in autoimmune settings. To further assess the response of ABCs to IL-2 in patients with SLE, we evaluated IL-2 receptor expression in different B cell populations and observed increased expression of CD25 and CD122 in ABCs compared to other subsets (Supplementary Fig S12A,B). We then profiled IL-2 receptor expression across B cell compartments using canonical surface markers (Fig 5B,C). Among all B cell subsets, ABCs exhibited the highest level of CD122 (Fig 5D). Moreover, CD122 expression was strongly correlated with the percentage of ABCs in patients with SLE (r = 0.6193, P = .0047; Fig 5E). We also found that ABCs showed the highest proportion of CD122$^+$ cells, and when we analysed the frequencies of CD122$^+$CD25$^-$ single-positive and CD122$^+$CD25$^+$ double-positive populations, ABCs again displayed the highest frequencies of both, further supporting their enhanced IL-2 receptor expression profile (Fig 5F-H and Supplementary Fig S12C). ABCs are known to be enriched with autoreactive clone, especially recognising nuclear-related antigen, and are strongly correlated with anti-Sm and anti-RNP autoantibodies [25,26]. We found that serum IL-2 levels positively correlated with titres of anti-Sm and anti-U1-ribonucleoprotein (RNP) antibodies, while the correlation with anti-dsDNA antibodies was less robust (Fig 5I). However, IL-2 levels did not correlate with overall disease activity indicators such as Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) or C3/C4 (Supplementary Fig S12D), further supporting that IL-2 plays multifaceted roles in SLE by expanding both regulatory cells and pathogenic cells.

Collectively, these results indicated that IL-2 may exert a positive effect on ABC-related disease activity, underscoring the need to account for B cell context when considering IL-2-based therapies.

DISCUSSION

ABCs represent a crucial pathogenic B cell subset in autoimmune diseases, yet microenvironment supporting their development and maintenance remains incompletely understood. In this study, we show that IL-2 pathway promotes ABC homeostasis. Through comprehensive multiomics analysis combining RNA-seq, scRNA-seq, and ATAC-seq, we demonstrated enhanced IL-2 signalling in ABCs across autoimmune diseases. Importantly, IL-2 administration promoted ABC accumulation in lupus-prone mice while simultaneously expanding Tregs, providing a

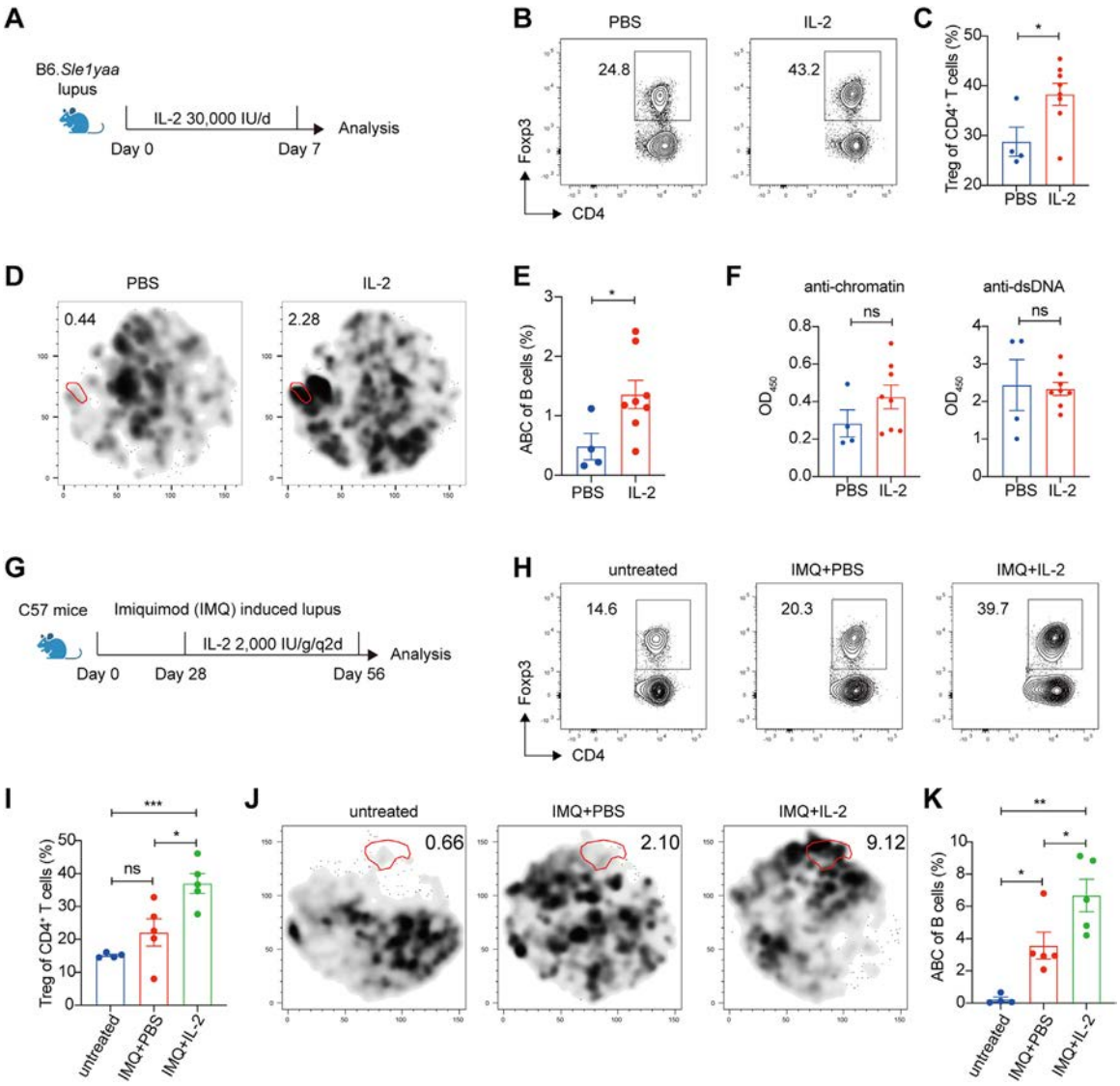


Figure 4. IL-2 promotes ABCs accumulation. (A) Schematic diagram showing that B6.*Sle1yaa* mice were administered with IL-2 (30,000 IU/d, n = 8) or PBS (n = 4) for 7 d by intraperitoneal injection (I.P.). (B, C) Representative flow cytometry plots (B) and quantitative analysis (C) of splenic Tregs ($CD4^+Foxp3^+$) frequency in B6.*Sle1yaa* mice treated with IL-2 or PBS. (D, E) Separated t-SNE plots (D) and quantitative analysis (E) of ABC frequency in B6.*Sle1yaa* mice treated with IL-2 or PBS. (F) Statistical analysis of anti-chromatin Ig and anti-dsDNA Ig levels in sera from mice described in (A). (G) Schematic diagram showing that C57 mice were induced by IMQ for a total 8 wk, with administration of IL-2 (2000 IU/g/q2d, n = 5) or PBS (n = 5) starting at day 28 and continuing for 4 wk. (H, I) Representative flow cytometry plots (H) and quantitative analysis (I) of splenic Tregs ($CD4^+Foxp3^+$) frequency in IMQ-induced lupus mice treated with IL-2 or PBS, and the age-matched C57 mice (untreated, n = 4). (J, K) Separated t-SNE plots (J) and quantitative analysis (K) of ABC frequency in IMQ-induced lupus mice treated with IL-2 or PBS, and untreated mice. Data are shown as mean ± SEM. $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$; ns, not significant. Unpaired Student's t test for (C, E, F, I and K). ABC, age-associated B cells; dsDNA, double-stranded DNA; IL, interleukin; IMQ, imiquimod; q2d, every second day; PBS, phosphate buffered saline.

potential explanation for the therapeutic heterogeneity to low-dose IL-2 treatment in patients with SLE.

Low-dose IL-2 therapy for SLE has shown promising efficacy with Systemic Lupus Erythematosus Responder Index 4 (SRI-4) response ranging from 50% to 89.5%, and British Isles Lupus Assessment Group–based Composite Lupus Assessment (BICLA) response ranging from 50% to 77.8% in small clinical cohorts from multiple open-label studies [13–15,54]. Meanwhile, treatment with low-dose IL-2 appears to be safe and tolerated well. However, the predefined primary endpoints for clinical efficacy were not met in 2 RCTs [16,17]. To date, the largest cohort of over 300 patients with SLE treated with low-dose IL-2 has revealed that only 43.3% (136/314) achieved the SRI-4 response, leaving more than half patients unresponsive to this treatment [55]. The inherent heterogeneity of SLE poses significant challenges in developing effective therapies. Sensitive measures for evaluating low-dose IL-2 treatment efficacy and robust predictive biomarkers have been proposed to help patients achieve stable disease remission [55,56]. From our studies, we observed that IL-2 consistently upregulates both protective Tregs and pathogenic ABCs. The balance between ABCs and Tregs may serve as a predictive biomarker for optimising IL-2-based treatment strategies.

Targeting IL-2 therapy has been previously investigated in 2 classical lupus mouse models: NZB/W F1 and MRL/lpr. While IL-2 supplementation consistently promotes Treg expansion, the overall outcomes, including serological improvements, vary depending on the drug duration and the lupus mouse strain used [52,53,57]. Given the complexity and heterogeneity of SLE aetiology, each model reflects specific subsets of attributes observed in human disease [58]. Extensive evidence also highlights the role of TLR7 in driving autoimmune responses [59]. B6.*Sle1yaa* and IMQ-induced mice, both TLR7-driven models, are uniquely

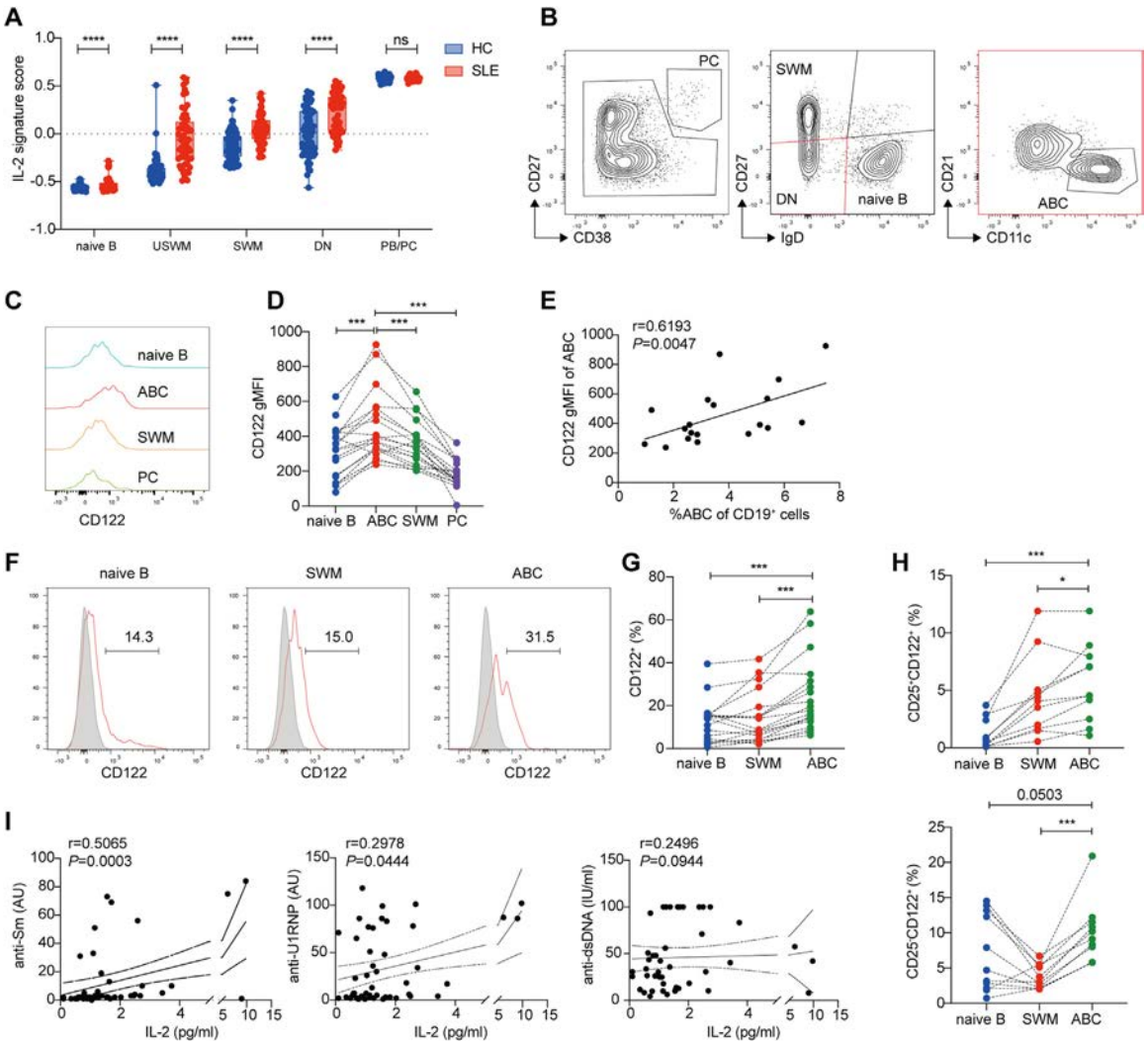


Figure 5. IL-2 responsiveness correlates with ABC-mediated effector B cell response in patients with systemic lupus erythematosus (SLE). (A) GSVA for comparing the indicated IL-2 signature scores in B cell subsets from patients with SLE and HC. (B) Representative flow cytometry plots of B cell subsets of PBMC from patients with SLE (cohort 1, n = 19), including $CD27^+CD38^+$ (PCs), $CD27^+IgD^-$ (SWM), $CD27^-IgD^+$ (naïve B), and $CD19^+CD27^-IgD^-CD21^-CD11c^+$ (ABC). (C) The histogram plot showed the CD122 expression levels of the indicated B cell subsets. (D) Line chart comparing the gMFI of CD122 in ABC with that in naïve B cells, SWM and PC from patients with SLE (cohort 1, n = 19). The dashed line indicated each individual patient. (E) Correlation analysis between the gMFI level of CD122 in ABC and the percentage of ABC in $CD19^+$ B cells. (F, G) Representative flow cytometry plots (F) and the statistics (G) of the frequency of CD122 positive portion among naïve B cells, SWM and ABC in PBMC from patients with SLE. (H) Statistical analysis of the fraction of $CD25^+CD122^+$ and $CD25^-CD122^+$ in the indicated naïve B cells, SWM, and ABC in patients with SLE. (I) The correlation analyses of the serum IL-2 levels with anti-Sm, anti-U1RNP and anti-dsDNA Ig titres in patients with SLE (cohort 2, n = 46). $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$; $^{****}P < .001$; ns, not significant. Unpaired Student's t test for (A), paired Student's t test for (D, G and H), Spearman r correlation analysis for (E and I). ABC, age-associated B cells; PBMC, peripheral blood mononuclear cell; AU, Arbitrary Units; DN, double negative; gMFI, geometric mean fluorescence intensity; GSVA, gene set variation analysis; HC, healthy controls; IL, interleukin; PB/PC, plasmablasts/plasma cells; SWM, switched memory; U1RNP, U1 ribonucleoprotein; USWM, unswitched memory.

suited to mimic the consequences of an overactive TLR7 pathway and its associated immune dysfunction, particularly the expansion of ABCs [36,60]. Using these ABC-dominant lupus models, we found that the promotion of pathogenic ABCs offsets the regulatory benefits of IL-2-mediated Treg boosting in TLR7-driven models. Our findings further underscore that the overall therapeutic outcomes of IL-2 depend heavily on the inherent heterogeneity of SLE.

ABCs are known to arise through extrafollicular pathways but still require T cell help, including CD40 costimulation and a cytokine milieu composed of IFN-γ and IL-21 [41]. Given that extrafollicular (EF) B cell responses lack the well-organised structure of GC, interactions between cognate T and B cells are often uncertain. IL-2 may also serve as an alternative survival cue, particularly under conditions of limited CD40 costimulation. Previous studies have demonstrated the distinct and stage-specific roles of IFN-γ and IL-21 in directing B cells towards an ABC fate. Our study adds another critical dimension to this extrafollicular niche, by showing that IL-2 contributes to the supportive microenvironment that promotes the extrafollicular development of ABCs, particularly during the maintenance stage. Together with existing findings, our data suggest that IL-2, in synergy with local microenvironmental cues, helps establish a specialised extrafollicular niche that sustains ABC populations outside of conventional GCs.

Several limitations of our study should be acknowledged. Our *in vivo* studies were conducted in lupus-prone mice, and the extent to which these findings translate to human disease requires further investigation. Additionally, the long-term consequences of IL-2-mediated ABC accumulation on disease progression have not been assessed. Future research should focus on identifying the molecular mechanisms distinguishing IL-2

responses in different B cell subsets, developing strategies to selectively target pathogenic vs regulatory effects of IL-2, establishing biomarkers to predict patient responses to IL-2 therapy, and investigating combination therapies that might enhance beneficial effects while minimising pathogenic B cell activation.

Overall, our study establishes IL-2 as a critical regulator of ABC homeostasis and provides a mechanistic explanation for the variable efficacy of IL-2 therapy in SLE. The multifaceted role of IL-2 on pathogenic B cells and regulatory T cells suggests that patient stratification and targeted therapeutic approaches will be essential for optimal clinical outcomes. These findings contribute to our understanding of cytokine networks in autoimmune diseases and may guide the development of more effective treatment strategies for SLE and related disorders. Moreover, this work highlights the importance of considering cell-type-specific responses when developing cytokine-based therapies, as the same mediator can have opposing effects on different immune cell populations.

Competing interests

Given his role as Associate Editor of *Annals of the Rheumatic Diseases*, and as the corresponding author of this manuscript, NS had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to another journal editor. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The computations in this work were performed on the π 2.0 and Siyuan Mark-I clusters, supported by the Center for High Performance Computing at Shanghai Jiao Tong University. We thank the Shanghai Jiao Tong University School of Medicine Library for providing access to the Ingenuity Pathway Analysis software (Qiagen). We thank the help from Peng Niu for animal experiments, Yunjie Chen for FACS sorting, Jialing Qin for sample collection, and Jie Qian and Yuqing Yu for reagents support.

Contributors

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. NS, DD, and JBH were responsible for study conception and design. XH, DD, YJ, SG, YS, SC, HD, and QF were responsible for the acquisition of data. DD, XH, YJ, NS, and QF were responsible for analysis and interpretation of data.

Funding

This study was supported by grants from the National Natural Science Foundation of China (82422037, 32441095, 82402099, 32141004 and 31930037), Innovative research team of high-level local universities in Shanghai. JBH was supported by R01 AI024717 from the National Institutes of Allergy and Infectious Diseases, NIH and I01 BX006254 a VA Merit Award from the US Department of Veterans Affairs. The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the National Institutes of Health, the Department of Veterans Affairs or the United States government.

Patient consent for publication

This research was done without direct patient involvement beyond sample collection. Patients did not participate in designing the study, analysing the data, or drafting the manuscript.

Ethics approval

This study involved human participants and was approved by Shanghai Renji Hospital Ethics Committee (KY-2022-023-B). Participants gave informed consent to participate in the study before taking part. This study involved animal experiments that were approved by Institutional Animal Care and Use Committee at Renji Hospital of Shanghai Jiaotong University School of Medicine (RJ-2022-0314).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

The RNA-seq data have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (PRJCA042407). Source data are available upon request.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.08.012.

REFERENCES

[1] Shouse AN, LaPorte KM, Malek TR. Interleukin-2 signaling in the regulation of T cell biology in autoimmunity and cancer. Immunity 2024;57(3):414–28.
[2] Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. Nat Rev Immunol 2012;12(3):180–90.
[3] Malek TR. The biology of interleukin-2. Annu Rev Immunol 2008;26:453–79.
[4] Chinen T, Kannan AK, Levine AG, Fan X, Klein U, Zheng Y, et al. An essential role for the IL-2 receptor in Treg cell function. Nat Immunol 2016;17(11):1322–33.
[5] Trinchieri G, Matsumoto-Kobayashi M, Clark SC, Seehra J, London L, Perussia B. Response of resting human peripheral blood natural killer cells to interleukin 2. J Exp Med 1984;160(4):1147–69.
[6] Ross SH, Cantrell DA. Signaling and function of interleukin-2 in T lymphocytes. Annu Rev Immunol 2018;36:411–33.
[7] Faliti CE, Mesina M, Choi J, Bélanger S, Marshall MA, Tipton CM, et al. Interleukin-2-secreting T helper cells promote extra-follicular B cell maturation via intrinsic regulation of a B cell mTOR-AKT-Blimp-1 axis. Immunity 2024;57 2772-89.e8.
[8] Dörner T, Furie R. Novel paradigms in systemic lupus erythematosus. Lancet 2019;393(10188):2344–58.
[9] Linker-Israeli M, Bakke AC, Kitridou RC, Gendler S, Gillis S, Horwitz DA. Defective production of interleukin 1 and interleukin 2 in patients with systemic lupus erythematosus (SLE). J Immunol 1983;130(6):2651–5.
[10] Solomou EE, Juang YT, Gourley MF, Kammer GM, Tsokos GC. Molecular basis of deficient IL-2 production in T cells from patients with systemic lupus erythematosus. J Immunol 2001;166(6):4216–22.
[11] Tsokos GC. Systemic lupus erythematosus. N Engl J Med 2011;365(22):2110–21.
[12] Lieberman LA, Tsokos GC. The IL-2 defect in systemic lupus erythematosus disease has an expansive effect on host immunity. J Biomed Biotechnol 2010;2010:740619.

[13] He J, Zhang X, Wei Y, Sun X, Chen Y, Deng J, et al. Low-dose interleukin-2 treatment selectively modulates CD4(+) T cell subsets in patients with systemic lupus erythematosus. Nat Med 2016;22(9):991–3.
[14] Humrich JY, von Spee-Mayer C, Siegert E, Bertolo M, Rose A, Abdirama D, et al. Low-dose interleukin-2 therapy in refractory systemic lupus erythematosus: an investigator-initiated, single-centre phase 1 and 2a clinical trial. Lancet Rheumatol 2019;1(1):e44–54.
[15] Raeber ME, Caspar DP, Zurbuchen Y, Guo N, Schmid J, Michler J, et al. Interleukin-2 immunotherapy reveals human regulatory T cell subsets with distinct functional and tissue-homing characteristics. Immunity 2024;57(9) 2232-50.e2210.
[16] He J, Zhang R, Shao M, Zhao X, Miao M, Chen J, et al. Efficacy and safety of low-dose IL-2 in the treatment of systemic lupus erythematosus: a randomised, double-blind, placebo-controlled trial. Ann Rheum Dis 2020;79(1):141–9.
[17] Humrich JY, Cacoub P, Rosenzwajg M, Pitoiset F, Pham HP, Guidoux J, et al. Low-dose interleukin-2 therapy in active systemic lupus erythematosus (LUPIL-2): a multicentre, double-blind, randomised and placebo-controlled phase II trial. Ann Rheum Dis 2022;81(12):1685–94.
[18] Simon Q, Grasseau A, Boudigou M, Le Pottier L, Bettachioli E, Cornec D, et al. A proinflammatory cytokine network profile in Th1/Type 1 effector B cells delineates a common group of patients in four systemic autoimmune diseases. Arthritis Rheumatol 2021;73(8):1550–61.
[19] Zhu H, Mi W, Luo H, Chen T, Liu S, Raman I, et al. Whole-genome transcription and DNA methylation analysis of peripheral blood mononuclear cells identified aberrant gene regulation pathways in systemic lupus erythematosus. Arthritis Res Ther 2016;18:162.
[20] Li B, Guo Q, Wang Y, Su R, Gao C, Zhao J, et al. Increased serum interleukin-2 levels are associated with abnormal peripheral blood natural killer cell levels in patients with active rheumatoid arthritis. Mediators Inflamm 2020; 2020:6108342.
[21] Hipp N, Symington H, Pastoret C, Caron G, Monvoisin C, Tarte K, et al. IL-2 imprints human naive B cell fate towards plasma cell through ERK/ELK1-mediated BACH2 repression. Nat Commun 2017;8(1):1443.
[22] Cancro MP. Age-associated B cells. Annu Rev Immunol 2020;38:315–40.
[23] Rubtsov AV, Rubtsova K, Fischer A, Meehan RT, Gillis JZ, Kappler JW, et al. Toll-like receptor 7 (TLR7)-driven accumulation of a novel CD11c(+) B-cell population is important for the development of autoimmunity. Blood 2011;118(5):1305–15.
[24] Liu Y, Zhou S, Qian J, Wang Y, Yu X, Dai D, et al. T-bet+CD11c+ B cells are critical for antichromatin immunoglobulin G production in the development of lupus. Arthritis Res Ther 2017;19(1):225.
[25] Wang S, Wang J, Kumar V, Karnell JL, Naiman B, Gross PS, et al. IL-21 drives expansion and plasma cell differentiation of autoreactive CD11c(hi)T-bet(+) B cells in SLE. Nat Commun 2018;9(1):1758.
[26] Jenks SA, Cashman KS, Zumaquero E, Marigorta UM, Patel AV, Wang X, et al. Distinct effector B cells induced by unregulated toll-like receptor 7 contribute to pathogenic responses in systemic lupus erythematosus. Immunity 2018;49 (4) 725-39.e726.
[27] Scharer CD, Blalock EL, Mi T, Barwick BG, Jenks SA, Deguchi T, et al. Epigenetic programming underpins B cell dysfunction in human SLE. Nat Immunol 2019;20(8):1071–82.
[28] Wu C, Fu Q, Guo Q, Chen S, Goswami S, Sun S, et al. Lupus-associated atypical memory B cells are mTORC1-hyperactivated and functionally dysregulated. Ann Rheum Dis 2019;78(8):1090–100.
[29] Hao Y, O'Neill P, Naradikian MS, Scholz JL, Cancro MP. A B-cell subset uniquely responsive to innate stimuli accumulates in aged mice. Blood 2011;118(5):1294–304.
[30] Dai D, Gu S, Han X, Ding H, Jiang Y, Zhang X, et al. The transcription factor ZEB2 drives the formation of age-associated B cells. Science 2024;383 (6681):413–21.
[31] Qin Y, Cai ML, Jin HZ, Huang W, Zhu C, Bozec A, et al. Age-associated B cells contribute to the pathogenesis of rheumatoid arthritis by inducing activation of fibroblast-like synoviocytes via TNF-alpha-mediated ERK1/2 and JAK-STAT1 pathways. Ann Rheum Dis 2022;81(11):1504–14.
[32] Naradikian MS, Myles A, Beiting DP, Roberts KJ, Dawson L, Herati RS, et al. Cutting edge: IL-4, IL-21, and IFN-gamma interact to govern T-bet and CD11c expression in TLR-activated B cells. J Immunol 2016;197(4):1023–8.
[33] Russell Knode LM, Naradikian MS, Myles A, Scholz JL, Hao Y, Liu D, et al. Age-associated B cells express a diverse repertoire of VH and Vkappa genes with somatic hypermutation. J Immunol 2017;198(5):1921–7.
[34] Levack RC, Newell KL, Popescu M, Cabrera-Martinez B, Winslow GM. CD11c (+) T-bet(+) B cells require IL-21 and IFN-gamma from type 1 T Follicular helper cells and intrinsic Bcl-6 expression but develop normally in the absence of T-bet. J Immunol 2020;205(4):1050–8.
[35] Manni M, Gupta S, Ricker E, Chinenov Y, Park SH, Shi M, et al. Regulation of age-associated B cells by IRF5 in systemic autoimmunity. Nat Immunol 2018;19(4):407–19.
[36] Brown GJ, Cañete PF, Wang H, Medhavy A, Bones J, Roco JA, et al. TLR7 gain-of-function genetic variation causes human lupus. Nature 2022;605 (7909):349–56.
[37] Stone SL, Peel JN, Scharer CD, Risley CA, Chisolm DA, Schultz MD, et al. T-bet transcription factor promotes antibody-secreting cell differentiation by limiting the inflammatory effects of IFN-gamma on B cells. Immunity 2019;50(5) 1172-87.e1177.
[38] Rubtsova K, Rubtsov AV, Thurman JM, Mennona JM, Kappler JW, Marrack P. B cells expressing the transcription factor T-bet drive lupus-like autoimmunity. J Clin Invest 2017;127(4):1392–404.
[39] Gao X, Shen Q, Roco JA, Dalton B, Frith K, Munier CML, et al. Zeb2 drives the formation of CD11c+ atypical B cells to sustain germinal centers that control persistent infection. Sci Immunol 2024;9(93):eadj4748.
[40] Rubtsov AV, Rubtsova K, Kappler JW, Jacobelli J, Friedman RS, Marrack P. CD11c-expressing B cells are located at the T Cell/B cell border in spleen and are potent APCs. J Immunol 2015;195(1):71–9.
[41] Song W, Antao OQ, Condiff E, Sanchez GM, Chernova I, Zembrzuski K, et al. Development of Tbet- and CD11c-expressing B cells in a viral infection requires T follicular helper cells outside of germinal centers. Immunity 2022;55(2) 290-307 e295.
[42] Song W, Sanchez GM, Mayer DP, Blackburn HN, Chernova I, Flavell RA, et al. Cutting edge: IL-21 and tissue-specific signals instruct Tbet+CD11c+ B cell development following viral Infection. J Immunol 2023;210(12):1861–5.
[43] Rochman Y, Spolski R, Leonard WJ. New insights into the regulation of T cells by γc family cytokines. Nat Rev Immunol 2009;9(7):480–90.
[44] Holla P, Dizon B, Ambegaonkar AA, Rogel N, Goldschmidt E, Boddapati AK, et al. Shared transcriptional profiles of atypical B cells suggest common drivers of expansion and function in malaria, HIV, and autoimmunity. Sci Adv 2021;7(22):eabg8384.
[45] Ma J, Wu Y, Ma L, Yang X, Zhang T, Song G, et al. A blueprint for tumor-infiltrating B cells across human cancers. Science 2024;384(6695):eadj4857.
[46] Malle L, Patel RS, Martin-Fernandez M, Stewart OJ, Philippot Q, Buta S, et al. Autoimmunity in Down's syndrome via cytokines, CD4 T cells and CD11c+ B cells. Nature 2023;615(7951):305–14.
[47] Yang Y, Chen X, Pan J, Ning H, Zhang Y, Bo Y, et al. Pan-cancer single-cell dissection reveals phenotypically distinct B cell subtypes. Cell 2024;187(17) 4790-811.e22.
[48] Wu C, Jiang S, Chen Z, Li T, Gu X, Dai M, et al. Single-cell transcriptomics reveal potent extrafollicular B cell response linked with granzyme K(+) CD8 T cell activation in lupus kidney. Ann rheum dis 2025;84(3):451–66.
[49] Zhang B, Zhang Y, Xiong L, Li Y, Zhang Y, Zhao J, et al. CD127 imprints functional heterogeneity to diversify monocyte responses in inflammatory diseases. J Exp Med 2022;219(2):e20211191.
[50] Arazi A, Rao DA, Berthier CC, Davidson A, Liu Y, Hoover PJ, et al. The immune cell landscape in kidneys of patients with lupus nephritis. Nat Immunol 2019;20(7):902–14.
[51] Zumaquero E, Stone SL, Scharer CD, Jenks SA, Nellore A, Mousseau B, et al. IFNgamma induces epigenetic programming of human T-bet^{hi} B cells and promotes TLR7/8 and IL-21 induced differentiation. Elife 2019;8:e41641.
[52] Liang K, He J, Wei Y, Zeng Q, Gong D, Qin J, et al. Sustained low-dose interleukin-2 therapy alleviates pathogenic humoral immunity via elevating the Tfr/Tfh ratio in lupus. Clin Transl Immunology 2021;10(6):e1293.
[53] Mizui M, Koga T, Lieberman LA, Beltran J, Yoshida N, Johnson MC, et al. IL-2 protects lupus-prone mice from multiple end-organ damage by limiting CD4-CD8- IL-17-producing T cells. J Immunol 2014;193(5):2168–77.
[54] Bader L, Boyman O, Raeber ME. A systematic review of low-dose interleukin-2 for the treatment of systemic lupus erythematosus. medRxiv. 2024:2022.2012.2002.22283038.
[55] Feng R, Xiao X, Huang B, Zhang K, Zhang X, Li Z, et al. Predictive biomarkers for low-dose IL-2 therapy efficacy in systemic lupus erythematosus: a clinical analysis. Arthritis Res Ther 2024;26(1):180.
[56] Gao X, He J, Sun X, Li F. Dynamically modeling the effective range of IL-2 dosage in the treatment of systemic lupus erythematosus. iScience 2022;25 (9):104911.
[57] Humrich JY, Morbach H, Undeutsch R, Enghard P, Rosenberger S, Weigert O, et al. Homeostatic imbalance of regulatory and effector T cells due to IL-2 deprivation amplifies murine lupus. Proc Natl Acad Sci U S A 2010;107(1):204–9.
[58] Li W, Titov AA, Morel L. An update on lupus animal models. Curr Opin Rheumatol 2017;29(5):434–41.
[59] Vinuesa CG, Shen N, Ware T. Genetics of SLE: mechanistic insights from monogenic disease and disease-associated variants. Nat Rev Nephrol 2023;19(9):558–72.
[60] Yokogawa M, Takaishi M, Nakajima K, Kamijima R, Fujimoto C, Kataoka S, et al. Epicutaneous application of toll-like receptor 7 agonists leads to systemic autoimmunity in wild-type mice: a new model of systemic lupus erythematosus. Arthritis Rheumatol 2014;66(3):694–706.

Ann Rheum Dis 85 (2026) 339–350

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Sjögren's syndrome

A proteomic profile correlates with salivary gland inflammation and disease-associated antibodies in Sjögren's disease

Albin Björk[1,2,#], Johannes Mofors[1,3,#], Lauro Meneghel[1], Marika Kvarnström[1,2], Elisabeth Maeland[4,5], Elina Richardsdotter Andersson[1], Roland Jonsson[4,6], Roald Omdal[6,7], Helena Forsblad-d'Elia[8], Sara Magnusson Bucher[9], Per Eriksson[10], Thomas Mandl[11], Peter Olsson[11], Marie Fischer[1,3], Katrine B Norheim[12,13], Svein Joar Auglænd Johnsen[12,13], Daniel Hammenfors[4,6], Kathrine Skarstein[14,15], Malin V Jonsson[5], Silke Appel[14], Ingrid Kockum[16], Juliana Imgenberg-Kreuz[17], Gunnel Nordmark[17], Marie Wahren-Herlenius[1,4,*]

[1] *Division of Rheumatology, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden*
[2] *Center for Rheumatology, Academic Specialist Center, Stockholm, Sweden*
[3] *Department of Gastro, Dermatology and Rheumatology, Karolinska University Hospital, Stockholm, Sweden*
[4] *Department of Rheumatology, Haukeland University Hospital, Bergen, Norway*
[5] *Department of Clinical Dentistry, University of Bergen, Bergen, Norway*
[6] *Broegelmann Research Laboratory, Department of Clinical Science, University of Bergen, Bergen, Norway*
[7] *Research Department, Stavanger University Hospital, Stavanger, Norway*
[8] *Department of Rheumatology and Inflammation Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*
[9] *Department of Rheumatology, Faculty of Medicine and Health, Örebro University, Örebro, Sweden*
[10] *Department of Biomedical and Clinical Sciences, Division of Inflammation and Infection/Rheumatology, Linköping University, Linköping, Sweden*
[11] *Department of Clinical Sciences Malmö, Division of Rheumatology, Lund University, Malmö, Sweden*
[12] *Department of Rheumatology, Stavanger University Hospital, Stavanger, Norway*
[13] *Department of Clinical Science, University of Bergen, Bergen, Norway*
[14] *Gade Laboratory for Pathology, Department of Clinical Medicine, University of Bergen, Bergen, Norway*
[15] *Department of Pathology, Haukeland University Hospital, Bergen, Norway*
[16] *Karolinska Neuroimmunology and Multiple Sclerosis Center, Department of Clinical Neuroscience, Karolinska Insitutet, Stockholm, Sweden*
[17] *Rheumatology and Science for Life Laboratory, Department of Medical Sciences, Uppsala University, Uppsala, Sweden*

ARTICLE INFO

Article history:
Received 12 July 2025
Received in revised form 14 November 2025
Accepted 15 November 2025

ABSTRACT

Objectives: To identify serum and salivary gland proteomic biomarkers in primary Sjögren's disease (SjD), evaluate their correlation with tissue inflammation, and relate findings to clinical, autoantibody, and genetic profiles.

*Correspondence to Dr Marie Wahren-Herlenius, Division of Rheumatology, Department of Medicine Solna, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden.

E-mail address: marie.wahren@ki.se (M. Wahren-Herlenius).

[#] Albin Björk and Johannes Mofors contributed equally as shared first authors.

https://doi.org/10.1016/j.ard.2025.11.013

Handling editor Josef S Smolen

Methods: Paired salivary gland extracts and serum samples from patients with SjD (n = 81) and sicca controls (n = 19), and serum from larger Swedish (SjD n = 456; controls n = 141) and Norwegian (SjD n = 233; controls n = 137) cohorts, were analysed using proximity extension assay technology. Patients were stratified by antinuclear antibodies (ANA), anti-Ro/SSA, anti-La/SSB autoantibodies, and extraglandular manifestations. Human leukocute antigen (HLA) alleles were integrated to assess genetic associations.

Results: Proteomic analysis revealed more pronounced alterations in the salivary gland extracts compared to serum. In serum, 27 proteins were associated with SjD in the Swedish discovery cohort, of which 16 proteins were validated in the Norwegian replication cohort. Several proteins including both described biomarkers C-X-C motif chemokine ligand (CXCL)10 and CXCL13 and more novel lysosome-associated membrane glycoprotein 3 and cytotoxic and regulatory T cell molecule correlated with extraglandular manifestations, particularly pulmonary involvement. Levels of the 16 validated proteins increased in a stepwise fashion across serological subgroups, from lowest in ANA negative to highest in anti-Ro/SSA and anti-La/SSB double-positive patients and were linked to risk alleles HLA-DRB1*03 and DRB1*15. A composite 'Sjögren protein score' based on the 16 validated proteins correlated with salivary gland focus score and successfully distinguished SjD from healthy controls (area under the curve 0.888; 89% specificity, 76% sensitivity).

Conclusions: We define a validated serum proteomic signature that reflects local glandular inflammation and correlates with genetic and serological features. The 'Sjögren protein score' holds promise as a noninvasive biomarker for the diagnosis of SjD.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Primary Sjögren's disease (SjD) is clinically and biologically heterogenous, and differences in proteomic profiles could aid the unmet need for better patient stratification and personalised management.
- Proximity extension assay technology enables highly sensitive and specific detection of proteomic abnormalities in both serum and tissue samples.

WHAT THIS STUDY ADDS

- Levels of inflammatory proteins exhibit a stepwise increase corresponding to the presence and specificity of disease-associated autoantibodies.
- The study identifies proteins associated with SjD, the focus score, extraglandular manifestations, and the frequency of human leukocyte antigen (HLA) risk alleles.
- The 'Sjögren protein score', based on validated serum biomarkers, correlates with salivary gland inflammation and effectively distinguishes patients from controls, including seronegative individuals.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- These findings enhance our understanding of proteomic dysregulation in SjD and reveal associations with autoantibody profiles, genetic risk factors (HLA alleles), and systemic disease features.
- The correlation between the 'Sjögren protein score' and histological inflammation in minor salivary glands suggests that a serum proxy score could serve as an alternative to tissue biopsies in disease assessment.

INTRODUCTION

Primary Sjögren's disease (SjD) is a chronic rheumatic disease characterised by inflammation and functional impairment of salivary and lacrimal glands, leading to dryness of the mouth and eyes. The disease manifests itself in a variety of forms; while subgroups of patients present with isolated glandular involvement, one-third of patients have a more systemic disease with extraglandular manifestations in various organs [1]. A subgroup of approximately 5% of patients eventually develops lymphoma. Furthermore, the disease occurs both with and without disease-associated autoantibodies in serum, such as antinuclear antibodies (ANA), anti-Ro/SSA, and anti-La/SSB. Consequently, both clinical and immunological disease heterogeneity result in challenges for understanding pathogenetic mechanisms, measuring disease activity, and therapeutic development [2]. Moreover, there is a discrepancy between measures of systemic disease activity and patient-reported symptoms and outcomes [3], which adds another layer of complexity.

The disease heterogeneity stresses the need for precision medicine in the treatment of SjD. Several approaches and symptom-based tools for patient stratification have been proposed [4,5], with indications that such tools may aid in the identification of patients responsive to specific treatments [4]. Still, objective biomarkers are needed to detect pathogenic mechanisms at play in individual patients, and to discover or follow molecular targets of new treatments. Analysis of serum and tissue proteomes holds the promise to reveal dysregulated biological pathways and processes that can be used to this end.

To achieve a more comprehensive understanding of disease subtypes of SjD, integration of genetics, serology, data on extraglandular manifestations, and tissue biomarkers is needed. Furthermore, a better comprehension of the connection between the local inflammation in the target tissue compared with that of the blood is desirable to indicate how efficient disease modulation may be achieved. Whether local inflammation of the salivary glands is mirrored in a similar manner by distinct changes of the circulating proteome in SjD remains to be examined.

In this study, we aimed to identify protein biomarkers that indicate minor salivary gland (MSG) inflammation and thus perform large-scale proteomic analyses of MSG extracts and serum samples. To discern disease subtypes associated with specific proteome dysregulation, we implement integrated analyses of proteomic data in relation to autoantibody status, genetics, presence of extraglandular manifestations, and lymphoma. In paired samples, we further analyse the relationship between proteomic profiles of MSGs and serum. To validate the results of the serum proteome, both a discovery and a replication cohort are used.

METHODS

Patients and controls

Patient samples were collected at the Departments of Rheumatology at the University Hospitals in Sweden in Gothenburg, Linköping, Malmö/Lund, Stockholm, Uppsala, and Örebro (n = 456 SjD); and in Norway in Bergen and Stavanger (n = 233 SjD). Most samples were collected in a cross-sectional manner, with 260/689 (38%) of the samples collected within 1 year of the SjD diagnosis. Information on disease-modifying antirheumatic drug (DMARD) treatment was incomplete and not included. Serum samples from healthy controls were collected in Stockholm and Uppsala, Sweden (n = 141), and Bergen and Stavanger, Norway (n = 137) (Table). Samples for paired analysis of MSG extracts and serum (SjD n = 81; sicca controls n = 19) were collected in Stockholm (Supplementary Table S1). Sicca controls were negative for anti-Ro/SSA and anti-La/SSB and had undergone a biopsy (focus score < 1) for investigation of SjD. All patients fulfilled the 2002 American European Consensus Group criteria [6]. Clinical characteristics and autoantibody status were registered at sample collection or in retrospect. For parts of the replication cohort (n = 57), results from salivary gland ultrasound examinations were available, graded according to the OMERACT scoring system [7]. Extraglandular manifestations were registered if they had ever been present, and were not defined for level of activity according to the European Alliance of Associations for Rheumatology Sjögren's Syndrome Disease Activity Index score (Supplementary Table S2). Blood sampling did not always coincide with extraglandular manifestation registration and was not performed in any specific temporal relation to lymphoma diagnosis. The study was approved by local ethics committees, and study subjects gave written informed consent.

Sample processing

MSG extracts were prepared by thawing frozen biopsies preserved in O.C.T. Compound (Tissue-Tek, Sakura), washing them repeatedly in phosphate buffered saline, and then homogenising the tissue with TissueLyser LT (Qiagen) in radioimmunoprecipitation assay lysis buffer (10% w/v) with protease and phosphatase inhibitors (P8340 and P0044, Sigma-Aldrich) on ice. After centrifugating at 10,000 × *g* for 10 minutes at + 4°C, supernatants were collected. Protein concentration was measured with DC Protein Assay (BioRad) and adjusted to 0.75 mg/mL. Tissue protein extracts and serum samples were stored at −80°C.

Proteomic assay

Protein levels were measured at the Clinical Biomarkers Facility at Science for Life Laboratory, Uppsala University, Uppsala, Sweden, using 6 Olink Target 96 proximity extension assay (PEA) panels (Supplementary Table S3). The PEA technology was selected due to previous experience of high sensitivity and specificity [8] and local availability. Protein levels are reported as normalised protein expression units, which represent log2-transformed data on an arbitrary scale. Samples failing quality control at the facility were excluded from analysis. Furthermore, proteins with missing values in more than 20% of the samples were omitted. For the remaining missing values, imputation was performed using group-specific means within cases and controls, respectively. Interassay reproducibility between the Olink PEA and enzyme-linked immunosorbent assay in serum and MSG extracts, respectively, was assessed in 40 samples for C-X-C motif chemokine ligand (CXCL)13 and CXCL10 (DCX130 and DIP100, R&D Systems) (Supplementary Fig S1).

Table
Clinical characteristics of patients with primary Sjögren's disease and controls

Cohort	Discovery cohort (Sweden)			Replication cohort (Norway)		
	SjD	Controls	*P* value	SjD	Controls	*P* value
Individuals, n	456	141	-	233	137	-
Women, n (%)	428 (93.9)	141 (100)	**.0009***	213 (91.4)	136 (99.3)	**.0008***
Age at inclusion, y (mean ± SD)	57.9 ± 13.9	53.2 ± 8.5	**.0001****	59.1 ± 12.5	58.6 ± 10.6	.7**
Age at diagnosis, y (mean ± SD)	53.5 ± 14.1	-	-	51.1 ± 12.6	-	-
Autoantibody frequency, n (%)						
ANA	334/455 (73.4)	-	-	182/231 (78.8)	-	-
Anti-Ro/SSA antibodies	322 (70.6)	-	-	172 (73.8)	-	-
Anti-La/SSB antibodies	187/453 (41.3)	-	-	99 (42.5)	-	-
Positive salivary gland biopsy, n (%)	306/315 (97.1)	-	-	171/205 (83.4)	-	-
Major salivary gland swelling, frequency, n (%)	104/433 (24.0)	-	-	59/162 (36.4)	-	-
Extraglandular manifestations, frequency, n (%)						
Raynaud's phenomenon	116/449 (25.8)	-	-	70/210 (33.3)	-	-
Arthritis	91/447 (20.4)	-	-	43/224 (19.2)	-	-
Cutaneous involvement	42/443 (9.5)	-	-	11/224 (4.9)	-	-
Pulmonary involvement	21/446 (4.7)	-	-	2/112 (1.8)	-	-
Renal involvement	14/441 (3.2)	-	-	0/112 (0)	-	-
Lymphadenopathy	39/441 (8.8)	-	-	19/224 (8.5)	-	-
Lymphoma	21/454 (4.6)	-	-	5/226 (2.2)	-	-
Laboratory findings, n (%)						
Anaemia	124/440 (28.2)	-	-	11/171 (6.4)	-	-
Leukopenia	162/438 (37.0)	-	-	56/226 (24.8)	-	-
Lymphopenia	75/297 (25.3)	-	-	14/161 (8.7)	-	-
Thrombocytopenia	19/436 (4.4)	-	-	1/169 (0.6)	-	-
Hypergammaglobulinaemia	186/354 (52.5)	-	-	90/218 (41.3)	-	-
C3 below normal limit	18/223 (8.1)	-	-	14/167 (8.4)	-	-
C4 below normal limit	37/165 (22.4)	-	-	23/167 (13.8)	-	-

ANA, antinuclear antibodies; SjD, Sjögren's disease.
P values calculated using *Fisher's exact test and **_t_ test.

A 'Sjögren protein score' was calculated as the *z*-score sum derived using the control group means and SDs of C-C motif chemokin ligand 19 (CCL19), cytotoxic and regulatory T cell molecule (CRTAM), CXCL10, CXCL11, CXCL13, CXCL9, galectin 9 (Gal9), granzyme H (GZMH), interferon gamma (IFNγ), interleukin (IL)-10, (IL-12, lymphocyte activating gene 3 prote (LAG3), soluble programmed cell death protein-1 (PDCD1), tumor necrosis factor (TNF), TNF receptor superfamily member (TNFRSF)4, and TNFRSF9, similar to previously reported protein scores [8].

Genotyping

Human leukocyte antigen (HLA) genotypes were defined by imputation using the HLA*IMP:02 software [9], with genotype data assayed with the Illumina OmniExpressExome array for patients [10] and the multiple sclerosis replication chip for out-of-study population controls [11,12].

Statistical analysis

Differential protein expression was evaluated using linear and logistic regression models, with adjustments for age and sex (male/female). Correction for multiple testing was applied using the Benjamini-Hochberg procedure to control the false discovery rate (FDR). Differences in the distribution of categorical variables were assessed with Fisher's exact test. The Kruskal-Wallis test followed by Dunn's post hoc test was used for multiple-group comparisons. Statistical significance was defined as an FDR of ≤5% and a ±0.5 log2 fold change. All statistical analyses and visualisations were performed using R (version 4.1.2).

Patient and public involvement

Patients and the public were not involved in planning the project.

RESULTS

Screening of the proteome in serum and salivary gland extracts

To perform a comprehensive screen of the circulating and MSG proteome, we analysed paired MSG extracts and serum samples from patients with SjD (n = 81), and sicca controls matched for age and sex (n = 19) using 6 different Olink PEA panels encompassing a total of 552 proteins, of which 528 were unique (Fig 1A, Supplementary Table S1). A list of proteins passing quality control is presented in Supplementary Table S3.

Protein levels in patients and controls for each of the 6 panels are visualised in heatmaps in Figure 1B, in which unsupervised hierarchical clustering analysis of rows (proteins) was performed within the sicca control and SjD groups. In Figure 1C, the proteins with statistically significant differential levels in MSG extracts and serum comparing patients and sicca controls are depicted. Notably, a more pronounced dysregulation of the proteome of patients with SjD was detected in MSG extracts than in serum. The highest log2 fold change values in SjD MSG extracts compared to sicca controls for each of the 6 panels were CXCL13, src homology 2 domain protein 1A (SH2D1A), dipeptidase 1 (DPEP1), IL-12, B cell scaffold protein with ankyrin repeats 1 (BANK1), and immunoglobulin lambda constant 2 (IGLC2) (Supplementary Table S3). Of special note, the SjD autoantigen Tripartite motif-containing protein 21 (TRIM21 also known as Ro52) was measured at lower levels in both MSG extracts and in the serum of patients. However, as higher expression of Ro52 is documented in salivary glands of patients with SjD [13], this was likely caused by a technical interference as PEA technology depends on the binding of the assay antibodies for the detection of proteins, and autoantibodies in patients with SjD can block epitopes on TRIM21. TRIM21 was therefore excluded from further analyses. Ro60 and La proteins were not included in the protein panels. As could be expected [8,14], several interferon (IFN)-induced proteins were among the most significant proteins both in serum and MSG extracts, including CXCL10, CXCL9, and sialoadhesin (SIGLEC1). In the MSG extracts, levels of interferon regulatory factor 9 (IRF9), which is a crucial mediator of type I IFN signalling, were also higher in SjD. We next investigated the association between protein levels in MSG extracts and the MSG focus score by analysing CXCL13 and CXCL10, the 2 proteins with the highest fold change, and observed a significant positive correlation (Supplementary Fig S2).

To find the most informative panel to be employed for investigation in larger patient cohorts, we generated *P* value density plots to indicate the relative *P* value frequencies in MSG extracts and serum (Fig 1D). With the aim of identifying target tissue biomarkers, the Immuno-Oncology panel was selected as the most relevant panel for further study since it contained the highest number of statistically significant proteins with the highest log2 fold change in salivary gland extracts.

Serum proteome profiles associated with primary SjD

To assess the serum protein profile in SjD, we measured the 92 proteins included in the Immuno-Oncology panel (of which 79 passed quality control and the predefined cut-off, Supplementary Table S4) in a discovery cohort consisting of 456 patients with SjD and 141 healthy controls, and in a replication cohort, encompassing 233 patients with SjD and 137 healthy controls (Table). In both cohorts, the control groups had higher proportions of females (both $P < .001$). Controls in the discovery cohort were somewhat younger compared to the patients ($P < .001$). All analyses were therefore adjusted for age and sex.

Principal component analysis (PCA) of the whole serum protein datasets revealed tendencies for separation between patient and control samples in both the discovery and the replication cohorts, as well as when PCA was performed on the pooled dataset (Fig 2A-C). Proteins in the full dataset are shown in a heatmap with annotation for laboratory parameters, also indicating which proteins are most likely to be coexpressed based on hierarchical clustering (Fig 2D).

We next performed case-control analyses to identify and validate proteins expressed at differential levels comparing patients and controls. In the discovery cohort, 70 proteins were detected at significantly different levels in SjD compared to controls at 5% FDR, including 27 proteins also passing a cut-off set at ±0.5 log2 FC (Fig 2E). Out of the 27 proteins, 16 could be validated at differential protein levels in patients vs controls in the replication cohort (Fig 2F). Notably, all proteins passing the cut-off in the smaller replication cohort were also detected in the discovery cohort, further strengthening the consistency of the findings. In a pooled analysis, 20 proteins were detected at higher levels in patients compared to controls (Fig 2G). Among the most significant proteins were Gal9, CXCL13, PDCD1, CCL19, and LAG3. As could be anticipated in a panel of mostly inflammatory markers, proteins were generally detected at higher levels in patients compared to controls.

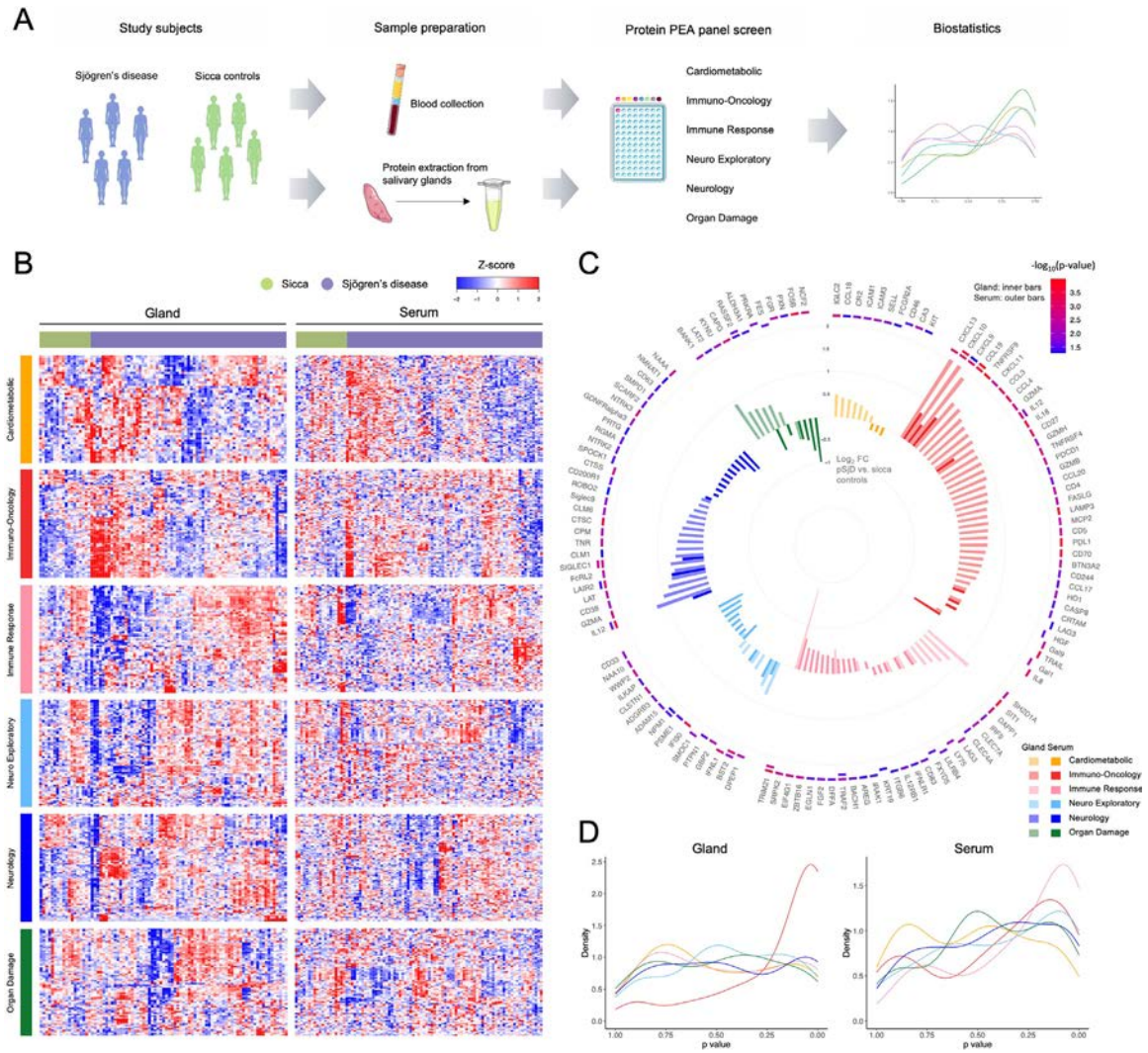


Figure 1. Analysis of the serum and salivary gland proteome comparing patients with Sjögren's disease and sicca controls. (A) Schematic overview of the study design. (B) Hierarchical clustering analysis of protein levels measured in each of the 6 Olink proximity extension assay panels. Hierarchical clustering of rows is unsupervised, and unsupervised clustering of columns is performed separately within each disease-status group. Data on each row are normalised by *z*-score transformation. Different panels are indicated by colours to the left. (C) Differential protein levels in salivary gland extracts (lighter bars) and serum (darker bars) comparing patients and sicca controls. Nominal *P* values are indicated in the outermost circle for salivary gland extracts (inner bars) and serum (outer bars). FDR corrected *P* values are presented in Supplementary Table S3. (D) Density plots showing smoothed relative frequency of nominal *P* values for each of the Olink PEA panels in serum and minor salivary gland extracts. FDR, false discovery rate; PEA, proximity extension assay.

Serum protein levels associated with extraglandular disease manifestations

To identify proteins associated with discrete disease manifestations, we categorised patients based on the ever-present or absence of different extraglandular manifestations as well as lymphoma. Some extraglandular manifestations were rare in the smaller replication cohort, and the analysis therefore included all patients in the study. Accordingly, future validation in an external cohort is warranted. Log2 fold change values for proteins associated with the clinical manifestations are plotted in Figure 3A.

Higher levels of CXCL10 were associated with lymphadenopathy, cutaneous, biological, and pulmonary involvement, as well as lymphoma. Similarly, CXCL11 was associated with cutaneous, renal, biological, and pulmonary involvement and lymphoma. Interestingly, higher levels of cluster of differentiation 8a (CD8A) and cluster of differentiation 27 (CD27) were associated only with renal involvement, thus indicating their specific involvement in renal pathology in SjD. Correspondingly, LAG3 was associated only with activity in the biological involvement. Pulmonary involvement was present in 23 patients and was associated with a striking upregulation of several inflammatory biomarkers. All proteins that are associated with ever lymphoma also associated with pulmonary involvement.

To better comprehend patterns of coregulation among the proteins associated with extraglandular manifestations, the proteins from Figure 3A are listed according to the correlation matrix in Figure 3B. A clustering of IFN-regulated proteins [15], including CXCL10, CCL19, and CXCL13 was apparent, whereas IL-8 and monocyte chemoattractant protein 3 (MCP3), which are associated with lymphoma and pulmonary involvement, formed a separate cluster.

HLA risk allele frequency and autoantibody profiles are linked to a gradual increase in inflammatory serum proteins

In SjD, autoantibodies have been suggested to drive immunopathogenesis and to mediate production of inflammatory

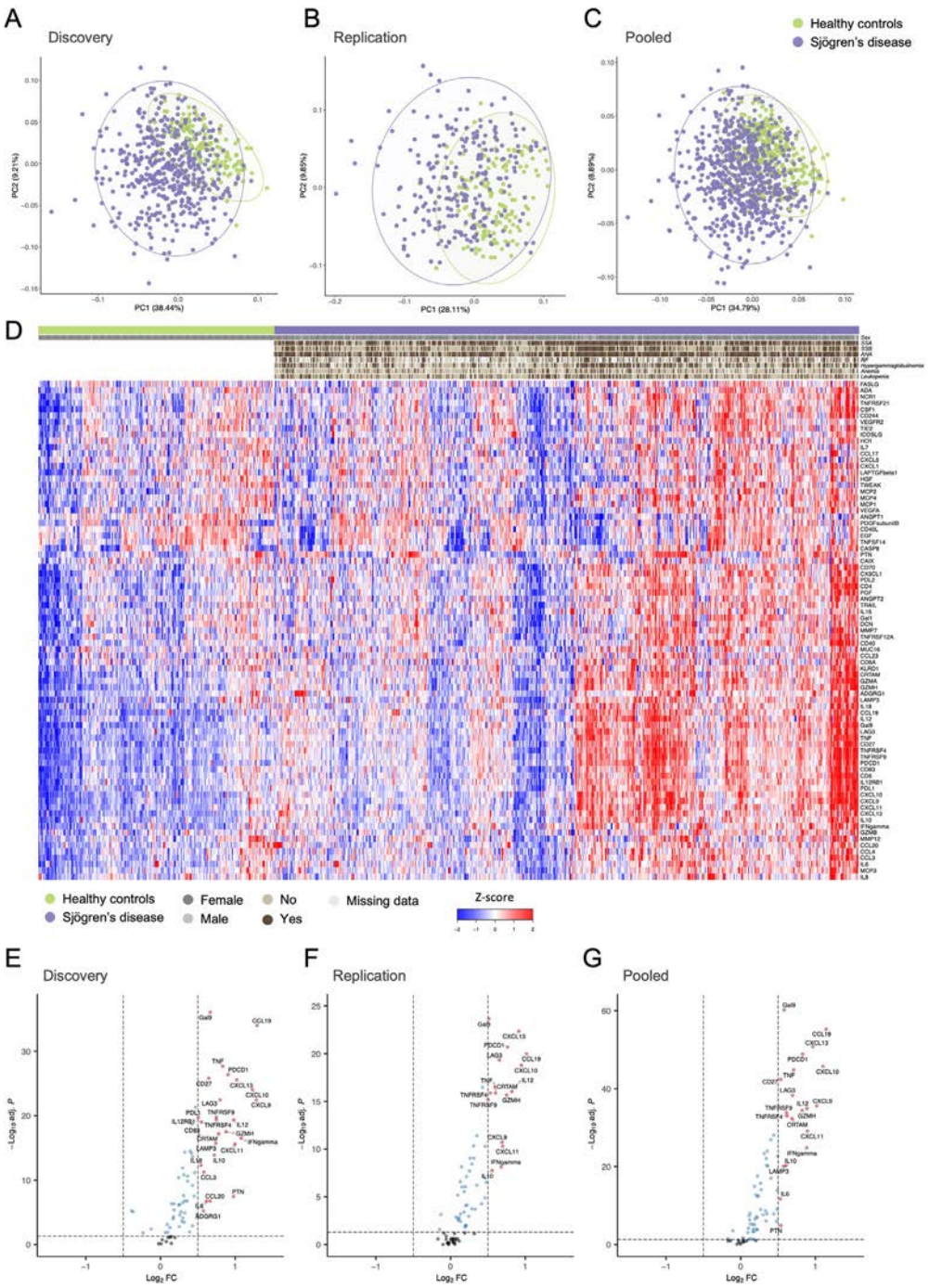


Figure 2. Discovery and validation of serum protein markers in the discovery and the replication cohort. (A-C) Principal component analysis (PCA) of the whole serum protein datasets in the discovery and replication cohort, and in a pooled analysis of both datasets. (D) Heatmap of the Immuno-Oncology panel of the pooled serum dataset with coloured annotations for clinical parameters and sex. Rows are clustered by unsupervised hierarchical clustering, and columns are clustered by unsupervised hierarchical clustering performed separately within each disease-status group. Data on each row are normalised by *z*-score transformation. (E-G) Volcano plots of differential protein levels in serum comparing patients and controls. The vertical dotted line indicates 0.5 log2 fold change, and the horizontal line FDR-adjusted *P* value <.05. FDR, false discovery rate.

proteins [16,17]. Moreover, previous studies have shown that HLA alleles that associate with increased risk of development of SjD are exclusively linked to the anti-Ro/SSA and/or anti-La/SSB positive subset of the disease [18–20]. To analyse proteomic profiles in SjD in relation to both autoantibody subset status as well as in relation to HLA risk alleles, we stratified the full patient cohort based on the presence or absence of ANA, anti-Ro/SSA, and anti-La/SSB autoantibodies according to Figure 4A.

Data on imputed HLA alleles were available for 628 of 689 (91%) patients. We analysed the SjD risk alleles HLA-DRB1*03 and DRB1*15 in relation to autoantibody status and found that their frequency did not differ comparing patients with anti-Ro/SSA and La/SSB-negative SjD (either ANA positive or negative) to controls, but differed significantly comparing ANA positive and the Ro/SSA or La/SSB single positive, as well as the anti-Ro/SSA and La/SSB double positive for HLA-DRB1*03 (Fig 4B). These observations indicate genetic differences in the mechanisms underlying the development of ANA vs anti-Ro/SSA and anti-La/SSB.

Analysis of the 16 proteins validated across both cohorts showed a striking gradual increase according to the autoantibody stratification as well as HLA-DRB*1:03, with the lowest

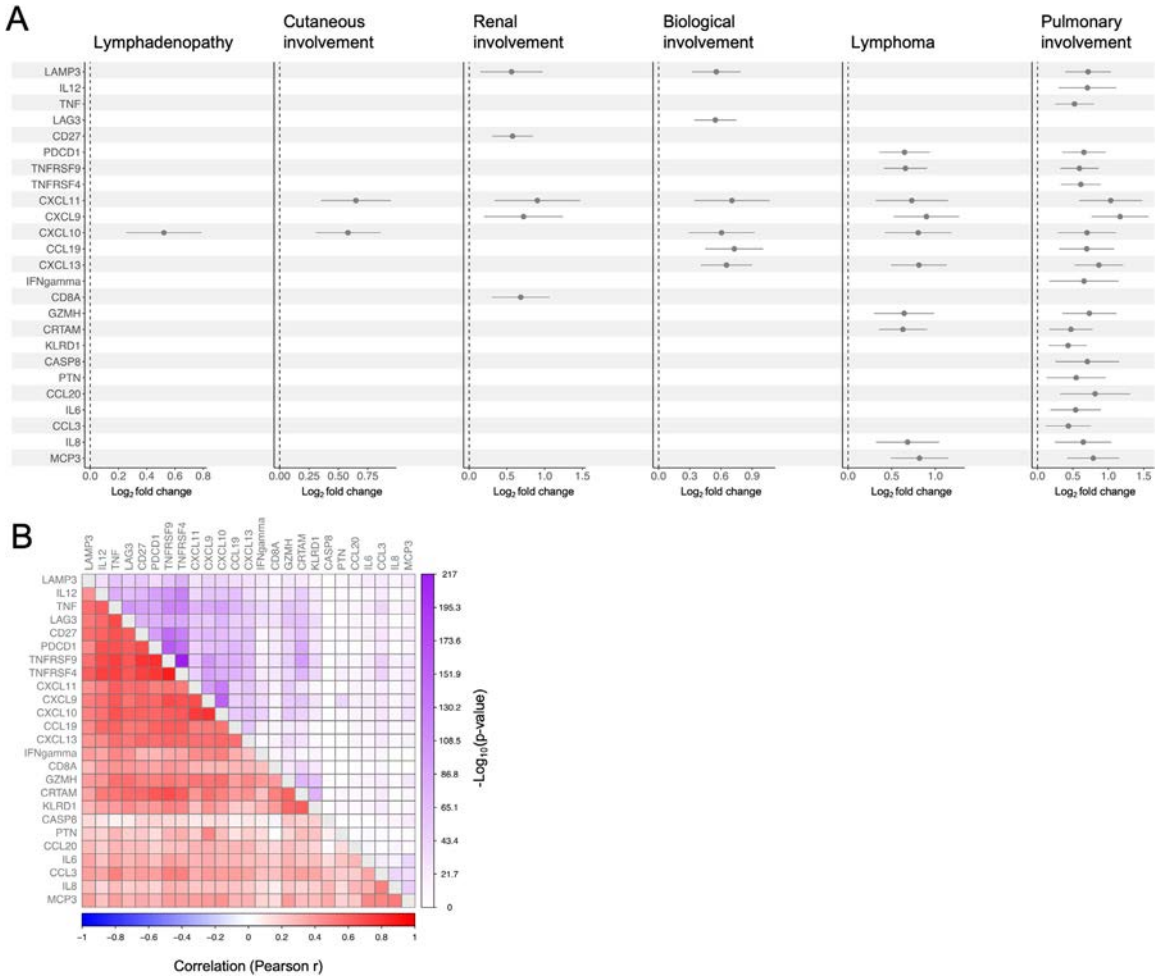


Figure 3. Associations between serum protein levels and extraglandular manifestations. (A) Proteins associating with disease manifestations at a cut-off at FDR-adjusted *P* value <.05 are shown as log2 fold change with 95% CIs. (B) Correlation matrix of proteins from panel (A). The lower triangle displays Pearson correlation coefficients (red), and the upper triangle shows corresponding *P* values (purple). FDR, false discovery rate.

levels in ANA-negative patients and the highest in anti-Ro/SSA and La/SSB double-positive patients (Fig 4C). The protein levels are plotted individually in Figure 4D, and statistical analysis of trend among the patient subgroups revealed that the gradients were significant for all 16 proteins ($P < .0001$, Fig 4D). Between-group comparisons revealed higher levels of the 16 validated proteins in all patient groups compared to controls (except ANA-negative patients compared to controls for CXCL11).

The salivary gland proteome in relation to autoantibody status and the serum proteome

Having observed the prominent gradient of 16 inflammatory proteins in serum, we wondered if a similar pattern could be present in MSG extracts. We thus analysed data from the initial proteome screen of MSG extracts. Of the 16 validated proteins, 12 of them were detectable in MSG extracts. Also in the salivary gland tissue, a similar pattern with the highest protein levels in anti-Ro/SSA and anti-La/SSB positive patients was detected for most proteins (Fig 5A), indicating that the inflammatory environment of the MSGs is mirrored in the circulation.

To understand the degree to which protein levels in serum and MSG extracts correspond to each other, we assessed the correlation of protein levels measured in serum versus MSG extracts from all 6 Olink PEA panels (Fig 5B). Interestingly, the protein with the highest correlation between serum and MSG was CD33, also known as Siglec-3, which is a protein in the immunoglobulin superfamily involved in negative regulation of cytokine production and cell activation, followed by leukocyte-associated immunoglobulin-like receptor 2 (LAIR2), which had the second highest correlation also belongs to the immunoglobulin superfamily (Fig 5C,D).

The Sjögren protein score and its correlation with salivary gland inflammation and autoantibody status

We next wanted to investigate whether the 16 validated proteins (Fig 4 C,D) could be useful to estimate inflammation of the salivary gland. Instead of an invasive MSG biopsy, a serum proxy score from the circulation would be much preferred. We thus generated a 'Sjögren protein score' based on the 16 validated proteins as a new biomarker and analysed its performance. The score gradually increased based on stratification by focus score, indicating that it mirrors the ongoing inflammatory process of the target tissue, although there was a substantial overlap at lower focus score levels (Fig 5E). Of note, the score was significantly higher in patients compared to controls for all SjD patient antibody strata (Fig 5F), including patients negative for ANA, anti-Ro/SSA, and anti-La/SSB. A high protein score was related to the presence of rheumatoid factor and hypergammaglobulinemia, as well as with the salivary gland ultrasound scores in n = 57 patients with available data (Supplementary Fig S3).

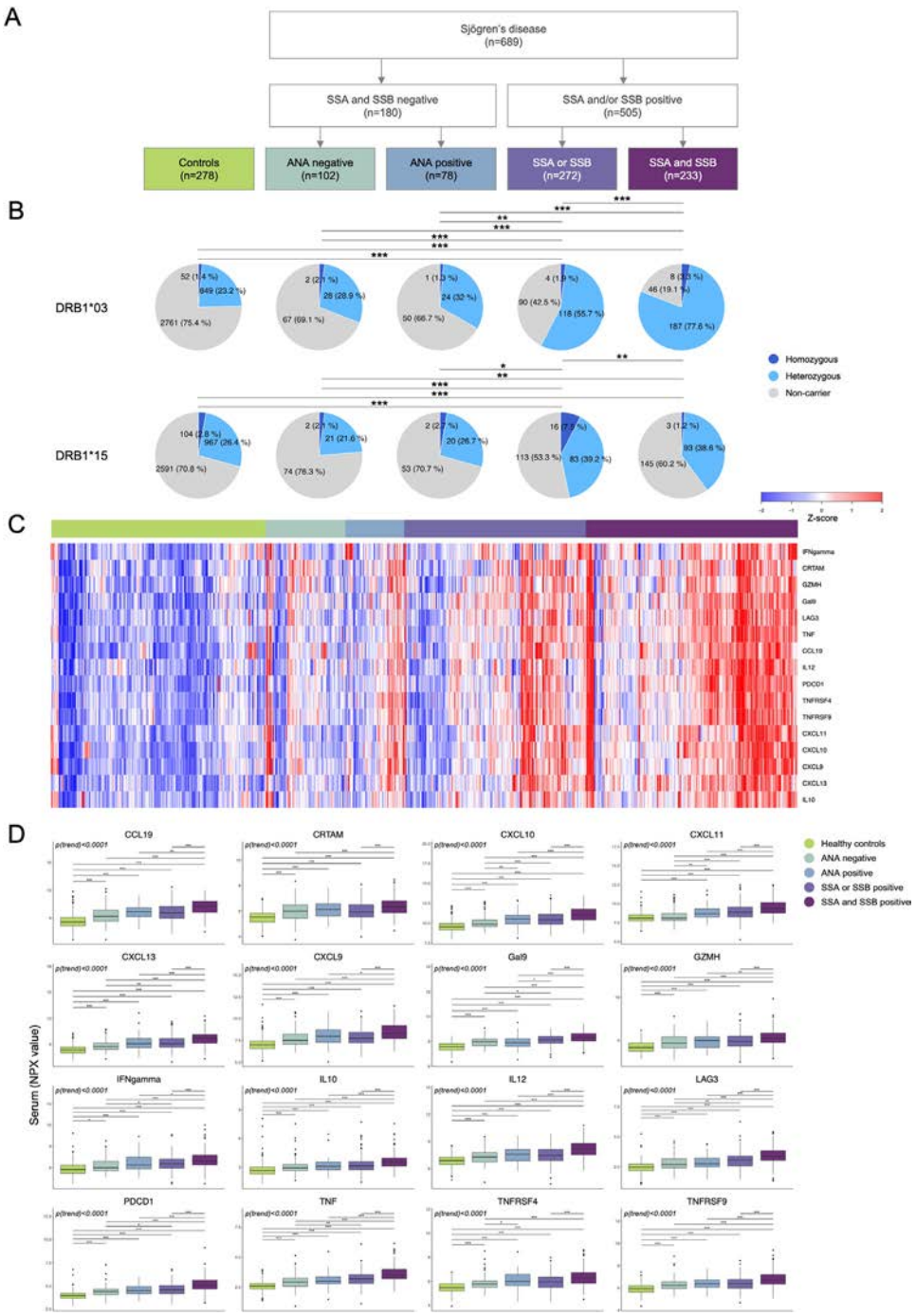


Figure 4. Serum levels of inflammatory proteins are associated with serological autoantibody status in patients with primary Sjögren's disease. (A) Patients stratified by autoantibody status of ANA, anti-Ro/SSA, and anti-La/SSB. The number of patients, and controls, in each group for serum protein analysis is indicated in the boxes. (B) Frequency of SjD risk-associated human leukocyte antigen (HLA) alleles HLA-DRB1*03 and *15 in a population controls (n = 3662), and in the stratified patient subgroups. (C) Heatmap of the 16 proteins validated in the discovery and replication cohort. Columns are clustered by unsupervised hierarchical clustering performed separately within each group. Rows are clustered by unsupervised hierarchical clustering. Data on each row are normalised by *z*-score transformation. (D) Protein NPX values of the 16 validated proteins plotted by subgroup. *P*(trend), *P* values calculated by linear regression of statistical trend in patient subgroups. Fischer's exact test and Kruskal-Wallis test with post hoc Dunn's test, * $P < .05$, ** $P < .01$, *** $P < .001$. ANA, antinuclear antibodies; NPX, normalised protein expression; SjD, Sjögren's disease.

Receiver operating characteristic analysis yielded an area under the curve of 0.888 and determined a score cut-off corresponding to 89% specificity and 76% sensitivity for distinguishing patients from controls (Fig 5G).

DISCUSSION

In this study, we investigated the serum and MSG proteome of SjD, revealing an inflammatory gradient of the serum and gland proteome related to serological autoantibody status as well as risk-associated HLA alleles. Connections between pathological inflammation in target tissue and alterations of the circulating proteome indicate the utility of a 'Sjögren protein score' to predict focus score and aid SjD classification.

Sixteen proteins upregulated in SjD were validated across the two separate cohorts. The identified proteins are implicated in processes such as leukocyte migration, regulation of immune effector processes, cytokine signalling, and anti-viral IFN

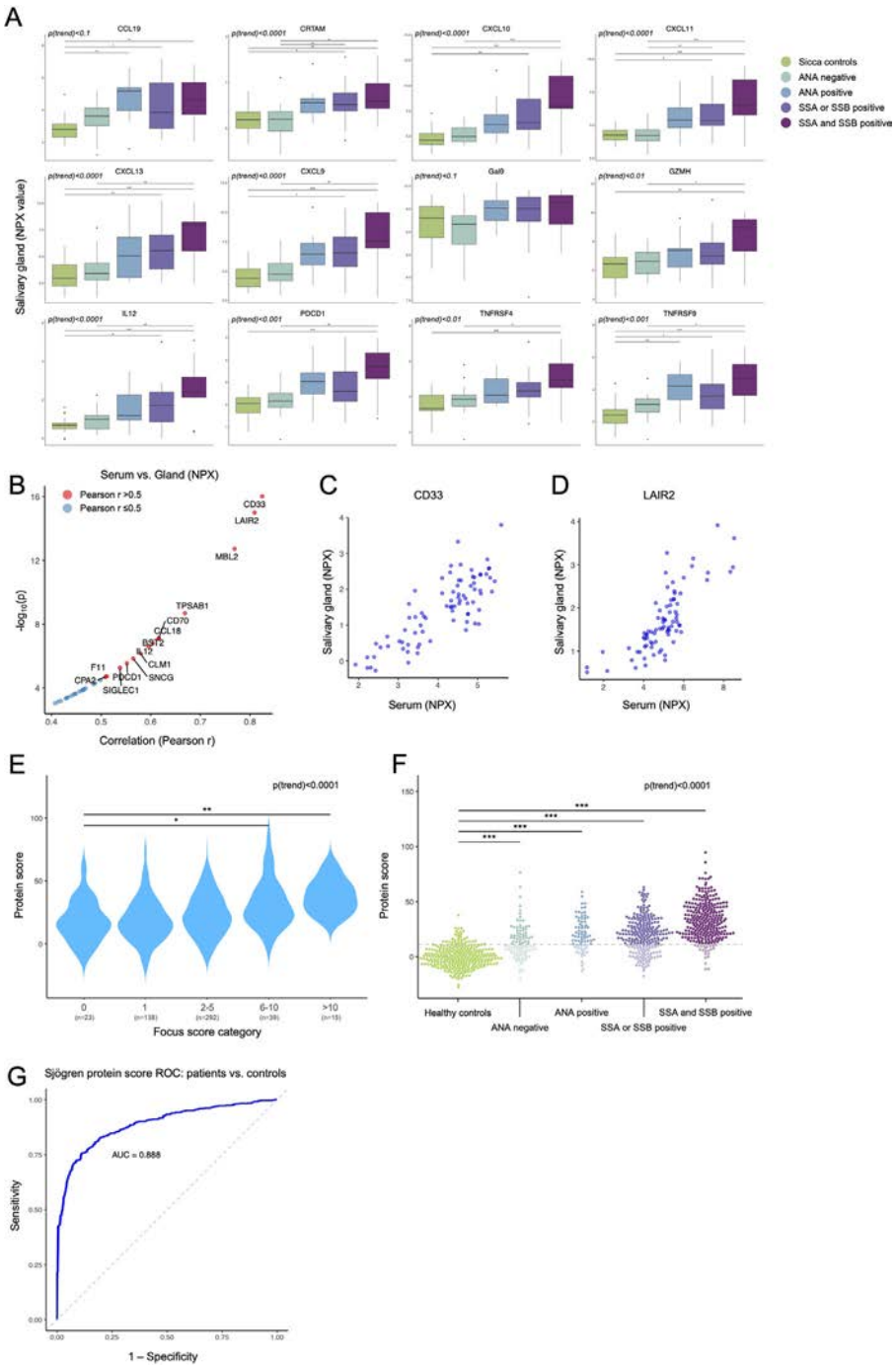


Figure 5. Protein levels in minor salivary gland extracts and their association with serum levels and focus score. (A) NPX values for 12 of the 16 validated proteins that were detectable in minor salivary gland, plotted by autoantibody-defined patient subgroups and sicca controls. (B) Correlation between protein levels in matched serum samples and salivary gland extracts. Pearson correlation coefficients are indicated on the X axis and corresponding *P* values on the Y axis. (C) Correlation between salivary gland lysate and serum NPX values for CD33 and (D) LAIR2. (E) The Sjögren protein score (based on the 16 validated proteins) in relation to focus score categories. (F) The Sjögren protein score stratified by autoantibody status. The dotted line indicates a cut-off at 11.2 determined by (G) receiver operating characteristic curve of the Sjögren protein score for distinguishing patients from controls (AUC = 0.888), yielding 89% specificity and 76% sensitivity. Kruskal-Wallis test with post hoc Dunn's test, $*P < .05$, $**P < .01$, $***P < .001$. For (E) and (F), inter-group comparisons against the control group are indicated in the figure while comparisons among the patient subgroups are not shown. P(trend), *P* values of trend calculated by linear regression for all focus score groups (E) and in patient subgroups (A, F). ANA, antinuclear antibodies; ROC; receiver operating characteristic; AUC, area under the curve; CD33, Siglec-3;; LAIR2, leukocyte-associated immunoglobulin-like receptor 2; NPX, normalised protein expression.

responses. Of special note, levels of CRTAM, a receptor expressed on activated $CD8^+$ T cells and natural killer/ natural killer T cells [21], were consistently elevated, which, to our knowledge, has not been previously described in SjD. Furthermore, LAG3, a potent immune checkpoint receptor targeted in the treatment of advanced melanoma [22,23], showed consistently high levels yet remains unexplored in SjD. Beyond the novel findings, several previously suggested biomarkers were confirmed at high levels, such as CXCL13 [24–26], Gal9 [27], and CXCL10 [8]. Of note, the type I IFN-induced protein Gal9 reached the highest level of statistical significance in both cohorts, and serum levels of Gal9 were recently shown to be predictive of response to treatment with leflunomide and hydroxychloroquine combination treatment [27].

In a pooled analysis of both cohorts, lysosome-associated membrane glycoprotein 3 (LAMP3), pleiotrophin (PTN), IL-6, and CD27 also passed the predefined cut-off. The involvement of LAMP3 in the pathogenesis of SjD, and regulation of this protein as a potential therapeutic approach, has gained significant attention in recent years [28–30], with indications that epithelial LAMP3 plays a role in the development of salivary gland hypofunction [31] as well as induction of ectopic TLR7 [29]. PTN is a growth hormone important for neural development during embryogenesis but is also associated with many other processes [32] and was previously reported at higher levels in patients with SjD and systemic lupus erythematosus compared to healthy controls [33]. Finally, CD27 is an important costimulatory protein expressed on subsets of CD4+ and CD8+ T cells as well as on memory B cells, which are generally reported at lower levels in patients with SjD compared to controls [34]. High levels of CD27 may reflect a shared immune activation, including both T and B cells. Shedding of soluble CD27 can occur upon prolonged immune activation, and the CD27-CD70

pathway has been suggested as a target for treatment of autoimmune diseases [35].

Levels of distinct proteins were higher in patients who at some point in their disease course had presented with various extraglandular manifestations. These results should be interpreted in light of the limitation that validation in an external cohort is still needed. Interestingly, some markers are associated only with one manifestation, including CD8A and CD27 for renal involvement, LAG3 for biological involvement, and numerous ones with pulmonary involvement. The finding that serum levels of CD8A and CD27 were higher in patients with renal involvement is interesting since both T cells and B cells infiltrate the tubulointerstitium at equal proportions in SjD-associated nephritis [36]. As such, one may speculate that kidney inflammation at the histological level is mirrored in the periphery by increased T and B cell markers. The LAG3 association with activity in the biological involvement provides a logical mirroring of activation of the humoral immune system since LAG3 is an major histocompatibility complex class II binding protein expressed on activated T and B cells. Thus far, LAG3 has not been discussed in the context of SjD, except for a genetic connection [37]. Interstitial lung disease is a potentially severe manifestation of SjD affecting approximately 9% to 20% of patients [38]. In the pooled analysis, pulmonary involvement was associated with the most pronounced changes in the proteome. In part, this was reflected by high levels of IFN-induced proteins such as CXCL9, CXCL10, and CXCL13. However, cytokines including IL-12 and IL-6 were specifically elevated in this group, suggesting that drugs such as ustekinumab (anti-IL-12/IL-23) and tocilizumab (anti-IL-6R) may be useful for this patient subgroup. Interestingly, our data indicated an overlap of the proteomic profile between patients with pulmonary involvement and those affected by lymphoma (only 3 patients had both manifestations), suggesting shared underlying immunopathological pathways.

This study confirms the link between autoantibodies targeting Ro/SSA and La/SSB and HLA alleles conferring increased risk of SjD [18]. However, ANA single positivity was not clearly associated with a higher frequency of risk-HLA. As such, this subpopulation is unlikely to represent an early SjD phenotype, which would be expected to progress into the development of positivity for anti-Ro/SSA. Still, both ANA-negative SjD as well as those single positive for ANA had significantly higher levels of inflammatory proteins in serum, revealing that the immune activation in this subgroup of patients can be detected not only as a focus score of MSG biopsies but also in serum.

Integration of HLA risk alleles into the analysis revealed a strong tendency for higher inflammatory status of the proteome in relation to frequency of risk-associated HLA. Whether levels of inflammatory proteins are directly linked to genetic factors, or rather a cause of pathogenic properties of anti-Ro/SSA and anti-La/SSB autoantibodies remains to be understood. Our data suggest that integration of a polygenic risk score including HLA [39] in combination with data on autoantibodies and proteomic profile may provide a phenotypic description to be used in clinically relevant patient stratification.

Analysis of MSG biopsy extracts revealed a more profound proteomic dysregulation between patients and sicca controls compared to that of serum samples. Among proteins displaying the highest fold change values, several proteins of importance to T-B cell interaction were noted, such as CXCL13, which is a chemokine important for B cell migration and formation of germinal centre-like structures, IL-12, which promotes differentiation of T cells and IFNγ production, and SH2D1A, which is a key regulator of immune cell signalling. Interestingly, mutations in SH2D1A are associated with X-linked lymphoproliferative disease, which leads to immune dysfunction [40]. The relevance SH2D1A in the pathogenesis of SjD remains to be explored.

Paired serum and MSG biopsy lysate samples allowed for the analysis of the correlation between the proteome in serum and gland, as well as the focal inflammation in the glands, measured by focus score. The inflammatory gradient observed when stratifying by autoantibody status was also evident in the MSG extracts, indicating that the serum proteome could potentially serve as a peripheral marker of the target tissue. Indeed, using the markers confirmed across the two large cohorts, we could calculate a 'Sjögren protein score' with 89% specificity and 76% sensitivity for the diagnosis. The Sjögren protein score also correlated with focal sialadenitis in the gland, salivary gland ultrasound scores, as well as with SjD classification, regardless of autoantibody status. The finding that the 'Sjögren protein score' is elevated also in Sjögren-patients without anti-Ro/SSA/anti-La/SSB antibodies compared to controls is important in the sense that it indicates that a biopsy may not be necessary in selected cases in the future.

Limitations of the study relate to the fact that the specificity of the score was not assessed for SjD in relation to sicca controls or SjD mimics such as active hepatitis C infection, sarcoidosis, or other rheumatic diseases including IgG4-related disease, which should be a focus for future studies. For now, the Sjögren protein score should therefore be considered only under this limitation. Also, the analysis of the two larger cohorts was limited to the 92 proteins in the selected panel. A more comprehensive screen could have detected additional proteins of relevance. Furthermore, data on organ manifestations indicated whether the patients had ever had these manifestations, and the activity of that organ system at the time of sampling could not be confirmed. Information on DMARD treatment was incomplete, but effects of treatment on the proteome measured by Olink PEA have been described [41]. Strengths of the study include inclusion of both serum and target tissue for proteome analysis, an initial comprehensive screen and that results were confirmed across two large cohorts, as well as the integration of HLA in the analysis.

In all, the data of the present study shed further light on the dysregulated proteome in SjD in relation to extraglandular manifestations and underlying HLA risk alleles. The importance of analysis and clinical consideration of positivity against anti-La/SSB autoantibodies, together with anti-Ro/SSA is supported by higher inflammatory activity reflected by the proteome. Finally, the novel 'Sjögren protein score' appears promising as a biomarker to indicate the inflammatory status of target tissue and also distinguishes patients with anti-Ro/La autoantibody-negative SjD from controls.

Acknowledgements

Images from Servier Medical Art by Servier which are licensed under a Creative Commons Attribution 3.0 Unported License, were used in some figures. An abstract was previously presented at the European Alliance of Associations for Rheumatology (EULAR) 2024 congress: https://ard.bmj.com/content/83/Suppl_1/42

Contributors

The study was conceptualised by MW-H, AB, and JM. Experimental work was performed by LM, AB, and ERA. Data analysis and interpretation were performed by JM, AB, MW-H, GN, JI-K,

and LM. AB, MK, RJ, RO, HF-dE, SMB, PE, TM, PO, MF, KBM, SJAJ, DH, EM, KS, MVJ, SA, IK, JI-K, and GN contributed with clinical characterisation of patients and samples. MW-H, AB, and JM obtained funding and drafted the original manuscript. All authors read, contributed to, and approved the final version of the manuscript. MW-H is responsible for the overall content as guarantor.

Funding

This project received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) NECESSITY under grant agreement No 806975. The JU receives support from the European Union's Horizon 2020 research and innovation program and European Federation of Pharmaceutical Industries and Associations. The present article reflects only the author's view, and the JU is not responsible for any use that may be made of the information it contains.

The project was also funded by grants from the Stockholm County Council, the King Gustaf V 80-year Foundation, the Swedish Research Council, the Norwegian Research Council, the Swedish Rheumatism Association, the Swedish HeartLung Foundation, the Knut and Alice Wallenberg Foundation, the Tore Nilson Foundation, the Magnus Bergvall Foundation, the Lars Hierta Foundation, the Rheumatology Foundations at Karolinska Institutet, the Professor Nanna Svartz Foundation, the Åke Wiberg Foundation, the Ulla and Roland Gustafsson Donation Fund, and the Alex and Eva Wallström Foundation.

Competing interests

TM is working as medical lead at Union Chimiqie Belge (UCB). All other authors declare they have no competing interests.

Patient consent for publication

Not applicable.

Ethics approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committees. Written informed consent was obtained from all participants.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

All data relevant to the study are included in the article or uploaded as supplementary information.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ard.2025.11.013.

Orcid

Marie Wahren-Herlenius: http://orcid.org/0000-0002-0915-7245

REFERENCES

[1] Mariette X, Criswell LA. Primary Sjögren's syndrome. N Engl J Med 2018;378(10):931–9.
[2] Baldini C, Fulvio G, La Rocca G, Ferro F. Update on the pathophysiology and treatment of primary Sjögren syndrome. Nat Rev Rheumatol 2024;20(8):473–91.
[3] Seror R, Theander E, Brun JG, Ramos-Casals M, Valim V, Dörner T, et al. Validation of EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient indexes (ESSPRI). Ann Rheum Dis 2015;74(5):859–66.
[4] Tarn JR, Howard-Tripp N, Lendrem DW, Mariette X, Saraux A, Devauchelle-Pensec V, et al. Symptom-based stratification of patients with primary Sjögren's syndrome: multi-dimensional characterisation of international observational cohorts and reanalyses of randomised clinical trials. Lancet Rheumatol 2019;1(2):e85–94.
[5] Nguyen Y, Nocturne G, Henry J, Ng WF, Belkhir R, Desmoulins F, et al. Identification of distinct subgroups of Sjögren's disease by cluster analysis based on clinical and biological manifestations: data from the cross-sectional Paris-Saclay and the prospective ASSESS cohorts. Lancet Rheumatol 2024;6(4):e216–25.
[6] Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American European Consensus Group. Ann Rheum Dis 2002;61(6):554–8.
[7] Jousse-Joulin S, D'Agostino MA, Nicolas C, Naredo E, Ohrndorf S, Backhaus M, et al. Video clip assessment of a salivary gland ultrasound scoring system in Sjögren's syndrome using consensual definitions: an OMERACT ultrasound working group reliability exercise. Ann Rheum Dis 2019;78(7):967–73.
[8] Björk A, Richardsdotter Andersson E, Imgenberg-Kreuz J, Thorlacius GE, Mofors J, Syvänen AC, et al. Protein and DNA methylation-based scores as surrogate markers for interferon system activation in patients with primary Sjögren's syndrome. RMD Open 2020;6(1):e000995.
[9] Dilthey A, Leslie S, Moutsianas L, Shen J, Cox C, Nelson MR, et al. Multi-population classical HLA type imputation. PLoS Comput Biol 2013;9(2):e1002877.
[10] Khatri B, Tessneer KL, Rasmussen A, Aghakhanian F, Reksten TR, Adler A, et al. Genome-wide association study identifies Sjögren's risk loci with functional implications in immune and glandular cells. Nat Commun 2022;13(1):4287.
[11] International Multiple Sclerosis Genetics Consortium. Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. Science 2019;365(6460):eaav7188.
[12] Hedström AK, Katsoulis M, Hössjer O, Bomfim IL, Oturai A, Sondergaard HB, et al. The interaction between smoking and HLA genes in multiple sclerosis: replication and refinement. Eur J Epidemiol 2017;32(10):909–19.
[13] Aqrawi LA, Kvarnström M, Brokstad KA, Jonsson R, Skarstein K, Wahren-Herlenius M. Ductal epithelial expression of Ro52 correlates with inflammation in salivary glands of patients with primary Sjögren's syndrome. Clin Exp Immunol 2014;177(1):244–52.
[14] Hjelmervik TO, Petersen K, Jonassen I, Jonsson R, Bolstad AI. Gene expression profiling of minor salivary glands clearly distinguishes primary Sjögren's syndrome patients from healthy control subjects. Arthritis Rheum 2005;52(5):1534–44.
[15] Rusinova I, Forster S, Yu S, Kannan A, Masse M, Cumming H, et al. Interferome v2.0: an updated database of annotated interferon-regulated genes. Nucleic Acids Res 2013;41:D1040–6 Database issue.
[16] Båve U, Nordmark G, Lövgren T, Rönnelid J, Cajander S, Eloranta ML, et al. Activation of the type I interferon system in primary Sjögren's syndrome: a possible etiopathogenic mechanism. Arthritis Rheum 2005;52(4):1185–95.
[17] Bodewes ILA, Björk A, Versnel MA. Wahren-Herlenius M. Innate immunity and interferons in the pathogenesis of Sjögren's syndrome. Rheumatology (Oxford) 2021;60(6):2561–73.
[18] Thorlacius GE, Björk A, Wahren-Herlenius M. Genetics and epigenetics of primary Sjögren syndrome: implications for future therapies. Nat Rev Rheumatol 2023;19(5):288–306.
[19] Thorlacius GE, Hultin-Rosenberg L, Sandling JK, Bianchi M, Imgenberg-Kreuz J, Pucholt P, et al. Genetic and clinical basis for two distinct subtypes of primary Sjögren's syndrome. Rheumatology 2021;60(2):837–48.
[20] Gottenberg JE, Busson M, Loiseau P, Cohen-Solal J, Lepage V, Charron D, et al. In primary Sjögren's syndrome, HLA class II is associated exclusively with autoantibody production and spreading of the autoimmune response. Arthritis Rheum 2003;48(8):2240–5.
[21] Takeuchi A, Badr Mel S, Miyauchi K, Ishihara C, Onishi R, Guo Z, et al. CRTAM determines the CD4+ cytotoxic T lymphocyte lineage. J Exp Med 2016;213(1):123–38.
[22] Munir AZ, Gutierrez A, Qin J, Lichtman AH, Moslehi JJ. Immune-checkpoint inhibitor-mediated myocarditis: CTLA4, PD1 and LAG3 in the heart. Nat Rev Cancer 2024;24(8):540–53.

[23] Aggarwal V, Workman CJ, Vignali DAA. LAG-3 as the third checkpoint inhibitor. Nat Immunol 2023;24(9):1415–22.
[24] Nocturne G, Seror R, Fogel O, Belkhir R, Boudaoud S, Saraux A, et al. CXCL13 and CCL11 serum levels and lymphoma and disease activity in primary Sjögren's syndrome. Arthritis Rheumatol 2015;67(12):3226–33.
[25] Aqrawi LA, Ivanchenko M, Björk A, Ramírez Sepúlveda JI, Imgenberg-Kreuz J, Kvarnström M, et al. Diminished CXCR5 expression in peripheral blood of patients with Sjögren's syndrome may relate to both genotype and salivary gland homing. Clin Exp Immunol 2018;192(3):259–70.
[26] Loureiro-Amigo J, Franco-Jarava C, Perurena-Prieto J, Palacio C, Martínez-Valle F, Soláns-Laqué R. Serum CXCL13, BAFF, IL-21 and IL-22 levels are related to disease activity and lymphocyte profile in primary Sjögren's syndrome. Clin Exp Rheumatol 2021;39(6):131–9 Suppl 133.
[27] Hamkour S, van der Heijden EH, Lopes AP, Blokland SLM, Bekker CPJ, Van Helden-Meeuwsen CG, et al. Leflunomide/hydroxychloroquine combination therapy targets type I IFN-associated proteins in patients with Sjögren's syndrome that show potential to predict and monitor clinical response. RMD Open 2023;9(3):e002979.
[28] Nakamura H, Tanaka T, Pranzatelli T, Ji Y, Yin H, Perez P, et al. Lysosome-associated membrane protein 3 misexpression in salivary glands induces a Sjögren's syndrome-like phenotype in mice. Ann Rheum Dis 2021;80(8):1031–9.
[29] Nakamura H, Tanaka T, Zheng C, Afione SA, Atsumi T, Noguchi M, et al. Amplified type I interferon response in Sjögren's disease via ectopic toll-like receptor 7 expression in salivary gland epithelial cells induced by lysosome-associated membrane protein 3. Arthritis Rheumatol 2024;76 (7):1109–19.
[30] Nakamura H, Tanaka T, Zheng C, Afione SA, Warner BM, Noguchi M, et al. Lysosome-associated membrane protein 3 induces lysosome-dependent cell death by impairing autophagic caspase 8 degradation in the salivary glands of individuals with Sjögren's disease. Arthritis Rheumatol 2023;75(9):1586–98.
[31] Nakamura H, Tanaka T, Zheng C, Afione SA, Warner BM, Noguchi M, et al. Correction of LAMP3-associated salivary gland hypofunction by aquaporin gene therapy. Sci Rep 2022;12(1):18570.
[32] Wang X. Pleiotrophin: activity and mechanism. Adv Clin Chem 2020;98:51–89.
[33] Wu GC, Yuan H, Pan HF. Ye DQ. Elevated plasma midkine and pleiotrophin levels in patients with systemic lupus erythematosus. Oncotarget 2017;8(25):40181–9.
[34] Hansen A, Daridon C, Dörner T. What do we know about memory B cells in primary Sjögren's syndrome? Autoimmun Rev 2010;9(9):600–3.
[35] Han BK, Olsen NJ, Bottaro A. The CD27-CD70 pathway and pathogenesis of autoimmune disease. Semin Arthritis Rheum 2016;45(4):496–501.
[36] François H, Mariette X. Renal involvement in primary Sjögren syndrome. Nat Rev Nephrol 2016;12(2):82–93.
[37] Wang Y, Chen S, Chen J, Xie X, Gao S, Zhang C, et al. Germline genetic patterns underlying familial rheumatoid arthritis, systemic lupus erythematosus and primary Sjögren's syndrome highlight T cell-initiated autoimmunity. Ann Rheum Dis 2020;79(2):268–75.
[38] La Rocca G, Ferro F, Sambataro G, Elefante E, Fonzetti S, Fulvio G, et al. Primary-Sjögren's-syndrome-related interstitial lung disease: a clinical review discussing current controversies. J Clin Med 2023;12(10):3428.
[39] Fugmann C, Reid S, Pucholt P, Kvarnström M, Björk A, Mofors J, et al. A high polygenic risk score is associated with SSA/SSB antibody positivity and early onset in primary Sjögren's disease. Rheumatology (Oxford) 2025;64(7):4341–6.
[40] Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. Nat Genet 1998;20(2):129–35.
[41] Baloche V, Bogers C, Leavis H, Van Roon J. OP0215 Immunome analysis indicates robust inhibition of proinflammatory mediators upon leflunomide-hydroxychloroquine combination therapy in Sjögren's syndrome: CXCR3 ligands as potential inflammatory endotype biomarkers. Ann Rheum Dis 2023;82(suppl 1):141.

Ann Rheum Dis 85 (2026) 351–359

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Myositis

Myositis-specific autoantibodies recognising Mi2 also target the AIRE protein at a shared PHD zinc finger

Jon Musai[1], Sahana Jayaraman[2], Katherine Pak[1], Iago Pinal-Fernandez[1,2], Sandra Muñoz-Braceras[1], Maria Casal-Dominguez[1], Eric Cho[1], Fa'alataitaua M. Fitisemanu[1], Peter D. Burbelo[3], Mariana J. Kaplan[4], Blake M. Warner[5], Adam I. Schiffenbauer[6], Albert Selva-O'Callaghan[7,8], José César Milisenda[9,10,11], Lisa G. Rider[6], H. Benjamin Larman[2], Andrew L. Mammen[1,2,*]

[1] *Muscle Disease Section, National Institute of Arthritis and Musculoskeletal and Skin Disease, National Institutes of Health, Bethesda, Maryland, USA*
[2] *Johns Hopkins University School of Medicine, Baltimore, Maryland, USA*
[3] *Adeno-Associated Virus Biology Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland, USA*
[4] *Systemic Autoimmunity Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, Maryland, USA*
[5] *Salivary Disorders Unit, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland, USA*
[6] *Environmental Autoimmunity Group, Clinical Research Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Bethesda, Maryland, USA*
[7] *Systemic Autoimmune Disease Unit, Vall d'Hebron Institute of Research, Barcelona, Spain*
[8] *Autonomous University of Barcelona, Barcelona, Spain*
[9] *Muscle Research Unit, Internal Medicine Service, Hospital Clinic, Barcelona, Spain*
[10] *Barcelona University, Barcelona, Spain*
[11] *CIBERER and IDIBAPS, Barcelona, Spain*

ARTICLE INFO

Article history:
Received 21 January 2025
Received in revised form 20 August 2025
Accepted 21 August 2025

ABSTRACT

Objectives: In patients with dermatomyositis with anti-Mi2 autoantibodies, autoantibodies can enter muscle cells, leading to the aberrant expression of genes normally repressed by the Mi2/nucleosome remodelling and deacetylation (NuRD) complex. However, the mechanism by which autoantibodies interfere with Mi2/NuRD function remains unclear. This study aimed to identify additional autoantibodies in anti−Mi2-positive patients and the epitopes recognised by these autoantibodies.

Methods: Phage immunoprecipitation sequencing (PhIP-Seq) was used to screen sera from patients with anti-Mi2 autoantibody-positive myositis. Enzyme-linked immunosorbent assays (ELISAs) and luciferase immunoprecipitation system (LIPS) immunoassays were used to detect autoantibodies in sera from healthy controls, patients with myositis, and those with other autoimmune diseases.

*Correspondence to: Dr. Andrew L Mammen, Muscle Disease Section, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, 50 South Drive, Room 1141, Building 50, MSC 8024, Bethesda, MD 20892, USA.

E-mail address: andrew.mammen@nih.gov (A.L. Mammen).

Jon Musai, Sahana Jayaraman, H Benjamin Larman, and Andrew L. Mammen contributed equally to this study.

Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.08.023

Results: PhIP-Seq identified autoantibodies recognising the autoimmune regulator (AIRE) in sera from anti-Mi2 autoantibody-positive patients. Both anti-AIRE and anti-Mi2 autoantibodies predominantly recognised a homologous region containing the plant homeodomain zinc finger type I (PHD1), which is critical for AIRE and Mi2/NuRD function. ELISA and LIPS showed that anti-Mi2 autoantibody-positive patients were positive for anti-AIRE autoantibodies, whereas AIRE reactivity was largely absent in healthy comparators, anti-Mi2 autoantibody-negative myositis, and other autoimmune diseases. Affinity-purified anti-Mi2 autoantibodies recognised both Mi2 and AIRE by ELISA, whereas anti−Mi2-depleted fractions did not recognise either protein.
Conclusions: Autoantibodies targeting Mi2 recognise AIRE at a shared PHD1 epitope − a conserved motif found in numerous transcriptional regulators. These findings support a model in which anti-Mi2 autoantibodies disrupt the Mi2/NuRD complex, and potentially other PHD1-containing proteins, by interfering with chromatin binding, although further studies are needed to directly demonstrate this mechanism *in vivo*.

KEY MESSAGES

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Anti-Mi2 autoantibodies can enter muscle cells and cause derepression of genes normally repressed by the Mi2/NuRD complex. The mechanism by which anti-Mi2 autoantibodies disrupt Mi2/NuRD function has remained unclear.

WHAT THIS STUDY ADDS

- Anti-Mi2 autoantibodies also recognize the autoimmune regulator (AIRE) at a shared immunodominant epitope, the PHD1 finger, which is critical for chromatin binding and transcriptional regulation.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- This study provides a mechanistic model in which anti-Mi2 autoantibodies disrupt chromatin regulation by targeting conserved PHD domains. The findings highlight the potential for broad dysregulation of PHD-containing transcriptional regulators in dermatomyositis.

INTRODUCTION

Myositis is a heterogeneous family of autoimmune diseases that includes dermatomyositis (DM), antisynthetase syndrome (ASyS), immune-mediated necrotising myopathy (IMNM), and inclusion body myositis (IBM) [1,2]. Approximately 70% of patients with DM have a known myositis-specific autoantibody (MSA) targeting 1 or more intracellular autoantigens. The most prevalent MSAs in DM target the chromodomain helicase DNA-binding (CHD) proteins (CHD3, ie, Mi2α or CHD4, ie, Mi2β), nuclear matrix protein (NXP2), transcriptional intermediary factor 1 γ (TIF1γ), or melanoma differentiation-associated protein 5 (MDA5) [1]. Importantly, each of these autoantibodies is associated with a unique disease phenotype. For instance, adult patients with anti-TIF1γ autoantibodies exhibit a significantly heightened risk of malignancy compared with other patients with DM but have a lower propensity for developing interstitial lung disease [3]. Conversely, those harbouring anti-MDA5 autoantibodies are prone to rapidly progressive interstitial lung disease, with a relatively low risk of cancer [4].

Compared with other patients with DM, those with anti-Mi2 autoantibodies have more severe muscle involvement, more extensive myofiber necrosis, higher muscle enzyme levels, and photosensitive rashes [5]. We recently reported that anti-Mi2 autoantibodies localise to the nuclei of muscle cells in patient biopsies and are associated with the overexpression of genes typically repressed by the Mi2/nucleosome remodelling and deacetylation (NuRD) complex [6–8]. These findings suggest a potential disruption of Mi2/NuRD function, although the precise mechanism by which anti-Mi2 autoantibodies exert this effect remains unclear.

The current study initially aimed to identify novel myositis autoantibodies. We performed phage immunoprecipitation sequencing (PhIP-Seq) using an oligonucleotide library encoding 274,207 overlapping peptides spanning the human proteome to screen patient sera for novel myositis autoantibodies [9,10]. Surprisingly, this revealed that sera from individuals with anti-Mi2 autoantibodies also recognise autoimmune regulator (AIRE), a protein whose dysfunction leads to autoimmunity. Anti-Mi2/AIRE autoantibodies recognise a homologous plant homeodomain (PHD) zinc finger that is critical for the function of both Mi2 and AIRE. In the case of Mi2, this domain is required for the Mi2/NuRD complex to interact with nucleosomes. Our findings raise the possibility that anti-Mi2 autoantibodies bind to this domain, thereby preventing the Mi2/NuRD complex from interacting with nucleosomes and causing the derepression of genes that are normally repressed by this complex.

METHODS

Patients and serum samples

To conduct PhIP-Seq, we established a discovery cohort comprising 10 anti-Mi2 autoantibody-positive myositis sera: 8 from patients with adult DM and 2 from patients with juvenile DM. Additionally, we utilised 804 serum samples from healthy controls obtained from the Vaccine Research Cohort [11,12].

To validate our PhIP-Seq findings, we first conducted enzyme-linked immunosorbent assays (ELISAs) to measure anti-AIRE and anti-Mi2β levels in a separate cohort we termed the 'validation cohort'. This validation cohort included sera from 26 anti-Mi2 autoantibody-positive patients, 3 of whom were present in the PhIP-Seq discovery cohort. Our validation cohort also included sera from 63 healthy controls and 44 patients with other prevalent forms of myositis (Table).

Since anti-Mi2 autoantibodies can target different CHD proteins, such as Mi2β (ie, CHD4) or Mi2α (ie, CHD3), we also used luciferase immunoprecipitation system (LIPS) immunoassays to measure anti-AIRE and anti-Mi2α levels in the same validation cohort used for the ELISAs. Additionally, LIPS immunoassays were conducted on 53 serum samples from patients with other

Table
Serum samples tested for anti-AIRE autoantibodies using ELISA and LIPS

Group	AIRE reactivity by ELISA, % (n/N)	AIRE reactivity by LIPS, % (n/N)
Healthy control	1.6 (1/63)	0 (0/63)
Myositis (n = 70)		
Anti-Mi2	92 (24/26)	100 (26/26)
Anti-NXP2	0 (0/7)	0 (0/7)
Anti-TIF1γ	0 (0/5)	0 (0/5)
Anti-MDA5	0 (0/9)	0 (0/9)
Anti-Jo1	0 (0/8)	0 (0/8)
Anti-HMGCR	0 (0/5)	0 (0/5)
Anti-SRP	0 (0/4)	0 (0/4)
IBM	0 (0/6)	0 (0/6)
Disease control (n = 53)		
SLE	-	5.0 (1/20)
SSc	-	0 (0/8)
Vasculitis	-	0 (0/5)
Sjögren's syndrome	-	0 (0/20)

Using ELISAs and LIPS immunoassays, the prevalence of anti-AIRE autoantibodies was measured in a validation cohort that included different types of myositis and other healthy and disease comparators.
AIRE, autoimmune regulator; ELISA, enzyme-linked immunosorbent assay; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; IBM, inclusion body myositis; Jo1, histidyl transfer RNA synthetase; LIPS; luciferase immunoprecipitation systems; MDA5, melanoma differentiation-associated protein 5; Mi2, chromodomain helicase DNA-binding protein; NXP2, nuclear matrix protein 2; SLE, systemic lupus erythematosus; SRP, signal recognition particle; SSc, systemic sclerosis; TIF1γ, transcriptional intermediary factor 1 γ.

prevalent systemic autoimmune diseases, classified according to their corresponding ACR/EULAR (American College of Rheumatology/European Alliance of Associations for Rheumatology) criteria. These included 20 with systemic lupus erythematosus (SLE), 8 with systemic sclerosis (SSc), 5 with vasculitis, and 20 with Sjögren syndrome (Table).

Readily available myositis serum samples from patients who either fulfilled Llyod's criteria for IBM [13] or the National Institutes of Health (NIH) criteria for other types of autoantibody-positive myositis [1] were included in the study. Patients were classified as autoantibody-positive if they tested positive for autoantibodies against Mi2, TIF1γ, NXP2, MDA5, histidyl transfer RNA synthetase (Jo1), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), or signal recognition particle (SRP) using at least 1 of the following immunological methods: ELISA, *in vitro* transcription and translation followed by immunoprecipitation, line blotting (EUROIMMUN EUROLINE Autoimmune Inflammatory Myopathies 16 Ag [IgG] test kit), or immunoprecipitation from ^{35}S-methionine-labeled HeLa cell lysates. All serum samples were obtained from patients enrolled in institutional review board (IRB)-approved cohorts at the NIH in Bethesda, MD, as well as Vall d'Hebron and the Clinic Hospitals in Barcelona.

MSA discovery and epitope mapping by PhIP-Seq

The standard PhIP-Seq procedure [9,10] was employed to screen sera from 10 patients with anti-Mi2 autoantibody-positive myositis. The immunoglobulin (Ig) G concentration of each serum sample was quantified using an ELISA to normalize the IgG input (2 μg per reaction) into the subsequent PhIP-Seq assay. Serum antibodies were incubated overnight with the human peptidome library, which consists of 274,207 peptides of 90 amino acids in length. Following incubation, antibody and antibody-bound T7 phage were isolated using protein A- and protein G-coated Dynal beads (Invitrogen, #10002D and #10004D). The immunoprecipitated phage library underwent polymerase chain reaction (PCR) amplification with sample-specific DNA barcodes, and the resultant amplicons were then pooled and sequenced using an Illumina NextSeq instrument. Samples were then demultiplexed and aligned using an informatics pipeline as previously described [11,14].

Anti-AIRE and anti-Mi2β ELISA

ELISA plates (Falcon, #351172) were precoated overnight at 4°C with 100 ng of AIRE Human Recombinant Protein (OriGene, #TP313497M) per well diluted in 100 μL of 1 × phosphate-buffered saline (PBS). After coating, ELISA plates were washed with PBS-0.05% Tween (PBS-T) and blocked with 300 μL of 5% bovine serum albumin (BSA) in PBS-T for 1 hour at 37°C. Plates were then washed again with PBS-T. Diluted human serum samples (100 μL, 1:400 in 1% BSA/PBS-T) were added to each well and incubated for 1 hour at 37°C and then washed with PBS-T. Diluted HRP-labeled goat anti-human IgG antibody (100 μL, 1:10,000 in 1% BSA/PBS-T; Jackson ImmunoResearch Lab, #109-036-088) was added and incubated for 30 minutes at 37°C. After washing the plate with PBS-T followed by PBS, 100 μL of Sure Blue Peroxidase Substrate (SeraCare, #52-00-03) was added. Reactions were stopped with 100 μL of 1 N hydrochloric acid. The absorbance at 450 nm was determined, and test sample absorbances were normalised to the sera of an arbitrary positive control sample used as a reference in the anti-AIRE ELISA. The cutoff for determining AIRE positivity was defined as 2 SDs above the mean normalised absorbance of healthy control sera. We followed this procedure to develop an anti-Mi2β ELISA, adapting by precoating with 50 ng of Mi2β protein (ProSpec, #PRO-112) per well.

Anti-AIRE and anti-Mi2α LIPS

LIPS immunoassays were performed as described elsewhere [15–18]. AIRE or Mi2α autoantigen was fused to a light-emitting luciferase and then immunoprecipitated. Synthetic DNA encoding AIRE (NP_000374.1, amino acids 2-545) and Mi2α (NP_005843.2, amino acids 2-596) were obtained from Twist Biosciences. These DNAs contain *BamH1* and *Xho1* restriction sites for directional cloning into the pREN2 eukaryotic expression vector, resulting in a C-terminal *Renilla* luciferase fusion protein. Both pREN2-AIRE and pREN2-Mi2α constructs were sequenced to verify their integrity. These mammalian expression vectors were then transfected into Cos1 cells using Lipofectamine 3000 (Invitrogen). Crude lysates were harvested 48 and 24 hours after transfection for AIRE and Mi2α, respectively. For testing, serum samples from patients with myositis, healthy controls, and other autoimmune disease subjects were diluted in assay buffer A (20 mM Tris, pH 7.5, 150 mM NaCl, 5 mM $MgCl_2$, 1% Triton X-100) and incubated in a 96-well microtiter plate for 1 hour with 1×10^7 light units (LUs) per well with either *Renilla* luciferase-AIRE or -Mi2α. Next, the serum-antigen mixture was transferred to a microtiter filter plate (Millipore) containing Protein A/G UltraLink beads (Invitrogen) and incubated for another hour. The filter plates containing the immune complexes were then washed 8 times with buffer A and twice with 1 × PBS to remove unbound antigens. After the final wash, Coelenterazine substrate (Promega) was added to detect the amount of immunoprecipitated *Renilla* luciferase fusion proteins in LU using a Centro LB 960 Microplate Luminometer (Berthold Technologies). Given the broader dynamic range of LIPS compared with

ELISA, the cutoff for determining AIRE positivity was defined as the mean signal of healthy control sera plus 5 SDs.

Purification of MSAs from patient serum

Human IgG was purified and concentrated from anti-Mi2 autoantibody-positive (n = 3) and anti-MDA5 autoantibody-positive (n = 1) sera using Protein G Agarose (Millipore, #16-266) and the Amicon Pro Purification System (Millipore, #ACS500024) with a 30 kDa molecular weight cutoff Amicon Ultra Centrifugal Filter (Millipore, #UFC503024). After isolating IgG from anti-Mi2 autoantibody-positive serum samples, we used Mi2β-coated magnetic beads to affinity purify anti-Mi2β autoantibodies while also preserving the nonbound fraction for further analysis. NHS-Activated Magnetic Beads (Thermo Fisher Scientific, #88826) were coupled with Mi2β Recombinant Human Protein (ProSpec, #PRO-112) during an overnight incubation at 4°C with continuous mixing. The unbound ligand was removed, and the beads were washed with quench buffer (100 mM Tris-HCl, 150 M NaCl, pH 8.0). For affinity purification, total IgG from anti−Mi2-positive sera was added to the Mi2β-coupled beads, followed by incubation with continuous mixing for 2 hours at room temperature. After incubation, the Mi2β-coupled beads were placed on a magnetic rack to separate them from the solution. The supernatant containing unbound IgGs was collected, while the beads were washed to remove residual impurities. Bound IgGs were then eluted using 0.1 M glycine at pH 2.0, with the pH neutralised afterwards using 10% 1 M Tris at pH 8.0, yielding the Mi2β-enriched fraction. To generate the Mi2-depleted fraction, the collected supernatant was reincubated with fresh Mi2-coupled beads, and this process was repeated 3 times. This same procedure was performed with purified IgG from the anti-MDA5 autoantibody-positive serum sample using MDA5 protein (ProSpec, #PRO-1505)-coupled NHS-Activated Magnetic Beads to obtain MDA5-enriched and MDA5-depleted fractions. Using both the Mi2β and MDA5 fractions, we then performed anti-Mi2β, antihuman IgG (Thermo Fisher Scientific, #359722-004), and anti-AIRE ELISAs to assess immunoreactivity.

Statistical analysis

PhIP-Seq analysis was performed between anti-Mi2 autoantibody-positive patients and healthy controls using phipCC as previously described by Dr. Larman and colleagues [11,14]. Dichotomous variables were expressed as percentages and absolute frequencies. Pairwise comparisons for categorical variables between groups were performed using Fisher's exact test using the R programming language (R Foundation for Statistical Computing). Clustal Omega was used to assess the homology between protein sequences. A 2-sided *P* value of .05 or less was considered statistically significant with no adjustment for multiple comparisons.

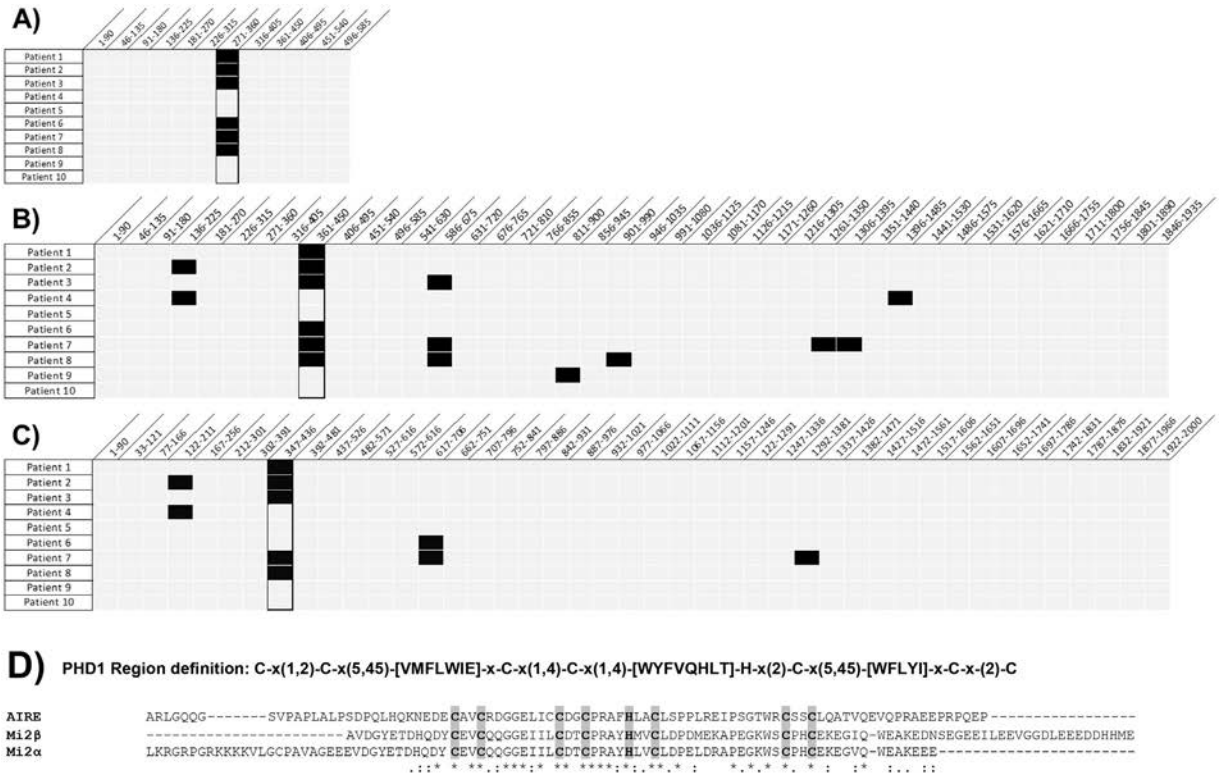


Figure 1. Identification of novel autoantibody specificities through phage immunoprecipitation sequencing (PhIP-Seq) analysis. (A) Antiautoimmune regulator (AIRE), (B) anti-Mi2β, and (C) anti-Mi2α reactivity as determined by PhIP-Seq from 10 patients with anti-Mi2 autoantibody-positive myositis. A−C, The peptide fragments of the protein, annotated by their starting and terminal amino acids, are arranged diagonally along the x-axis. Black squares show that the patient has autoantibodies against the corresponding peptide fragment, whereas grey squares indicate that the patient does not have autoantibodies against that peptide. Panel (A) highlights the immunogenic peptides of the AIRE protein, panel (B) showcases those of the Mi2β protein, and panel (C) displays the immunogenic peptides of the Mi2α protein. D, Sequence alignment of the immunodominant epitopes of AIRE (amino acids 271-360), Mi2β (amino acids 361-450), and Mi2α (amino acids 347-436) using Clustal Omega. The plant homeodomain zinc finger type I (PHD1) finger is characterised by a distinct motif featuring a combination of 4 cysteine residues, 1 histidine residue, and 3 additional cysteine residues, which are highlighted in grey. A detailed characterisation of PHD1 is provided and can also be found on PROSITE (https://prosite.expasy.org/PS01359).

RESULTS

Novel autoantibody reactivity against AIRE using PhIP-Seq

Among the 10 anti-Mi2 autoantibody-positive myositis samples tested with PhIP-Seq, 6 showed reactivity to AIRE (Fig 1A). This reactivity was specific to the AIRE peptide spanning amino acids 271 to 360 (Fig 1A). These same 6 patients who were positive for anti-AIRE also reacted to the Mi2β peptide spanning amino acids 361 to 450 (Fig 1B), and 5 of these patients also showed reactivity to the Mi2α peptide spanning amino acids 347 to 436 (Fig 1C). PhIP-Seq also detected reactivity to other Mi2 peptides, but these were found in 3 or fewer of the 10 anti-Mi2 autoantibody-positive patients. These immunodominant epitopes identified by PhIP-Seq in AIRE, Mi2β, and Mi2α corresponded to the PHD zinc finger type I (PHD1), which plays a crucial role in chromatin recognition and transcriptional regulation [19−22]. The immunodominant PHD1 epitopes of the 3 proteins revealed a high degree of similarity (Fig 1D).

Only patients with myositis with anti-Mi2 autoantibodies have anti-AIRE reactivity

We designed an ELISA with the full-length AIRE protein to evaluate AIRE reactivity in a validation cohort that consisted of serum samples from 26 anti-Mi2 autoantibody-positive patients, 44 patients with myositis with other MSAs or IBM, and 63 healthy comparators (Table). All anti-Mi2 patients who tested positive by 1 or more immunologic screening methods showed no reactivity to any other MSA and were subsequently confirmed by both anti-Mi2α LIPS and anti-Mi2β ELISA. Among the anti−Mi2-positive patients, all (25/25; 100%) were positive for anti-Mi2α, and 19 of 26 (73%) were also positive for anti-Mi2β.

Among these samples, autoantibodies recognising AIRE were present in 24/26 (92%) anti-Mi2 autoantibody-positive patients and 0/44 (0%) patients with anti-Mi2 autoantibody-negative myositis (P < 2.2e-16; Fig 2A). Only 1 (1.6%) of the 63 healthy control serum samples had anti-AIRE reactivity (P < 2.2e-16; Fig 2A).

To validate these data, we used a LIPS immunoassay with an AIRE antigen genetically fused to *Renilla* luciferase to measure autoantibody levels. We tested the same sera used in the ELISA, along with samples from 53 patients with different systemic autoimmune diseases. Among these samples, autoantibodies recognising AIRE were detected in all 26 (100%) of the anti-Mi2 autoantibody-positive patients and none (0%) of the anti-Mi2 autoantibody-negative myositis patients or healthy controls (P < 2.2e-16; Fig 2B). The 2 anti-Mi2 autoantibody-positive samples that were borderline negative for AIRE reactivity by ELISA were identified as positive by LIPS (Fig 2C), likely due to the broader dynamic range of LIPS. Additionally, only 1 (5%) of the 20 SLE sera showed reactivity against AIRE, whereas none (0%) of the sera from SSc (0/8), vasculitis (0/5), or Sjögren syndrome (0/20) patients exhibited AIRE reactivity (P < 2.2e-16; Fig 2B). The single healthy comparator who tested positive for anti-AIRE by ELISA tested negative for anti-AIRE using LIPS and anti-Mi2, suggesting a false positive (anti-Mi2 vs healthy comparators: P < 2.2e-16). Furthermore, the sole SLE patient who tested positive for anti-AIRE by LIPS did not show anti-Mi2α reactivity by LIPS.

We also compared the levels of AIRE-reactive autoantibodies measured by ELISA and LIPS immunoassays, which revealed a strong correlation between the 2 methods (Fig 2C). This underscores the reliability of both techniques in detecting AIRE reactivity.

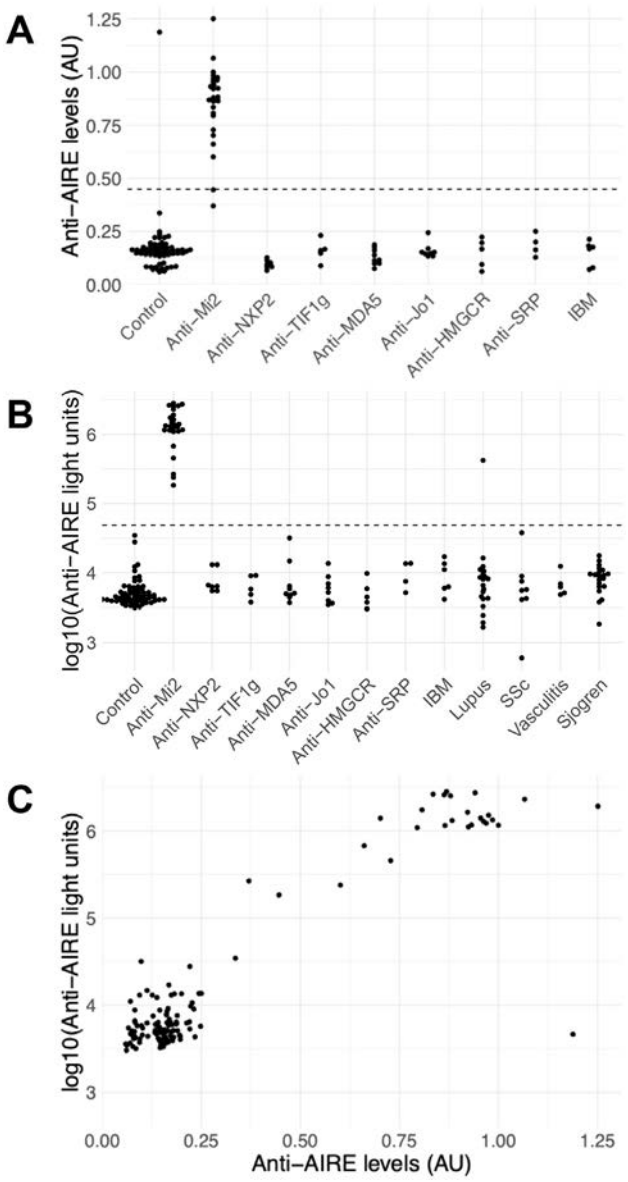


Figure 2. Detection of antiautoimmune regulator (AIRE) autoantibodies in healthy controls, myositis patients, and individuals with other systemic autoimmune diseases. Anti-AIRE autoantibody levels were measured using (A) enzyme-linked immunosorbent assay (ELISA) (measured in arbitrary units [AUs]) and (B) luciferase immunoprecipitation system (LIPS) (measured in light units) in sera from healthy controls, patients with myositis, and patients with other systemic autoimmune diseases (the latter only for LIPS). The dashed line indicates the cutoff for defining anti-AIRE positivity, with the cutoff set at 2 SDs for the anti-AIRE ELISA and 5 SDs for the anti-AIRE LIPS immunoassay. C, The correlation of anti-AIRE levels measured by ELISA and LIPS in 26 anti-Mi2 autoantibody-positive serum samples, 44 samples with other myositis-specific autoantibodies or inclusion body myositis (IBM), and 63 healthy controls by both ELISA (x-axis) and LIPS (y-axis). HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; Jo1, histidyl transfer RNA synthetase; MDA5, melanoma differentiation-associated protein 5; Mi2, chromodomain helicase DNA-binding protein; NXP2, nuclear matrix protein 2; SRP, signal recognition particle; SSc, systemic sclerosis; TIF1g, transcriptional intermediary factor 1 γ.

Anti-Mi2 autoantibodies also recognise AIRE

Due to the specificity of anti-AIRE reactivity in sera containing anti-Mi2 autoantibodies and the similarity in the immunodominant epitopes between AIRE and Mi2 proteins, we then

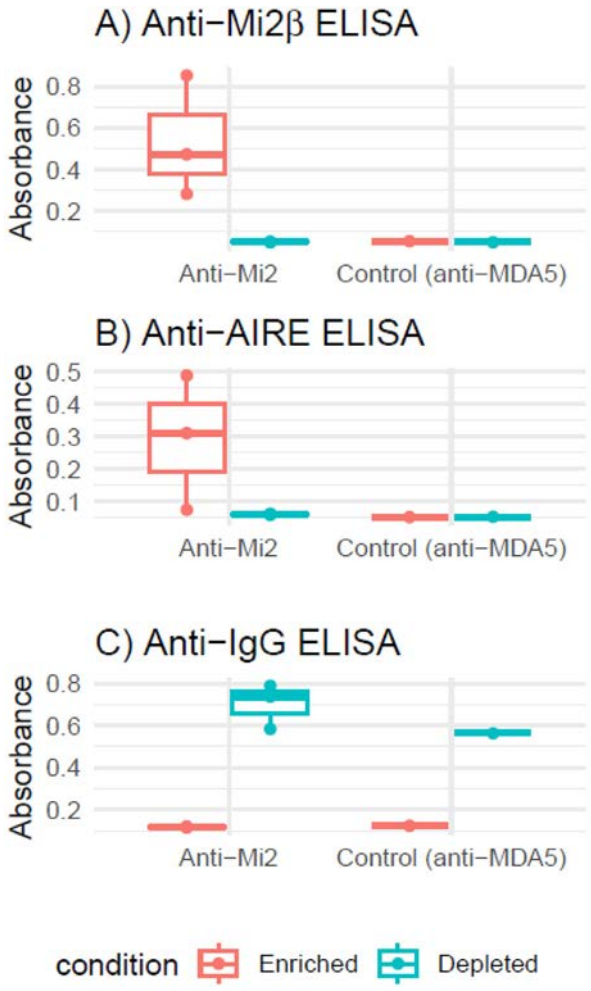


Figure 3. Detection of antiautoimmune regulator (AIRE) reactivity in affinity-purified anti-Mi2 autoantibody fractions. Purified immunoglobulin G (IgG) of anti-Mi2 autoantibody-positive sera (n = 3) underwent affinity purification using Mi2β-bound magnetic beads, which produced an Mi2β-enriched and Mi2β-depleted fraction. As a control, purified IgG of 1 antimelanoma differentiation-associated protein 5 (MDA5) autoantibody-positive serum sample underwent affinity purification using MDA5-bound magnetic beads, which produced MDA5-enriched and MDA5-depleted fractions. These fractions were subjected to an enzyme-linked immunosorbent assay (ELISA) to detect immunoreactivity against (A) Mi2β and (B) AIRE. C, The total concentration of immunoglobulin was determined by ELISA in the 2 sets of samples.

explored whether anti-Mi2 autoantibodies target both AIRE and Mi2β. To this end, we affinity-purified anti-Mi2 autoantibodies using full-length Mi2β protein conjugated to magnetic beads from 3 serum samples that were positive for both anti-Mi2 and anti-AIRE autoantibodies by ELISA. We also collected the non-bound antibodies from this purification process to obtain the anti−Mi2-depleted fraction. We detected AIRE recognition only in the anti−Mi2-enriched fractions, whereas depletion of anti-Mi2 autoantibodies resulted in a loss of AIRE reactivity (Fig 3). These results demonstrate that autoantibodies that target Mi2β also recognise AIRE.

Anti-AIRE levels do not correlate with anti-Mi2 levels

Given that nearly all anti-Mi2 autoantibody-positive patients were also anti−AIRE-positive, it was not feasible to conduct a comparative analysis of the clinical characteristics between anti−AIRE-positive and anti−AIRE-negative patients within anti-Mi2 autoantibody-positive myositis. However, we evaluated the association between anti-AIRE and anti-Mi2β levels within the 26 anti-Mi2 DM patient sera in our validation cohort. We observed a poor correlation between anti-AIRE and anti-Mi2β levels, as determined by ELISA (Fig 4A). As noted earlier, anti-Mi2 autoantibodies can also recognise Mi2α (ie, CHD3). Therefore, we employed LIPS immunoassays to assess whether anti-AIRE levels correlate with anti-Mi2α levels. We also observed a weak correlation between anti-AIRE and anti-Mi2α levels by LIPS (Fig 4B).

DISCUSSION

In this study, we used PhIP-Seq to identify novel autoantibodies in the serum of patients with DM with anti-Mi2 autoantibodies. We found that sera from individuals with autoantibodies against Mi2 also recognise the AIRE protein. Importantly, PhIP-Seq allowed us to identify PHD1 as a shared immunodominant epitope of the AIRE and Mi2 autoantigens. This finding is consistent with a previous study defining the immunogenic regions of Mi2 [23]. We also demonstrated that affinity-purified anti-Mi2 autoantibodies can recognise AIRE. When these autoantibodies are removed from the total Ig fraction, AIRE reactivity is lost. This highlights that the same autoantibodies bind both Mi2 and AIRE at PHD1. PHD1 is distinguished by a unique motif consisting of 4 cysteine residues, 1 histidine residue, and 3 additional cysteine residues. This region is critical for both AIRE and Mi2 to recognise histone modifications and regulate target gene expression by coordinating with transcriptional and chromatin-remodelling machinery [19−22,24].

AIRE is a pivotal transcriptional regulator that maintains central immune tolerance by controlling the expression of tissue-restricted self-antigens in the thymus [22,25]. This is crucial for eliminating autoreactive T cells or redirecting them towards a regulatory T cell lineage [22,25]. AIRE's role also extends beyond the thymus [26,27]. Peripheral AIRE is mainly expressed in 'extra-thymic AIRE-expressing cells', which can also demonstrate antigen-presenting capabilities and are hypothesised to assist in peripheral immune tolerance [28−35].

Mutations in *AIRE* lead to a rare monogenic disorder called autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), and mono-allelic mutations in PHD1 of AIRE have been found to associate with a broader range of autoimmune phenotypes [36−41]. Moreover, mutations in PHD1 of *AIRE* have been shown to significantly reduce the expression of AIRE-dependent genes and impair AIRE's binding to its promoters in the thymus [19,20,38,41]. This highlights PHD1 as a critical domain of AIRE's function. It is intriguing to consider the possibility that anti-Mi2 autoantibodies could enter thymic cells, bind to PHD1 of AIRE, and promote autoimmunity by disrupting AIRE's role in regulating tissue-restricted antigen expression. While we found no clinical reports indicating APECED-related manifestations in either paediatric [42] or adult [5] populations of anti-Mi2 autoantibody-positive patients, we cannot exclude the possibility that autoantibody-mediated thymic dysfunction contributes to autoimmunity in anti−Mi2-positive DM. However, exploring this mechanism further would require studying thymic tissue from patients with anti−Mi2-positive DM, and such samples are not currently available.

We, and more recently others [43], have reported that MSAs can be internalised by muscle cells and may interfere with the function of their target autoantigens. Similar mechanisms have also been proposed in other autoimmune diseases [44−48]. In muscle biopsies from anti-Mi2 autoantibody-positive patients,

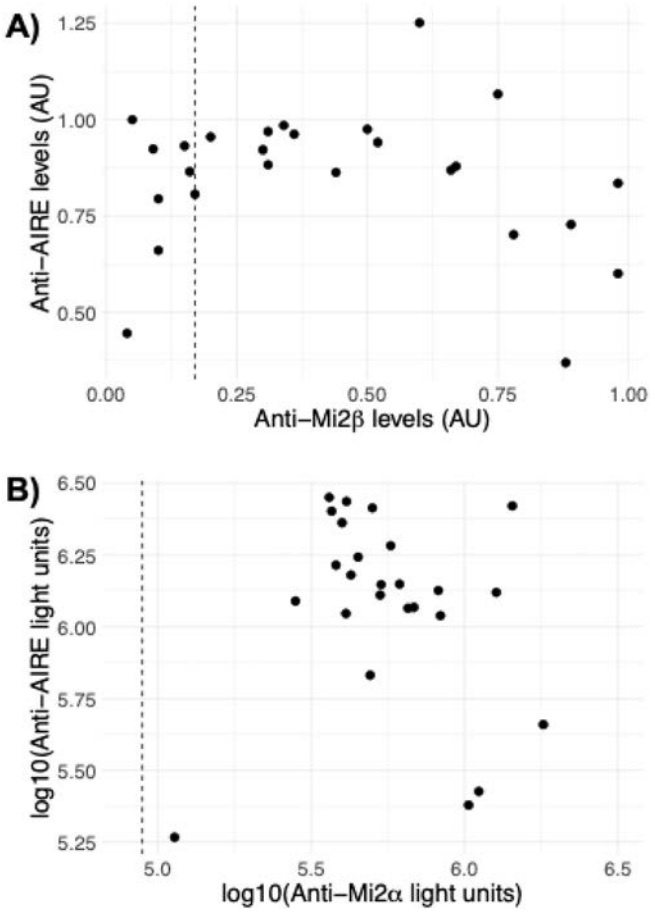


Figure 4. Correlation between antiautoimmune regulator (AIRE) and anti-Mi2 autoantibody reactivity levels by enzyme-linked immunosorbent assay (ELISA) and luciferase immunoprecipitation system (LIPS). A, Reactivity levels measured by ELISA of anti-AIRE and anti-Mi2β autoantibodies and (B) reactivity levels measured by LIPS of anti-AIRE and anti-Mi2α autoantibodies. Vertical dashed lines indicate the cutoff for defining positivity for each Mi2 assay. Due to limited serum availability, only 25 of the 26 anti-Mi2 samples were tested for anti-Mi2α by LIPS. AU, arbitrary units.

we observed nuclear localisation of IgG and gene expression patterns consistent with Mi2/NuRD complex disruption [6,7]. Given that PHD fingers mediate histone tail binding, which is critical for Mi2/NuRD function [24], our current findings raise the possibility that anti-Mi2 autoantibodies impair chromatin binding by targeting PHD1, leading to derepression of normally silenced genes in muscle cells. Further studies are needed to directly test this model.

In addition to Mi2 and AIRE, more than 70 human proteins contain a PHD domain, many of which are transcription factors that regulate the expression of other transcriptional regulators [49]. In a separate study, we investigated whether anti-Mi2 autoantibodies also recognise other PHD-containing proteins. We found robust evidence that, similar to AIRE, these autoantibodies also bind the PHD domain of SP140L and the PHD-containing region of TRIM33 (also known as TIF1γ), both of which are key transcriptional regulators [50]. Previously, we showed that 54% of anti−Mi2-specific genes in affected muscle biopsies are associated with Mi2/NuRD complex function [6]. Our data suggest that the remaining 46% may be driven by dysregulation of other PHD-containing proteins targeted by anti-Mi2 autoantibodies. Dissecting the individual contributions of each of these targets, however, presents a considerable challenge given their number and functional complexity.

The discovery that anti-Mi2 autoantibodies also recognise AIRE suggests that testing for anti-AIRE reactivity may provide an improved detection method, especially for patients who test borderline negative in assays utilising Mi2 protein. The lack of a correlation between anti-Mi2 and anti-AIRE antibody levels may result from autoantibodies targeting regions of Mi2 outside PHD1 (Fig 1). Therefore, detecting anti-AIRE autoantibodies in an anti-Mi2 autoantibody-positive patient can confirm that these autoantibodies are targeting the functionally relevant PHD1 of Mi2, as this is the only shared epitope between both proteins. Alternatively, functional assays [7] or assays specifically designed to target PHD1 [50] may provide a more physiologically relevant detection compared with currently available methods. However, larger studies, including those addressing false positives in anti-Mi2 results, are needed to robustly support these findings.

Our study has several limitations. Firstly, although the sample size and control groups were sufficient to demonstrate a strong association between anti-Mi2 and anti-AIRE autoantibodies, some subgroups remained relatively small. Secondly, although PhIP-Seq is a powerful tool for autoantibody discovery, it lacks sensitivity [10], as illustrated by the fact that some patients with anti-Mi2 autoantibodies tested negative using this method. Finally, although the cross-reactivity between AIRE and Mi2 was demonstrated in a limited number of samples, the PhIP-Seq data support the presence of a shared epitope between these proteins. In a separate study, we also found evidence that anti-Mi2 autoantibodies specifically recognise other PHD-containing proteins, such as SP140L and TRIM33, which further validates the existence of a shared epitope [50].

In summary, we found that anti-Mi2 autoantibodies target the PHD domains of multiple proteins, including Mi2α, Mi2β, and AIRE. These findings suggest that anti-Mi2 autoantibodies disrupt the function of the Mi2/NuRD complex by interfering with its chromatin-binding capacity via the PHD domain. Given the key role of the PHD finger in regulating global transcriptional factors, further investigation is needed to understand how autoantibodies targeting this region affect PHD-containing proteins, such as AIRE. Exploring the muscle-specific and systemic effects of this reactivity could reveal how the dysregulation of target autoantigens, such as Mi2 and AIRE, interacts to drive the development of myositis and other autoantibody-mediated diseases.

CONTRIBUTORS

All authors contributed to the development of the manuscript, including interpretation of results, substantive review of drafts, and approval of the final draft for submission.

FUNDING

This study was funded, in part, by the Intramural Research Program of the National Institute of Dental and Craniofacial Research, the National Institute of Environmental Health Sciences, and the National Institute of Arthritis and Musculoskeletal and Skin Diseases, all part of the National Institutes of Health.

Competing interests

HBL is a founder of Infinity Bio, which provides antibody reactome profiling services. All other authors declare that they have nothing to declare.

Patient consent for publication

Patients and/or the public were not involved in the design, conduct, reporting, or dissemination plans of this research. Written informed consent was obtained from each participant.

Ethics approval

This study was approved by the Institutional Review Boards (IRBs) at the National Institutes of Health, Vall d'Hebron, and Clinic Hospitals in Barcelona.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Any anonymised data not published within the article will be shared upon request from any investigator.

Orcid

Jon Musai: http://orcid.org/0000-0002-6541-4998
Sahana Jayaraman: http://orcid.org/0000-0003-3413-2530
Iago Pinal-Fernandez: http://orcid.org/0000-0001-6338-9218
Eric Cho: http://orcid.org/0009-0008-8185-8688
Fa'alataitaua M. Fitisemanu: http://orcid.org/0000-0002-9449-1327
Peter D. Burbelo: http://orcid.org/0000-0003-1717-048X
Blake M. Warner: http://orcid.org/0000-0002-4961-018X
Albert Selva-O'Callaghan: http://orcid.org/0000-0003-2823-9761
José César Milisenda: http://orcid.org/0000-0003-0151-7872
Andrew L. Mammen: http://orcid.org/0000-0003-3732-3252

REFERENCES

[1] Casal-Dominguez M, Pinal-Fernandez I, Pak K, Huang W, Selva-O'Callaghan A, Albayda J, et al. Performance of the 2017 European Alliance of Associations for Rheumatology/American College of Rheumatology classification criteria for idiopathic inflammatory myopathies in patients with myositis-specific autoantibodies. Arthritis Rheumatol 2022;74(3):508–17.
[2] Selva-O'Callaghan A, Pinal-Fernandez I, Trallero-Araguas E, Milisenda JC, Grau-Junyent JM, Mammen AL. Classification and management of adult inflammatory myopathies. Lancet Neurol 2018;17(9):816–28.
[3] Trallero-Araguas E, Rodrigo-Pendas JA, Selva-O'Callaghan A, Martinez-Gomez X, Bosch X, Labrador-Horrillo M, et al. Usefulness of anti-p155 autoantibody for diagnosing cancer-associated dermatomyositis: a systematic review and meta-analysis. Arthritis Rheum 2012;64(2):523–32.
[4] Sato S, Hoshino K, Satoh T, Fujita T, Kawakami Y, Fujita T, et al. RNA helicase encoded by melanoma differentiation-associated gene 5 is a major autoantigen in patients with clinically amyopathic dermatomyositis: association with rapidly progressive interstitial lung disease. Arthritis Rheum 2009;60(7):2193–200.
[5] Pinal-Fernandez I, Mecoli CA, Casal-Dominguez M, Pak K, Hosono Y, Huapaya J, et al. More prominent muscle involvement in patients with dermatomyositis with anti-Mi2 autoantibodies. Neurology 2019;93(19):e1768–77.
[6] Pinal-Fernandez I, Milisenda JC, Pak K, Munoz-Braceras S, Casal-Dominguez M, Torres-Ruiz J, et al. Transcriptional derepression of CHD4/NuRD-regulated genes in the muscle of patients with dermatomyositis and anti-Mi2 autoantibodies. Ann Rheum Dis 2023;82(8):1091–7.
[7] Pinal-Fernandez I, Munoz-Braceras S, Casal-Dominguez M, Pak K, Torres-Ruiz J, Musai J, et al. Pathological autoantibody internalisation in myositis. Ann Rheum Dis 2024;83(11):1549–60.
[8] Pinal-Fernandez I, Casal-Dominguez M, Derfoul A, Pak K, Miller FW, Milisenda JC, et al. Machine learning algorithms reveal unique gene expression profiles in muscle biopsies from patients with different types of myositis. Ann Rheum Dis 2020;79(9):1234–42.
[9] Larman HB, Zhao Z, Laserson U, Li MZ, Ciccia A, Gakidis MA, et al. Autoantigen discovery with a synthetic human peptidome. Nat Biotechnol 2011;29(6):535–41.
[10] Larman HB, Laserson U, Querol L, Verhaeghen K, Solimini NL, Xu GJ, et al. PhIP-Seq characterization of autoantibodies from patients with multiple sclerosis, type 1 diabetes and rheumatoid arthritis. J Autoimmun 2013;43:1–9.
[11] Angkeow JW, Monaco DR, Chen A, Venkataraman T, Jayaraman S, Valencia C, et al. Phage display of environmental protein toxins and virulence factors reveals the prevalence, persistence, and genetics of antibody responses. Immunity 2022;55(6) 1051-66.e4.
[12] Venkataraman T, Valencia C, Mangino M, Morgenlander W, Clipman SJ, Liechti T, et al. Analysis of antibody binding specificities in twin and SNP-genotyped cohorts reveals that antiviral antibody epitope selection is a heritable trait. Immunity 2022;55(1) 174-84.e5.
[13] Lloyd TE, Mammen AL, Amato AA, Weiss MD, Needham M, Greenberg SA. Evaluation and construction of diagnostic criteria for inclusion body myositis. Neurology 2014;83(5):426–33.
[14] Morgenlander WR, Chia WN, Parra B, Monaco DR, Ragan I, Pardo CA, et al. Precision arbovirus serology with a pan-arbovirus peptidome. Nat Commun 2024;15(1):5833.
[15] Burbelo PD, Ching KH, Klimavicz CM, Iadarola MJ. Antibody profiling by Luciferase immunoprecipitation systems (LIPS). J Vis Exp 2009(32):1549.
[16] Burbelo PD, Riedo FX, Morishima C, Rawlings S, Smith D, Das S, et al. Sensitivity in detection of antibodies to nucleocapsid and spike proteins of severe acute respiratory syndrome coronavirus 2 in patients with coronavirus disease 2019. J Infect Dis 2020;222(2):206–13.
[17] Burbelo PD, Iadarola MJ, Keller JM, Warner BM. Autoantibodies targeting intracellular and extracellular proteins in autoimmunity. Front Immunol 2021;12:548469.
[18] Burbelo PD, Huapaya JA, Khavandgar Z, Beach M, Pinal-Fernandez I, Mammen AL, et al. Quantification of autoantibodies using a luminescent profiling method in autoimmune interstitial lung disease. Front Immunol 2024;15:1462242.
[19] Koh AS, Kuo AJ, Park SY, Cheung P, Abramson J, Bua D, et al. Aire employs a histone-binding module to mediate immunological tolerance, linking chromatin regulation with organ-specific autoimmunity. Proc Natl Acad Sci U S A 2008;105(41):15878–83.
[20] Org T, Chignola F, Hetenyi C, Gaetani M, Rebane A, Liiv I, et al. The autoimmune regulator PHD finger binds to non-methylated histone H3K4 to activate gene expression. EMBO Rep 2008;9(4):370–6.
[21] Zhong Y, Moghaddas Sani H, Paudel BP, Low JKK, Silva APG, Mueller S, et al. The role of auxiliary domains in modulating CHD4 activity suggests mechanistic commonality between enzyme families. Nat Commun 2022;13(1):7524.
[22] Peterson P, Org T, Rebane A. Transcriptional regulation by AIRE: molecular mechanisms of central tolerance. Nat Rev Immunol 2008;8(12):948–57.
[23] Ge Q, Nilasena DS, O'Brien CA, Frank MB, Targoff IN. Molecular analysis of a major antigenic region of the 240-kD protein of Mi-2 autoantigen. J Clin Invest 1995;96(4):1730–7.
[24] Musselman CA, Ramirez J, Sims JK, Mansfield RE, Oliver SS, Denu JM, et al. Bivalent recognition of nucleosomes by the tandem PHD fingers of the CHD4 ATPase is required for CHD4-mediated repression. Proc Natl Acad Sci U S A. 2012;109(3):787–92.
[25] Anderson MS, Venanzi ES, Chen Z, Berzins SP, Benoist C, Mathis D. The cellular mechanism of Aire control of T cell tolerance. Immunity 2005;23(2):227–39.
[26] Zhao B, Chang L, Fu H, Sun G, Yang W. The role of autoimmune regulator (AIRE) in peripheral tolerance. J Immunol Res 2018;2018:3930750.
[27] Anderson MS, Su MA. AIRE expands: new roles in immune tolerance and beyond. Nat Rev Immunol 2016;16(4):247–58.
[28] Huo F, Li D, Zhao B, Luo Y, Zhao B, Zou X, et al. Deficiency of autoimmune regulator impairs the immune tolerance effect of bone marrow-derived dendritic cells in mice. Autoimmunity 2018;51(1):10–7.
[29] Gardner JM, Devoss JJ, Friedman RS, Wong DJ, Tan YX, Zhou X, et al. Deletional tolerance mediated by extrathymic Aire-expressing cells. Science 2008;321(5890):843–7.
[30] Zhu W, Yang W, He Z, Liao X, Wu J, Sun J, et al. Overexpressing autoimmune regulator regulates the expression of toll-like receptors by interacting with their promoters in RAW264.7 cells. Cell Immunol 2011;270(2):156–63.
[31] Lindh E, Lind SM, Lindmark E, Hassler S, Perheentupa J, Peltonen L, et al. AIRE regulates T-cell-independent B-cell responses through BAFF. Proc Natl Acad Sci U S A. 2008;105(47):18466–71.
[32] Lindmark E, Chen Y, Georgoudaki AM, Dudziak D, Lindh E, Adams WC, et al. AIRE expressing marginal zone dendritic cells balances adaptive immunity and T-follicular helper cell recruitment. J Autoimmun 2013;42:62–70.

[33] Yamano T, Dobes J, Voboril M, Steinert M, Brabec T, Zietara N, et al. Aire-expressing ILC3-like cells in the lymph node display potent APC features. J Exp Med 2019;216(5):1027–37.
[34] Dobes J, Ben-Nun O, Binyamin A, Stoler-Barak L, Oftedal BE, Goldfarb Y, et al. Extrathymic expression of Aire controls the induction of effective T(H) 17 cell-mediated immune response to *Candida albicans*. Nat Immunol 2022;23(7):1098–108.
[35] Lee JW, Epardaud M, Sun J, Becker JE, Cheng AC, Yonekura AR, et al. Peripheral antigen display by lymph node stroma promotes T cell tolerance to intestinal self. Nat Immunol 2007;8(2):181–90.
[36] Peterson P, Nagamine K, Scott H, Heino M, Kudoh J, Shimizu N, et al. APECED: a monogenic autoimmune disease providing new clues to self-tolerance. Immunol Today 1998;19(9):384–6.
[37] Saugier-Veber P, Drouot N, Wolf LM, Kuhn JM, Frebourg T, Lefebvre H. Identification of a novel mutation in the autoimmune regulator (AIRE-1) gene in a French family with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. Eur J Endocrinol 2001;144(4):347–51.
[38] Oftedal BE, Hellesen A, Erichsen MM, Bratland E, Vardi A, Perheentupa J, et al. Dominant mutations in the autoimmune regulator AIRE are associated with common organ-specific autoimmune diseases. Immunity 2015;42 (6):1185–96.
[39] Halonen M, Kangas H, Ruppell T, Ilmarinen T, Ollila J, Kolmer M, et al. APECED-causing mutations in AIRE reveal the functional domains of the protein. Hum Mutat 2004;23(3):245–57.
[40] Finnish-German APECED Consortium. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. Nat Genet 1997;17(4):399–403.
[41] Oftedal BE, Assing K, Baris S, Safgren SL, Johansen IS, Jakobsen MA, et al. Dominant-negative heterozygous mutations in AIRE confer diverse autoimmune phenotypes. iScience 2023;26(6):106818.
[42] Rider LG, Shah M, Mamyrova G, Huber AM, Rice MM, Targoff IN, et al. The myositis autoantibody phenotypes of the juvenile idiopathic inflammatory myopathies. Medicine (Baltimore) 2013;92(4):223–43.
[43] Giannini M, Quiring G, Oulad-Abdelghani M, Lannes B, Allenbach Y, Benveniste O, et al. In immune-mediated necrotising myopathy, anti-HMGCR antibodies inhibit HMGCR activity, leading to the sarcoplasmic accumulation of lipid droplets and myofibres necrosis. Ann Rheum Dis 2025 in press. doi: 10.1016/j.ard.2025.04.027.
[44] Padgett RA, Mount SM, Steitz JA, Sharp PA. Splicing of messenger RNA precursors is inhibited by antisera to small nuclear ribonucleoprotein. Cell 1983;35(1):101–7.
[45] Alarcon-Segovia D, Ruiz-Arguelles A, Fishbein E. Antibody to nuclear ribonucleoprotein penetrates live human mononuclear cells through Fc receptors. Nature 1978;271(5640):67–9.
[46] Rivadeneyra-Espinoza L, Ruiz-Arguelles A. Cell-penetrating anti-native DNA antibodies trigger apoptosis through both the neglect and programmed pathways. J Autoimmun 2006;26(1):52–6.
[47] Malmborg AC, Shultz DB, Luton F, Mostov KE, Richly E, Leung PS, et al. Penetration and co-localization in MDCK cell mitochondria of IgA derived from patients with primary biliary cirrhosis. J Autoimmun 1998;11(5):573–80.
[48] Chen X, Tang X, Xie Y, Cuffari BJ, Tang C, Cao F, et al. A lupus-derived autoantibody that binds to intracellular RNA activates cGAS-mediated tumor immunity and can deliver RNA into cells. Sci Signal 2025;18(879):eadk3320.
[49] Jain K, Fraser CS, Marunde MR, Parker MM, Sagum C, Burg JM, et al. Characterization of the plant homeodomain (PHD) reader family for their histone tail interactions. Epigenetics Chromatin 2020;13(1):3.
[50] Pinal-Fernandez I, Musai J, Casal-Dominguez M, Pak K, Kaplan M, Warner BM, et al. Anti-Mi2 autoantibodies target the PHD fingers of SP140L and TIF1γ, while anti-TIF1γ autoantibodies primarily recognize epitopes outside the PHD region of TIF1γ. Rheumatology (Oxford) 2025:keaf426.

Ann Rheum Dis 85 (2026) 360–369

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Myositis

Autoreactive T cells identified in patients with anti-Jo1 + antisynthetase syndrome recognise a new epitope on histidyl t-RNA synthetase

Angeles S. Galindo-Feria[1,2,3], Ravi Kumar Sharma[1,2,4], Anatoly Dubnovitsky[1,2], Christina Gerstner[1,2], Genadiy Kozhukh[1], Annika Van Vollenhoven[1,2], Juan Sebastian Diaz Boada[1], Daniel Ramsköld[5], Adnane Achour[6,7], Maryam Dastmalchi[2,3], Hugh H. Reid[8], Tatyana Sandalova[6,7], Jamie Rossjohn[8,9], Karine Chemin[1,2], Vivianne Malmström[1,2], Ingrid E. Lundberg[1,2,3], Begum Horuluoglu[1,2,*]

[1] *Division of Rheumatology, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden*
[2] *Center for Molecular Medicine, Stockholm, Sweden*
[3] *Department of Gastro, Dermatology and Rheumatology, Karolinska University Hospital, Stockholm, Sweden*
[4] *Department of Clinical Immunology and Rheumatology, All India Institute of Medical Sciences, Bilaspur, (H.P), India 174001*
[5] *Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden*
[6] *Division of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden*
[7] *Science for Life Laboratory, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden*
[8] *Infection and Immunity Program and Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia*
[9] *Institute of Infection and Immunity, Cardiff University, School of Medicine, Heath Park, Cardiff, UK*

ARTICLE INFO

Article history:
Received 1 August 2025
Received in revised form 26 September 2025
Accepted 28 September 2025

ABSTRACT

Objectives: Anti-Jo1 + antisynthetase syndrome (ASyS) is characterised by autoantibodies targeting histidyl t-RNA synthetase (HisRS), association with *HLA-DRB1*03:01* and a distinct clinical phenotype including interstitial lung disease, myositis, arthritis, and mechanic's hands. Previous studies of autoreactive HisRS-specific $CD4^+$T cells point to yet undiscovered T cell epitopes. We aimed to identify new epitopes on HisRS to investigate the presence of autoreactive T cells and their corresponding T-cell receptor (TCR) repertoire from patients with ASyS.

Methods: Peptides from HisRS N-terminal region with appropriate major histocompatibility complex (MHC) anchor residues were selected for *in vitro* binding assays. The peptide ($HisRS_{41-55}$) with the highest HLA-DRB1*03:01 binding affinity was selected for studies with HLA-class II tetramers. Peripheral blood mononuclear cells (PBMCs) from patients with ASyS with *HLA-DRB1*03:01* (n = 12) were stimulated *in vitro* with peptide and peptide-HLA-DRB1*03:01 tetramers were used to detect $HisRS^+CD4^+$T cells. Single TCR sequencing of captured T cells allowed analyses of the underlying TCR repertoire.

Results: We identified a new T cell epitope on HisRS with high affinity for HLA-DRB1*03:01. Autoreactive $HisRS^+CD4^+$T cells were detected in PBMCs of patients (n = 6/12). TCR repertoire analysis of $HisRS^+CD4^+$T cells revealed shared gene V-alpha and beta usages. Moreover,

*Correspondence to Dr. Begum Horuluoglu, Karolinska Institute, Stockholm, Sweden.
E-mail address: begum.horuluoglu@ki.se (B. Horuluoglu).
Vivianne Malmström and Ingrid E. Lundberg contributed equally.
Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.09.015

HisRS$^+$CD4$^+$T cells persisted after treatment in 2 patients (P2 and P4) and 2 identical T cell clones were detected between the initial and follow-up time points in 1 patient (P2).
Conclusions: Autoreactive T-cells targeting a new HisRS epitope were identified indicating T cell reactivity to diverse epitopes of the HisRS protein in patients with anti-Jo1 autoantibodies. Furthermore, we demonstrated the TCR repertoire of autoreactive HisRS + CD4 + T cells in patients. Persistence of these T-cells and specific clones may be contributing to disease.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Association of *HLA-DRB1*03:01* with antisynthetase syndrome (ASyS) indicates the contribution of CD4$^+$T cells in disease.
- CD4$^+$T cells from peripheral blood mononuclear cell and bronchoalveolar lavage fluid cells from patients with ASyS are activated upon stimulation with the full-length histidyl t-RNA synthetase (HisRS) and HisRS-derived peptide indicating the presence of HisRS-reactive T cells.
- Shared T cell clones are found between muscle and blood with a cytotoxic phenotype. These clonally expanded T cells persist with a similar phenotype in a patient with ASyS despite treatment.

WHAT THIS STUDY ADDS

- A new T-cell epitope identified on HisRS that has a high affinity to bind HLA-DRB1*03:01.
- Autoreactive HisRS$^+$CD4$^+$T cells were identified using HLA-class II tetramers in 6 patients with ASyS upon *in vitro* stimulation.
- T-cell receptor (TCR) repertoire analysis of autoreactive T cells showed shared alpha or beta chain CDR3 amino acid sequences and V gene usages for both alpha and beta chains among patients with ASyS.
- Persistence of antigen-specific T cells and identical T cell clones were detected after up to 12 months with immunosuppressive treatment which might contribute to disease chronicity.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Presence of autoreactive T cells could be used as a tool to follow the effects of conventional treatments.
- Identification of antigen-specific T cells has implications for future development of therapies including tolerising and targeting pathogenic TCR signatures.

INTRODUCTION

Antisynthetase syndrome (ASyS) is a subgroup of idiopathic inflammatory myopathies characterised by the presence of autoantibodies against aminoacyl t-RNA synthetases, myositis, and extramuscular features such as interstitial lung disease (ILD), and is associated with high morbidity and mortality [1–3]. The most frequent anti-tRNA synthetase autoantibodies are the anti-Jo-1 autoantibodies targeting histidyl-transfer RNA synthetase (HisRS), and there is a genetic association with *HLA-DRB1*03:01* implicating the recognition of autoantigens by CD4$^+$ T cells [4,5]. T cells have been reported at the sites of inflammation in the muscle and lungs of patients with ASyS where they are proposed to contribute to disease pathogenesis [6–8].

HisRS, which is a cytoplasmic enzyme involved in protein translation, may trigger innate and adaptive immune responses leading to cell-mediated damage in tissues such as muscle and lung [9,10]. Our group has shown that CD4$^+$T cells from peripheral blood mononuclear cells (PBMCs) and bronchoalveolar lavage fluid (BALF) from patients with anti-Jo-1$^+$ ASyS with the *HLA-DRB1*03:01* haplotype were activated upon stimulation with HisRS protein. Increased levels of CD40L were detected on CD4$^+$T cells, along with enhanced interferon γ (IFNγ) production, suggesting the presence of T cells recognising the HisRS protein [11]. Interestingly, while almost all patients' T cells responded to the full-length HisRS protein, not all responded to stimulation with the peptide used for T cell stimulation ($HisRS_{11\text{-}23}$) suggesting that multiple epitopes may contribute to the T cell response. These findings highlight the potential for epitope spreading and the importance of identifying new epitopes on HisRS.

The recombination of T-cell receptor (TCR) gene segments is the basis for the generation of a diverse T cell repertoire where each T cell expresses a unique TCR sequence. Restricted TCR gene usage in the lungs and muscles of patients with myositis has been shown suggesting antigen-induced T cell responses in these organs [12]. More recently, we showed using single-cell RNA sequencing the presence of clonally expanded cytotoxic CD4$^+$T cell clones shared between muscle and blood of a patient with anti-Jo1 + ASyS suggesting the possibility to detect pathogenic T cells in blood [13]. These clonally expanded cytotoxic CD4$^+$ T cells persisted with the same phenotype in both blood and muscle after 9 months of conventional immunosuppressives treatment, further supporting the contribution of CD4$^+$T cells to disease chronicity [13].

In this study, we aimed to identify autoreactive HisRS-specific CD4$^+$T cells in the peripheral blood of patients with ASyS using HLA-class II tetramers loaded with a HisRS peptide, to detect low-frequency antigen-specific CD4$^+$T cells with high efficiency. We further performed single TCR sequencing to study the TCR repertoire of autoreactive HisRS-specific T cells and investigated their persistence at different disease stages.

METHODS

Patients and healthy controls

Peripheral blood samples from 2 cohorts (n = 33 in total) with anti-Jo1 autoantibodies were collected from Karolinska University Hospital Rheumatology Clinic at the time of diagnosis and at annual follow-up between 2014 and 2023. The diagnosis of ASyS was based on the presence of anti-Jo1 autoantibodies in addition to one of the following features: ILD, myositis, arthritis, Raynaud's phenomenon, fever, or mechanic's hands [14]. First cohort consisted of 19 patients who were included in peptide stimulation assays, whereas the second cohort with 14 patients was included for the HLA-class II tetramer assays. Disease activity was assessed using the International Myositis Assessment and Clinical Studies core set measures, including the Myositis Disease Activity Assesment Visual Analogue Scale (MYOACT), manual muscle testing 8 (MMT8), and the physician global assessment. A detailed, comprehensive summary of demographic, clinical, laboratory, immunological treatment, and genetics for both cohorts can be found in the supplementary methods and Supplementary Tables S1 and S2. HLA-

DRB1 alleles were genotyped using the DR low Olerup SSP kits. Peripheral blood cells from *HLA-DRB1*03*-positive healthy controls were kindly provided by the Uppsala Bioresource, Uppsala University Hospital.

Ethics

The research ethics committees at Region Stockholm (DNR: 2005/792-31/4, 2018/1198-32) and Uppsala University (DNR: 2009/013) approved the study. Written informed consent was obtained from all participants. This study was conducted in accordance with the Declaration of Helsinki.

RESULTS

Antigen-specific CD4$^+$ T cells are detected using in-house produced and assembled peptide-HLA-class II tetramers

First, we evaluated the efficacy of our tetramer assay to detect antigen-specific CD4$^+$T cells *in vitro* using HLA-DRB1*03:01 tetramers (Tmr hereafter) loaded with the positive control tetanus peptide ($Tet_{506-525}$). PBMCs from HLA-DRB1*03:01 positive healthy donors (HC1, HC2, and HC3) were stimulated with $Tet_{506-525}$ along with interleukin(IL)7 and IL15 cytokines to support T cell survival (Fig 1A). The cells were kept in culture for up to 21 days and stained for the presence of tetanus-reactive T cells on different days. Tetanus-HLA-DRB1*03:01 Tmrs conjugated with 2 different fluorophores were used to gate on double Tmr-positive T cells in order to minimise false positive events from the cultured cells using flow cytometry (Supplementary Fig S1A). We observed that the number of tetanus-specific CD4$^+$T cells increased with the number of incubation days and decided to continue with day 13 cultures for the detection of antigen-specific CD4$^+$T cells (Fig 1B,C). To further confirm the presence of $Tet_{506-525}{}^+$CD4$^+$T cells, we measured IFNγ secretion following stimulation of PBMC from 2 individuals (HC 1 and patient with ASyS). $Tet_{506-525}$ Tmr$^+$ cells were detected in HC1, while no Tmr$^+$ cells were detected in the patient (Fig 1D). FluoroSpot results showed a significantly higher number of IFNγ^+ cells (spots) in culture containing tetanus Tmr-positive CD4$^+$T cells as compared to their unstimulated counterparts. No such increase in the IFNγ^+ cells was observed in the cultures without tetanus Tmr-positive cells (Fig 1E). Altogether, our results indicate that our experimental set-up enabled us to detect

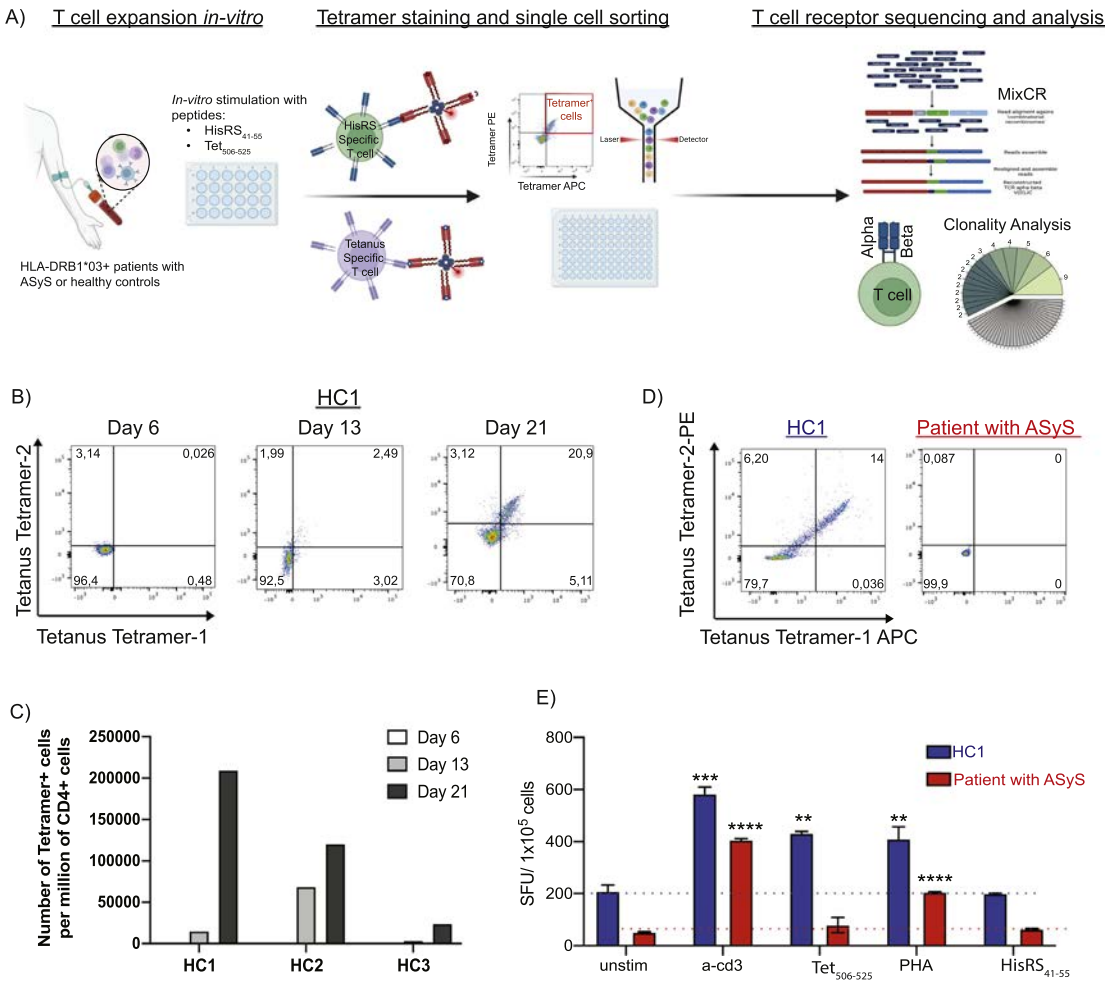


Figure 1. Tetanus tetramer staining of antigen-specific CD4$^+$T cells from human peripheral blood samples (A) Pipeline workflow starting from expansion of human peripheral blood mononuclear cells with respective peptides ($HisRS_{41-55}$ and $Tet_{506-525}$) along with IL7 and IL15, Tmr staining, single T-cell sorting and sequencing. (B) Representative flow plots showing tetanus-specific cells using $Tet_{506-525}$ tetramers conjugated with 2 different fluorophores on days 6, 13, and 21 following stimulation with $Tet_{506-525}$ from healthy control 1 (HC1). (C) Bar plot showing the number of $Tet_{506-525}$ Tmr$^+$ CD4$^+$T cells per million of CD4$^+$T cells on days 6, 13, and 21 from 3 healthy control (HC1, HC2, and HC3) peripheral blood sample following stimulation with $Tet_{506-525}$. (D) Flow plots showing presence (HC1, blue) and absence (patient with ASyS, red) of tetanus Tmr-positive CD4$^+$T cells, and (E) fluorospot staining from cells of these individuals which are positive for IFNγ upon stimulation with $Tet_{506-525}$ or a-CD3 or PHA as positive controls and $HisRS_{41-55}$ and unstimulated as negative controls. Number of spots per well was normalised to spots in 100,000 cells/well for plotting purposes. ASyS, antisynthetase syndrome; IFNγ, interferon γ; IL, interleukin; TMR, tetramer; HisRS, histidyl t-RNA synthetase; PHA, phytohemagglutinin.

antigen-specific T cells using in-house assembled peptide-HLA-DRB1*03:01 tetramers.

*A new T cell epitope on HisRS identified and loaded on peptide-HLA-DRB1*03:01 tetramer*

Given the known binding preferences for peptides to HLA-DRB1*03:01 and the previous reports on HisRS T cell reactivity being confined to the granzyme cleaved part of HisRS, we predicted tentative T cell epitopes for testing their binding to HLA-DRB1*03:01 [11]. A new epitope $HisRS_{41-55}$ was identified (LKAQLGPDESKQKFV, predicted binding residues underlined) with a higher binding affinity to HLA-DRB1*03:01 compared to the previously reported epitope $HisRS_{11-23}$ (Fig 2A). Binding assays comparing the $HisRS_{41-55}$ and $HisRS_{41-55}$-mut peptides (LKAALGPDASKAKFV, mutations underlined) showed no difference in HLA-binding, supporting that the identified anchor residues are indeed responsible for the interaction (Fig 2A). We also compared the stimulatory effects of these peptides by incubating PBMCs from patients with ASyS (n = 19, Supplementary Table S1). An increased CD40L expression was detected on $CD4^+$ T cells in 53% of the patients stimulated with $HisRS_{41-55}$, whereas 32% of patients had an increase upon $HisRS_{11-23}$ stimulation compared to unstimulated (Supplementary Fig S1B,C). There was no significant difference in CD40L upregulation between peptide stimulations (Supplementary Fig S1C). Thus, the new epitope identified ($HisRS_{41-55}$) was chosen for the assembly of peptide-HLA-DRB1*03:01 tetramers.

Autoreactive HisRS-specific $CD4^+$ T cells are detected in PBMC of patients with anti-$Jo1^+$ ASyS upon in vitro expansion

Next, we stimulated PBMCs from patients with anti-$Jo1^+$ ASyS (n = 12/14 with an *HLA-DRB1*03* haplotype, Supplementary Table S2) with $HisRS_{41-55}$ and $Tet_{506-525}$ [15] peptides, to investigate the presence of antigen-specific T cells using tetramers. We included antibodies against cell surface receptors previously described to be upregulated on antigen-specific T cells (CD69, CD25, PD1, and CD137) [16,17] and a cytotoxicity-related receptor (GPR56) [18]. Between days 10 to 14 of stimulation, we detected HisRS Tmr double ($Live^+CD3^+CD4^+$ Tmr-APC^+/Tmr-PE^+) positive T cells in 6 of 12 patients, and

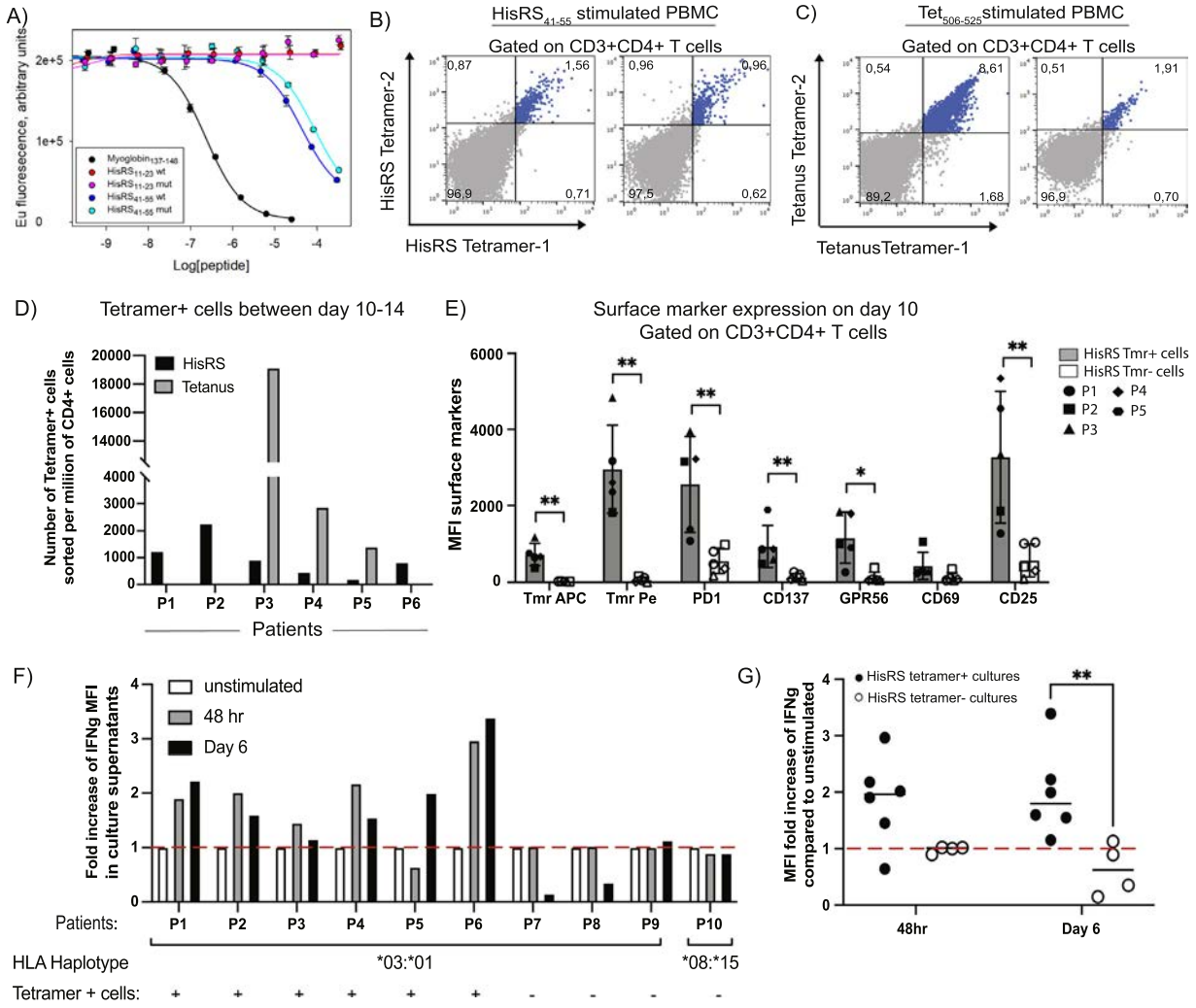


Figure 2. HisRS-specific $CD4^+$ T cells detected in peripheral blood of patients with ASyS. (A) Binding of HisRS peptides $HisRS_{10-25}$ (red), $HisRS_{10-25}$ mutated (pink), $HisRS_{41-55}$ (blue), $HisRS_{41-55}$ mutated (light blue) and myoglobulin (black) to MHC II. Representative flow cytometry plots showing (B) HisRS Tmr and (C) tetanus Tmr-positive cells (blue) among all $CD4^+$ T cells (grey) upon stimulation with $HisRS_{41-55}$ and $Tet_{506-525}$, respectively, using patient PBMCs. (D) Number of HisRS and tetanus Tmr-positive $CD4^+$ T cells per million of $CD4^+$ T cells in 6 patients P1-P6. (E) MFI of activation markers (PD1, CD69, CD137, and GPR56) from HisRS Tmr-positive (grey bar) vs HisRS Tmr-negative (clear bar) from 5 patients. Fold increase of IFNγ levels measured in supernatants of cultures at 48 h and 6 d after stimulation with $HisRS_{41-55}$ shown (F) per patient and (G) grouped based on presence of Tmr-positive cells. ASyS, antisynthease syndrome; HisRS, histidyl t-RNA synthetase; MFI, mean fluorescent intensity; P1-P6, patients 1 to 6; PBMCs, peripheral blood mononuclear cells; TMR, tetramer; MHC, major histocompatibility complex.

tetanus double Tmr-positive (Live CD3$^+$CD4$^+$ Tmr-APC$^+$/Tmr-PE) T cells in 3 of 12 patients (Fig 2B,C, Supplementary Fig S1A). There was no difference in clinical parameters in patients with detectable HisRS-specific CD4$^+$T and those without (Supplementary Table S3). The median number of HisRS Tmr-positive cells per million CD4$^+$T cells was 1044 (IQR 366-2087) in 6 patients most likely reflecting the number of HisRS-specific T cells among the seeded PBMC. We detected a higher number of Tet-Tmr$^+$ cells from the Tet$_{506\text{-}525}$ stimulated cultures compared to HisRS-Tmr$^+$ cells from HisRS$_{41\text{-}55}$ stimulated cultures due to a possible higher number of circulating tetanus-specific CD4$^+$ T cells in vaccinated adults (Fig 2D). Furthermore, HisRS Tmr-positive cells displayed significantly higher mean fluorescent intensity (MFI) of PD1, CD137, GPR56, and CD25 supporting their activation with HisRS$_{41\text{-}55}$ peptide stimulation as compared to HisRS Tmr-negative cells (Fig 2E). In the cultures from 48 hours and 6 days where HisRS Tmr-positive T cells were observed (6 patients; P1-P6), IFNγ levels were increased as compared to their unstimulated cultures (Fig 2F). Moreover, IFNγ levels were significantly higher in HisRS Tmr-positive cultures compared to Tmr-negative cultures on day 6, again supporting the antigen-specific T cell activation by HisRS$_{41\text{-}55}$ peptide in the Tmr-positive cultures (Fig 2G). Among other cytokines that were measured (IL6, IL10, tumour necrosis factor α [TNFα], and IL17A) in the supernatants TNFα levels were also significantly higher in tetramer-positive cultures at day 6 (Supplementary Fig S1D). Altogether, our data show that HisRS-specific CD4$^+$T cells are detectable in PBMC of patients with anti-Jo1$^+$ ASyS upon *in vitro* expansion.

TCR repertoire analysis revealed shared gene usages among patients

Single HisRS- and tetanus-specific CD4$^+$T cells were sorted between days 12 and 14 of peptide stimulation cultures for TCR sequencing. TCR alpha/ beta (α/β) chains of HisRS-specific CD4$^+$T cells (n = 520 T cells) and tetanus-specific CD4$^+$T cells (n = 130 T cells) were sequenced from 6 and 2 patients, respectively. T cells sharing identical complementarity determining region 3 (CDR3) amino acid sequences on both the α and β chains were considered to be a clone. Clonality analysis revealed the presence of expanded T cell clones in 5 of 6 patients for HisRS and 2 patients for tetanus (Fig 3A, Supplementary Fig S1E). The MFI values for tetramer staining of expanded T cells were confirmed from the index sorting (Fig 3B). Interestingly, we detected shared single α or β chain CDR3 sequences among different patients (Supplementary Fig S2A,B). We did not identify any clone (both α and β chain CDR3 identical) with public TCR sequences among all the tested patients with anti-Jo1$^+$ ASyS (Supplementary Table S4A). We used TCR_explore, a web-tool to further perform a TCR repertoire analysis to investigate shared patterns among TCR sequences between patients [19]. There was no difference in CDR3 lengths between patients (P1-P6) or antigens (HisRS vs Tetanus) (Supplementary Fig S2C,D). We observed a slightly higher diversity in CDR3 sequences of HisRS Tmr$^+$ cells compared to Tetanus Tmr$^+$ cells, although this difference did not reach any significance (Supplementary Fig S2E). Next, we evaluated the variable (V) and joining (J) gene pairs for both α and β chains (Fig 3C,D). We found shared gene pair usages for both α and β chains between patients for both HisRS and tetanus (Fig 3C-F, Supplementary Fig S2D,E). In addition, alpha chain V gene usages varied among individuals with AV13-1 and AV12-2 being in the top 3 gene usages observed in patients P1, P3, P5 and patients P2, P4, P5, P6, respectively, among HisRS$^+$ CD4$^+$T cells (Fig 3C, E, Supplementary Fig S2F, Supplementary Table S4B). Beta chain V gene usages showed a bias against BV20-1 among HisRS$^+$ CD4$^+$T cells which was shared between patients P1, P2, P5, and P6 (Fig 3D,F, Supplementary Fig S2G, Supplementary Table S4B). Among the tetanus$^+$CD4$^+$T cells, there were 3 CDR3α and one CDR3β sequences shared among the 2 patients. In addition, there were also 3 AVJ and 2 BVJ gene usage pairings shared between these patients (Supplementary Fig S3A-D). Thus, our results identified expanded T cell clones among HisRS$^+$ CD4$^+$T cells further supporting their specificity against HisRS$_{41\text{-}55}$ with shared alpha and beta VJ gene pairs with high diversity among HisRS-specific CD4$^+$T cells.

Persisting HisRS-specific T cells are detected in the blood of patients despite treatment

We investigated the presence of HisRS-specific CD4$^+$T cells among PBMC from follow-up samples for 4 available patients (Supplementary Table S3). In follow-up blood samples after up to 1-year with immunosuppressive treatment, we detected HisRS Tmr-positive cells upon stimulation with HisRS$_{41\text{-}55}$ peptide in 2 of these patients (P2 and P4) (Fig 4A,B). The number of HisRS Tmr$^+$ T cells was lower in the 1-year follow-up samples, compared to the samples from the first time point for both patients (Fig 4C). Similarly to the first time point, the MFI of surface markers PD1, CD137, GPR56, and CD69 as well as the IFNγ levels in the cultures were increased in HisRS Tmr-positive cells compared to the Tmr-negative cells in both patients, although this did not reach statistical significance (Fig 4D,E). Upon TCR repertoire analysis of the HisRS Tmr-positive T cells, we observed expanded T cell clones in both patients (Fig 4F). Interestingly, we detected 2 identical T cell clones between the first and second time point in patient P2 (Fig 4G,H, Supplementary Fig S3E). These data suggest that HisRS-specific T cells persist in the blood of patients despite treatment with conventional immunosuppressants. The persistence of autoreactive TCR clones may suggest their role in disease progression.

DISCUSSION

In this study, we identified a new T cell epitope on the HisRS protein with a high binding affinity to the HLA-DRB1*03:01. Autoreactive T cells against this epitope were detected using peptide-HLA tetramers in blood samples of 6 of 12 patients with *HLA-DRB1*03* and anti-Jo1$^+$ ASyS upon *in vitro* peptide stimulation. TCR repertoire analysis of HisRS-specific T cells demonstrated the presence of expanded T cell clones supporting their specificity for this epitope. No public clones were detected (sharing both alpha and beta chain CDR3 sequences) between patients indicating the diversity of the TCR repertoire. Further analysis of TCR repertoire revealed shared alpha or beta CDR3 amino acid sequences as well as variable (V) and joining (J) gene pairings for both alpha (α) and beta (β) chains among patients. Finally, we observed persisting HisRS-specific T cells in 2 of 3 patients in follow-up samples (7-16 months) despite conventional immunosuppressive treatment. Overall, our study confirms the presence of autoreactive T cells targeting HisRS-derived peptide in anti-Jo1$^+$ patients and their persistence suggests that they may be contributing to disease chronicity.

Peptide-HLA multimer techniques have identified antigen-specific CD4$^+$T cells in several autoimmune diseases, such as rheumatoid arthritis, type 1 diabetes, systemic lupus erythematosus, celiac disease, Parkinson's disease, and

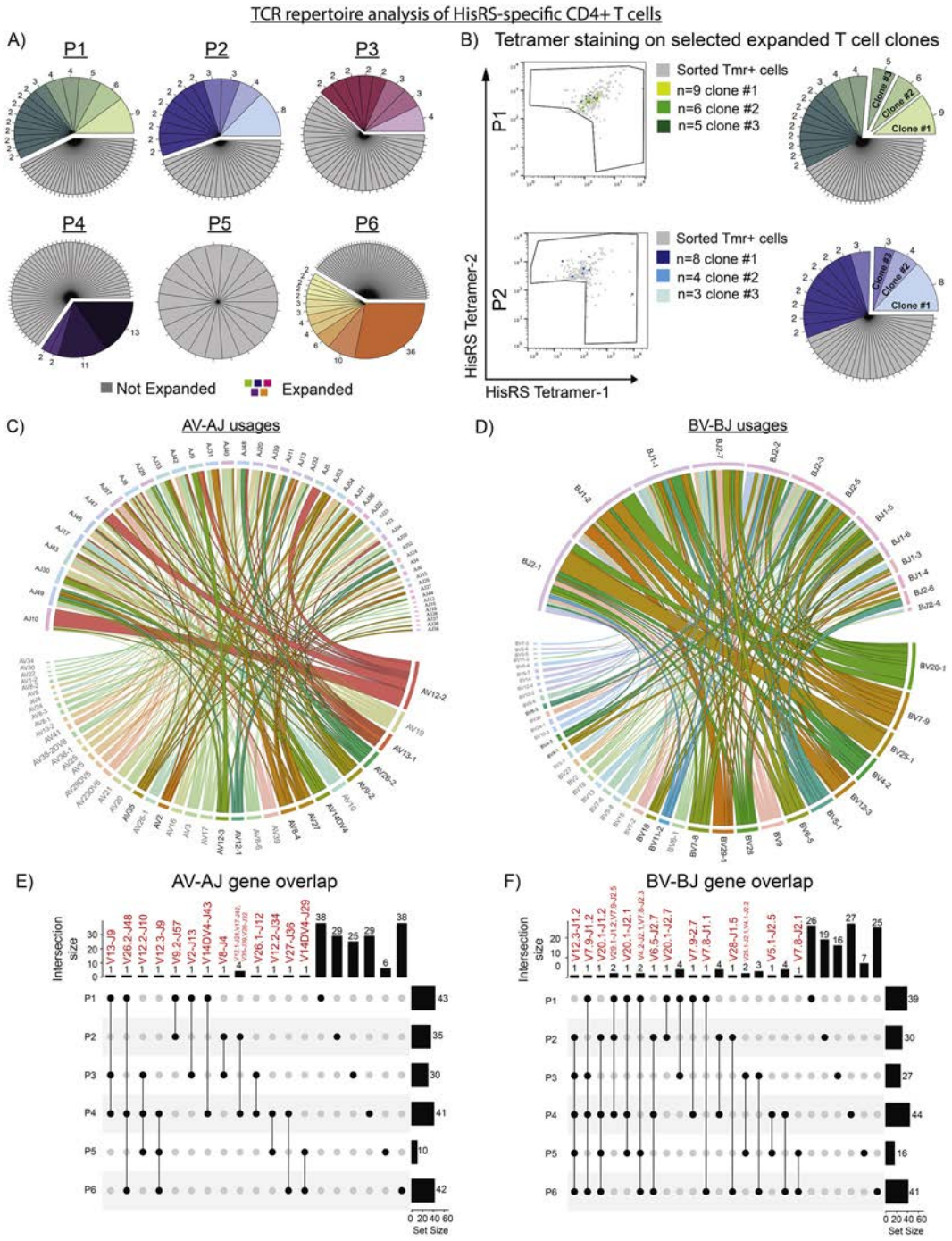


Figure 3. TCR sequencing analysis reveals expanded clones and shared VJ gene usages. (A) Pie charts showing expanded T cell clones after stimulation with $HisRS_{41-55}$ sorted with tetramers between days 12 and 14. A clone is defined as having an identical alpha and beta CDR3 amino acid sequence. Colourful slices (green, blue, pink, purple, and orange) indicate expanded CDR3 sequences, whereas grey shows unique CDR3 sequences. The number at slices corresponds to how many times a CDR3 sequence is repeated, not shown for unique sequences which is 1 for all grey slices. (B) Representative flow cytometry plots showing top 3 expanded clones from P1 and P2, for HisRS Tmr-APC and HisRS-Tmr PE. Colourful dots are corresponding to expanded T cells; grey dots are all $CD4^+Tmr^+$ cells sorted. Circos plots showing (C) alpha and (D) beta chain VJ gene pairings, each sequence is represented once. The connecting lines that have black borders are used by multiple patients. UpSet plots showing the shared gene pairings for (E) alpha and (F) beta chain. Connected dots correspond to shared usages between patients. Intersection size corresponds to how many different pairings are shared and set size corresponds to the total number of VJ pairs present in each patient. HisRS, histidyl t-RNA synthetase; MFI, mean fluorescent intensity; P1-P6, patients 1 to 6; TCR, T-cell receptor; TMR, tetramer, V, variable; J, joining.

atherosclerosis [20–28]. Presence of these T cells has been linked to disease progression, supporting their contribution to disease [25]. However, despite these advances, the α/β TCR identity of autoreactive $CD4^+$T cells remains uncharacterised in many autoimmune diseases, including ASyS. Previously, we reported the presence of antigen-reactive T cells in PBMC and BALF cells against the full-length HisRS protein in patients with anti-$Jo1^+$ ASyS. The reactivity of $CD4^+$T cells against the previously reported epitope $HisRS_{11-23}$ was limited to a subset of the patients suggesting the presence of additional immunogenic HisRS epitopes or due to epitope spreading [11]. Here, we identified a new epitope that has a higher binding affinity to HLADR1*03:01 than the previously described epitope and demonstrated the presence of

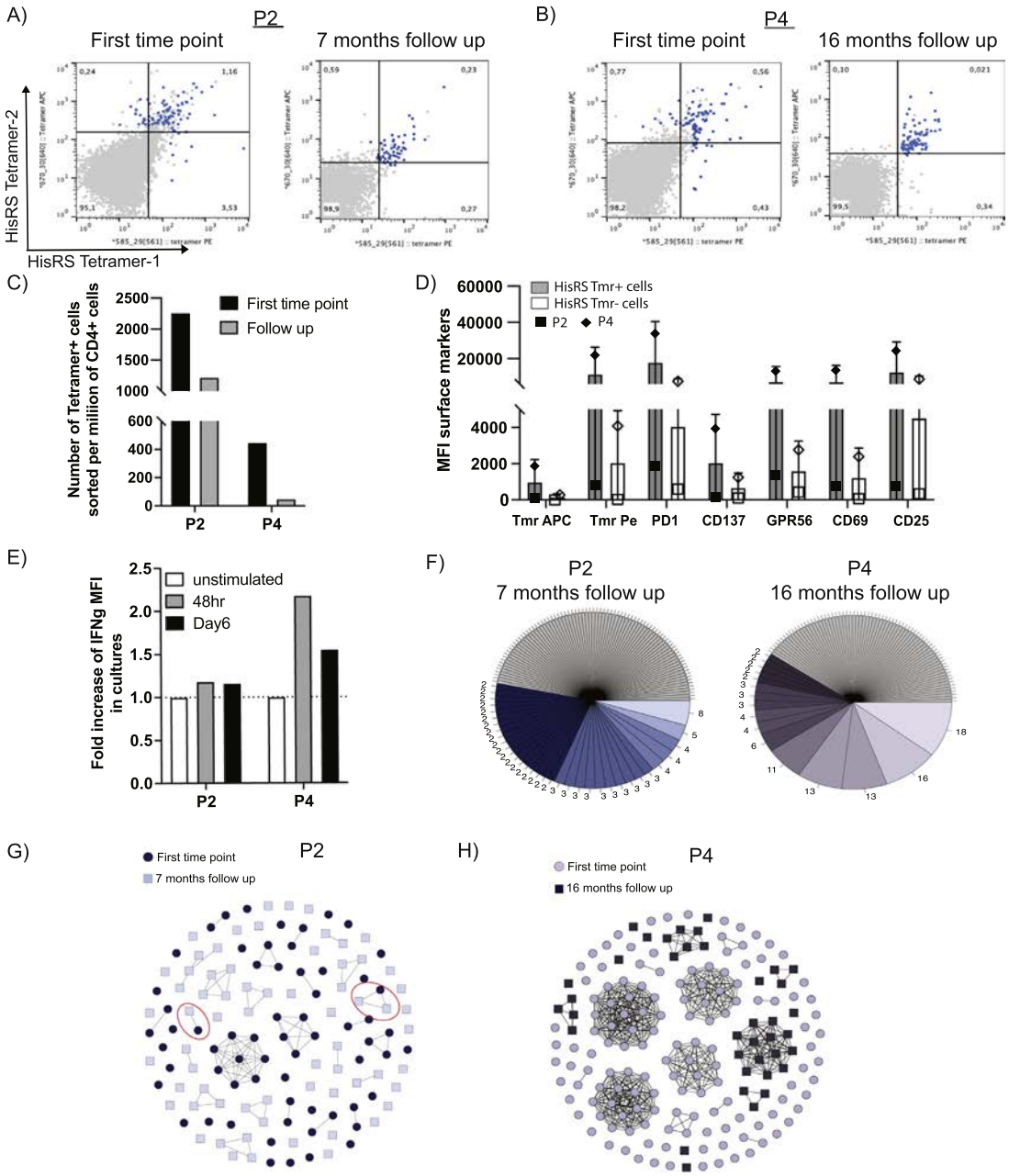


Figure 4. HisRS-specific CD4$^+$T cells detected in 1-y follow-up samples of two patients. Flow cytometry plots showing HisRS Tmr-positive cells (blue) among CD4$^+$T cells (grey) in (A) P2 and (B) P4 left panel first time point, right panel 1-year follow-up sample. (C) Number of HisRS-Tmr-postive CD4$^+$T cells per million of CD4$^+$T cells first time point (black bar) and follow up (grey bar) for two patients. (D) MFI of activation markers (PD1, CD69, CD137, and GPR56) of HisRS Tmr-positive (grey) vs HisRS tetramer negative (clear) cells (square: P2, diamond: P4). (E) Fold increase of IFNγ levels measured in supernatants of cultures 48 h and 6 d after stimulation with HisRS$_{41\text{-}55}$ shown per patient compared to unstimulated (clear bars) condition. (F) Pie charts showing expanded CDR3 sequences (expanded clones: blue for P2, purple for P4, unique clones: grey), the numbers on the slices indicate the number of cells that have corresponding sequences. Network plot showing shared CDR3 sequences between first time point (dark blue circle or dark purple circle) and 1-y follow-up (light blue square or lilac square) for (G) P2 and (H) P4. Connected shapes mean shared CDR3 sequences. IFNγ, interferon γ; HisRS, histidyl t-RNA synthetase; MFI, mean fluorescent intensity; P2-P4, patients 2 and 4; TMR, tetramer.

autoreactive CD4$^+$T cells targeting this epitope in 6 of 12 HLA-DRB1*03:01-positive patients with ASyS.

In our study, the TCR repertoire we report reflects both antigen-experienced HisRS-specific T cells as well as naïve T cells capable of responding to the HisRS$_{41\text{-}55}$ epitope, both very relevant to understand the pathology of the disease. Heterogeneity within the naïve T cell compartment has been described with certain subsets more prone to respond to specific antigens [29,30]. In support of this, Gerstner et al [28] showed that a clear proportion of the autoreactive T cells detected in patients with rheumatoid arthritis displayed a naïve phenotype, and these cells are likely to be activated *in vivo* due to high chronicity in autoimmune diseases. Taken together, this suggests that naïve T cells that can respond to autoantigens may be one of the target populations for tolerance therapies for patients with autoimmunity.

Previously, shared variable TCR gene usages in lung and muscle of patients with ASyS have been reported for selected V genes, suggesting a connection in the development and similar antigen-recognition of these cells [12]. More recently, using single-cell sequencing, we showed that cytotoxic CD4$^+$T cell clones were shared between blood and muscle in a patient with anti-Jo1$^+$ ASyS further supporting their developmental connection leading to tissue damage and demonstrating that these

potentially pathogenic T cells can be detected in peripheral blood [13].

Our TCR repertoire analysis of autoreactive HisRS-specific T cells from blood showed shared single alpha or beta CDR3 sequences among individuals, but no public clones (alpha and beta CDR3 sequences shared). However, we detected shared gene usages for both chains, with a bias towards increased variable TRAV-12, TRAV-13, and TRBV-20 gene usages. Biased usage of TCR gene segments has been reported in the context of infection or in autoimmunity [31]. Single-cell RNA sequencing of T cells from COVID-19-infected individuals showed a preference for TRAV1-2 and TRBV20 among $CD8^+$T cells [32]. More recently, TRBV20-1 was shown to be the highest used gene segment among citrullinated peptide-specific $CD4^+$T cells from patients with rheumatoid arthritis suggesting the preference towards specific gene usages in T cell responses [33–35]. Similarly, TCR alpha chain usage (TRAV12-1) was shown to be overrepresented among $CD8^+$T cells upon yellow fever virus infection and TRAV-26-1 in celiac disease [36,37]. Our observed bias in TRAV and -BV-usages suggests a similar structural recognition mechanism.

Furthermore, the persistence of tetramer-positive $CD4^+$T cells and specific T cell clones after treatment with conventional immunosuppressive treatment suggests that the persistence of T cells with identical TCRs might explain the high risk of relapses in patients with anti-$Jo1^+$ ASyS, in support of our previous findings [13]. Future functional studies are needed to study the contribution of these antigen-specific T cells in disease progression and relapses.

One limitation of the present study is the small sample size due to the rarity of anti-$Jo1^+$ ASyS. There were also delays in patient recruitment due to the COVID-19 pandemic. Another limitation of this study was the need for *in vitro* expansion of cells before repertoire analysis, due to the rarity of autoreactive T cells. Our data reflect the part of the TCR repertoire which responded to the stimulation *in vitro*. As confirmed by our studies, there are possibly several, some yet to be discovered, immunogenic peptides/antigens on HisRS in patients with anti-$Jo1^+$ ASyS [11]. On the contrary, utilisation of HLA-class II tetramers makes it possible to identify very rare antigen-specific $CD4^+$T cells; in patients with *HLA-DRB1*03:01* with T cell reactivity against the $HisRS_{41\text{-}55}$ peptide in this case and provides a proof of concept on the detection of T cells specific for our target peptide using HLA-class II tetramers in 50% of the patients included with *HLA-DRB1*03:01* haplotype.

To the best of our knowledge, this is the first study to report the detection of autoreactive HisRS-specific T cells using peptide-HLA-class II multimer technology and paired with α/β-TCR sequencing in patients with ASyS. Identification of antigen-specific T cells has clear implications for the future development of improved therapies including tolerising and targeting pathogenic TCR signatures. Moreover, targeting shared TCR signatures might also be tested as a therapeutic approach. Finally, our tetramers could also be used as valuable tools to study the effects of treatment on presumably pathogenic antigen-specific T cells in individual patients both under conventional immunosuppressive as well as new more targeted therapies.

Competing interests

IEL has received honorarium for lecture from Janssen Pharmaceutica NV, research grant from AstraZeneca and Janssen Pharmaceutica NV, and has been serving on the advisory board for Argenx, Astra-Zeneca, EMD Serono. Research & Development Institute, Chugai, Galapagos, Novartis, Pfizer and Janssen Pharmaceutica NV and has stock shares in Roche and Novartis.

CRediT authorship contribution statement

Angeles S. Galindo-Feria: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Ravi Kumar Sharma:** Writing – review & editing, Visualization, Methodology, Data curation. **Anatoly Dubnovitsky:** Writing – review & editing, Methodology, Data curation. **Christina Gerstner:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Genadiy Kozhukh:** Methodology. **Annika Van Vollenhoven:** Writing – review & editing, Methodology. **Juan Sebastian Diaz Boada:** Writing – review & editing, Software, Methodology. **Daniel Ramsköld:** Writing – review & editing, Software. **Adnane Achour:** Writing – review & editing, Methodology. **Maryam Dastmalchi:** Resources. **Hugh H. Reid:** Writing – review & editing, Methodology. **Tatyana Sandalova:** Methodology. **Jamie Rossjohn:** Writing – review & editing, Methodology. **Karine Chemin:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Vivianne Malmström:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization. **Ingrid E. Lundberg:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Begum Horuluoglu:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Acknowledgements

We thank the patients and medical staff, especially Dr Antonella Notarnicola and nurse Helene Sandlund at the Karolinska Rheumatology Clinic. Special thanks to Gloria Rostvall, Eva Lindroos, Julia Norkko, and Lucymary Okechukwu for biobanking and sample handling. We are grateful to Maija-Leena Eloranta and Lars Rönnbloom (Uppsala University Hospital) for providing HLA-matched control samples. We thank Anneli Dihkan for bringing the patient perspective to the research, and Barbro Larsson and Leonid Padyukov for support with HLA genotyping. We also appreciate Lars Klareskog for valuable discussions. We want to thank the CMM flow cytometry core and Lina Marcela Diaz Gallo for TCR demultiplexing scripts. We thank Timmy Hellström and Daniel Uvehag for support with scripts and containers for the bioinformatic pipeline and Alexandra Argyriou for artistic review of final figures. The authors acknowledge support from the National Genomics Infrastructure in Stockholm funded by Science for Life Laboratory, the Knut and Alice Wallenberg Foundation and the Swedish Research Council, and SNIC/Uppsala Multidisciplinary Center for Advanced Computational Science for assistance with massively parallel sequencing and access to the UPPMAX computational infrastructure.

Funding

This study was supported by The Myositis Association (BH), Professor Nanna Svartz Foundation (BH, 2024-074), Karolinska Institutet Research Foundations (BH, 2024-0021), Alex and Eva Wallström foundation (BH, 2023-00467), King Gustaf V 80th Year Foundation (BH FAI-2024-1083 and IL FAI-2024-1092),

Åke Wiberg Foundation (BH M24-00659 and RKS M23-0164), the Swedish Rheumatism Association (BH R-1013410 and IL R-1013188), The Swedish Research Council (IL, 2020–01378), Swedish Heart and Lung Foundation (IL 20220127 and BH 20241216), Region Stockholm (ALF project, IL, FoUI–985960), the Anna Hedin donation (IL), Konung Gustaf V:s och Drottning Victorias Frimurarstiftelse (IL). JR is supported by an NHMRC Investigator Award to 2008981.

Patient consent for publication

Not applicable.

Ethics approval

The study was approved by local ethics review boards and followed the Helsinki declaration.

Provenance and peer review

Not commissioned; externally peer reviewed.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.09.015.

Orcid

Angeles S. Galindo-Feria: http://orcid.org/0000-0001-7095-211X
Ravi Kumar Sharma: http://orcid.org/0000-0002-2658-0002
Anatoly Dubnovitsky: http://orcid.org/0000-0002-5350-4531
Christina Gerstner: http://orcid.org/0009-0003-4342-4241
Daniel Ramsköld: http://orcid.org/0000-0003-2892-673X
Adnane Achour: http://orcid.org/0000-0003-0432-710X
Tatyana Sandalova: http://orcid.org/0000-0002-7694-6420
Karine Chemin: http://orcid.org/0000-0002-2597-7809
Vivianne Malmström: http://orcid.org/0000-0001-9251-8082
Ingrid E. Lundberg: http://orcid.org/0000-0002-6068-9212
Begum Horuluoglu: http://orcid.org/0000-0003-2241-5170

REFERENCES

[1] Lundberg IE, de Visser M, Werth VP. Classification of myositis. Nat Rev Rheumatol 2018;14(5):269–78.
[2] Furst DE, Amato AA, Iorga ŞR, Gajria K, Fernandes AW. Epidemiology of adult idiopathic inflammatory myopathies in a U.S. managed care plan. Muscle Nerve 2012;45(5):676–83.
[3] Svensson J, Arkema EV, Lundberg IE, Holmqvist M. Incidence and prevalence of idiopathic inflammatory myopathies in Sweden: a nationwide population-based study. Rheumatology (Oxford) 2017;56(5):802–10.
[4] Chinoy H, Adimulam S, Marriage F, New P, Vincze M, Zilahi E, et al. Interaction of HLA-DRB1*03 and smoking for the development of anti-Jo-1 antibodies in adult idiopathic inflammatory myopathies: a European-wide case study. Ann Rheum Dis 2012;71(6):961–5.
[5] Miller FW, Chen W, O'Hanlon TP, Cooper RG, Vencovsky J, Rider LG, et al. Genome-wide association study identifies HLA 8.1 ancestral haplotype alleles as major genetic risk factors for myositis phenotypes. Genes Immun 2015;16(7):470–80.
[6] Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. I: Quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibers invaded by T cells. Ann Neurol 1984;16(2):193–208.
[7] Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. III: Immunoelectron microscopy aspects of cell-mediated muscle fiber injury. Ann Neurol 1986;19(2):112–25.
[8] Malmström V, Venalis P, Albrecht I. T cells in myositis. Arthritis Res Ther 2012;14(6):230.
[9] Howard OMZ, Dong HF, Yang D, Raben N, Nagaraju K, Rosen A, et al. Histidyl-tRNA synthetase and asparaginyl-tRNA synthetase, autoantigens in myositis, activate chemokine receptors on T lymphocytes and immature dendritic cells. J Exp Med 2002;196(6):781–91.
[10] Ascherman DP. Role of Jo-1 in the Immunopathogenesis of the Anti-synthetase Syndrome. Curr Rheumatol Rep 2015;17(9):56.
[11] Galindo-Feria AS, Albrecht I, Fernandes-Cerqueira C, Notarnicola A, James EA, Herrath J, et al. Proinflammatory histidyl-transfer RNA synthetase-specific CD4+ T cells in the blood and lungs of patients with idiopathic inflammatory myopathies. Arthritis Rheumatol 2020;72(1):179–91.
[12] Englund P, Wahlström J, Fathi M, Rasmussen E, Grunewald J, Tornling G, et al. Restricted T cell receptor BV gene usage in the lungs and muscles of patients with idiopathic inflammatory myopathies. Arthritis Rheum 2007;56(1):372–83.
[13] Argyriou A, Horuluoglu B, Galindo-Feria AS, Diaz-Boada JS, Sijbranda M, Notarnicola A, et al. Single-cell profiling of muscle-infiltrating T cells in idiopathic inflammatory myopathies. EMBO Mol Med 2023;15(10):e17240.
[14] Connors GR, Christopher-Stine L, Oddis CV, Danoff SK. Interstitial lung disease associated with the idiopathic inflammatory myopathies: what progress has been made in the past 35 years? Chest 2010;138(6):1464–74.
[15] Faner R, James E, Huston L, Pujol-Borrel R, Kwok WW, Juan M. Reassessing the role of HLA-DRB3 T-cell responses: evidence for significant expression and complementary antigen presentation. Eur J Immunol 2010;40(1):91–102.
[16] Yan Z-H, Zheng X-F, Yi L, Wang J, Wang X-J, Wei P-J, et al. CD137 is a useful marker for identifying CD4(+) T cell responses to Mycobacterium tuberculosis. Scand J Immunol 2017;85(5):372–80.
[17] Simon S, Labarriere N. PD-1 expression on tumor-specific T cells: friend or foe for immunotherapy? Oncoimmunology 2017;7(1):e1364828.
[18] Argyriou A, Wadsworth 2nd MH, Lendvai A, Christensen SM, Hensvold AH, Gerstner C, et al. Single cell sequencing identifies clonally expanded synovial CD4(+) T(PH) cells expressing GPR56 in rheumatoid arthritis. Nat Commun 2022;13(1):4046.
[19] Mullan KA, Zhang JB, Jones CM, Goh SJR, Revote J, Illing PT, Purcell AW, La Gruta NL, Li C, Mifsud NA. TCR_Explore: A novel webtool for T cell receptor repertoire analysis. Comput Struct Biotechnol J 2023;21:1272–82.
[20] Schwenzer A, Jiang X, Mikuls TR, Payne JB, Sayles HR, Quirke A-M, et al. Identification of an immunodominant peptide from citrullinated tenascin-C as a major target for autoantibodies in rheumatoid arthritis. Ann Rheum Dis 2016;75(10):1876–83.
[21] Boddul SV, Sharma RK, Dubnovitsky A, Raposo B, Gerstner C, Shen Y, et al. In vitro and ex vitro functional characterization of human HLA-DRB1 *04 restricted T cell receptors. J Transl Autoimmun 2021;4:100087.
[22] Mitchell AM, Alkanani AA, McDaniel KA, Pyle L, Waugh K, Steck AK, et al. T-cell responses to hybrid insulin peptides prior to type 1 diabetes development. Proc Natl Acad Sci USA 2021;118(6):e2019129118.
[23] Wolf D, Gerhardt T, Winkels H, Michel NA, Pramod AB, Ghosheh Y, et al. Pathogenic autoimmunity in atherosclerosis evolves from initially protective apolipoprotein B(100)-reactive CD4(+) T-regulatory cells. Circulation 2020;142(13):1279–93.
[24] Abdirama D, Tesch S, Grießbach A-S, von Spee-Mayer C, Humrich JY, Stervbo U, et al. Nuclear antigen-reactive CD4(+) T cells expand in active systemic lupus erythematosus, produce effector cytokines, and invade the kidneys. Kidney Int 2021;99(1):238–46.
[25] Lindestam Arlehamn CS, Dhanwani R, Pham J, Kuan R, Frazier A, Dutra JR, et al. α-Synuclein-specific T cell reactivity is associated with preclinical and early Parkinson's disease. Nat Commun 2020;11(1):1875.
[26] Buitinga M, Callebaut A, Sodré FMC, Crèvecoeur I, Blahnik-Fagan G, Yang M-L, et al. Inflammation-induced citrullinated glucose-regulated protein 78 elicits immune responses in human type 1 diabetes. Diabetes 2018;67(11):2337–48.
[27] Dendrou CA, Petersen J, Rossjohn J, Fugger L. HLA variation and disease. Nat Rev Immunol 2018;18(5):325–39.
[28] Gerstner C, Turcinov S, Hensvold AH, Chemin K, Uchtenhagen H, Ramwadhdoebe TH, et al. Multi-HLA class II tetramer analyses of citrulline-reactive T cells and early treatment response in rheumatoid arthritis. BMC Immunol 2020;21(1):27.
[29] Yoon J-W, Kim KM, Cho S, Cho M-J, Park S, Hwang D, et al. Th1-poised naive CD4 T cell subpopulation reflects anti-tumor immunity and autoimmune disease. Nat Commun 2025;16(1):1962.
[30] Martin B, Auffray C, Delpoux A, Pommier A, Durand A, Charvet C, et al. Highly self-reactive naive CD4 T cells are prone to differentiate into regulatory T cells. Nat Commun 2013;4:2209.

[31] Turner SJ, Doherty PC, McCluskey J, Rossjohn J. Structural determinants of T-cell receptor bias in immunity. Nat Rev Immunol 2006;6(12):883–94.
[32] Luo L, Liang W, Pang J, Xu G, Chen Y, Guo X, et al. Dynamics of TCR repertoire and T cell function in COVID-19 convalescent individuals. Cell Discov 2021;7(1):89.
[33] Sharma RK, Boddul SV, Yoosuf N, Turcinov S, Dubnovitsky A, Kozhukh G, et al. Biased TCR gene usage in citrullinated Tenascin C specific T-cells in rheumatoid arthritis. Sci Rep 2021;11(1):24512.
[34] Turcinov S, Klint EA, Van Schoubroeck B, Kouwenhoven A, Mia S, Chemin K, et al. Diversity and clonality of T cell receptor repertoire and antigen specificities in small joints of early rheumatoid arthritis. Arthritis Rheumatol 2023;75(5):673–84.
[35] Lim JJ, Jones CM, Loh TJ, Ting YT, Zareie P, Loh KL, et al. The shared susceptibility epitope of HLA-DR4 binds citrullinated self-antigens and the TCR. Sci Immunol 2021;6(58):eabe0896.
[36] Frick R, Gunnarsen KS, Dahal-Koirala S, Risnes LF, Sollid LM, Sandlie I, et al. A TRAV26-1-encoded recognition motif focuses the biased T cell response in celiac disease. Eur J Immunol 2020;50(1):142–5.
[37] Bovay A, Zoete V, Dolton G, Bulek AM, Cole DK, Rizkallah PJ, et al. T cell receptor alpha variable 12-2 bias in the immunodominant response to Yellow fever virus. Eur J Immunol 2018;48(2):258–72.

Ann Rheum Dis 85 (2026) 370–378

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Vasculitis

Disease Trajectories and Glucocorticoid Exposure in VEXAS Syndrome Treated with Cytokine-Directed Therapies

Benjamin A. Turturice[1], Alice Fike[1], Bhavisha A. Patel[2], Emma M. Groarke[2], Fernanda Gutierrez-Rodriguez[2], Karyssa Stonick[1], Hannah Berrett[1], Shanni Liu[1], Ivana Darden[2], Kaitlin A. Quinn[1], Neal S. Young[2], Peter C. Grayson[1,*]

[1] *National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD, USA*
[2] *National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA*

ARTICLE INFO

Article history:
Received 11 March 2025
Received in revised form 25 May 2025
Accepted 28 May 2025

ABSTRACT

Objectives: To establish the long-term impact of cytokine-directed therapies on glucocorticoid use and clinical outcomes in Vacuoles, E1-enzyme, X-linked, Autoinflammatory, Somatic (VEXAS).

Methods: Patients with VEXAS were prospectively followed for events of transfusion dependence, haematopoietic stem cell transplantation or death. Laboratory results, glucocorticoid exposure and clinical measures were retrospectively assessed in relationship to treatment initiation with interleukin-6-directed therapies (anti-IL6R) or Janus kinase inhibitors (JAKi). Patients were stratified by *UBA1* variants and presence of typical clonal haematopoiesis with variant allele fraction ≥ 10% ($CH^{VAF\geq10\%}$).

Results: In 71 VEXAS patients (81.7% with anti-IL6R or JAKi exposure), event-free survival differed by genotype and presence of concomitant $CH^{VAF\geq10\%}$: p.M41V (HR [95% confidence interval (CI)]: 5.7 [1.5-20.4]) or p.M41L/T with $CH^{VAF\geq10\%}$ (hazard ratio [HR]: 5.7 [1.6-20.8]) compared to p.M41L. No association between event rates and exposure to anti-IL6R or JAKi was observed. The p.M41V genotype had the highest risk of anaemia, elevated C-reactive protein (CRP) levels, and monocytopenia. Over a median follow-up of 4.8 (interquartile range [IQR] 3.0, 8.1) years, the patients' mean glucocorticoid dose was >15 mg/day prednisone regardless of variant or disease duration. At prospective visits, clinical remission on ≤10 mg/day prednisone was observed in only 2.7% of visits. Treatment with anti-IL6R or JAKi showed no clinically meaningful reduction (<5 mg/day difference) in steroid exposure at 1 year post-treatment. No attenuation in the progression of anaemia was observed in response to anti-IL6R and JAKi.

Conclusions: Cytokine-directed therapies alone do not alter the risk of haematologic disease progression or significantly reduce glucocorticoid exposure in VEXAS. These data provide benchmarks for future interventional studies.

INTRODUCTION

Vacuoles, E1-enzyme, X-linked, Autoinflammatory, Somatic (VEXAS) syndrome is an acquired genetic disease caused by variants that affect the E1-ubiquitin activating enzyme UBA1 [1–3]. Inflammatory and haematologic processes have been attributed to VEXAS. Differences in inflammatory manifestations (eg, pattern of organ involvement) have been associated with specific *UBA1* variants [4–6]. Although dysfunctional haematopoiesis is common in VEXAS, longitudinal associations with

*Correspondence to Dr. Peter C Grayson.
E-mail address: peter.grayson@nih.gov (P.C. Grayson).
Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.05.021

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Glucocorticoids and cytokine-directed therapies (eg, anti-interleukin-6 receptor (IL6R) monoclonal antibodies and Janus kinase (JAK) inhibitors) are frequently used as immunosuppression in treatment of Vacuoles, E1-enzyme, X-linked, Autoinflammatory, Somatic (VEXAS) syndrome.

WHAT THIS STUDY ADDS

- This study demonstrates that exposure to cytokine-directed therapies in combination with moderate- to high-dose steroids does not alter the trajectory of progressive anaemia in patients with VEXAS. Further, clinical remission on ≤ 10 mg/day prednisone is rare and unlikely to be improved by the addition cytokine-directed therapy alone.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- This study sets historical benchmarks for cytopenia progression, expected glucocorticoid exposure and disease activity over time. It also informs that the glucocorticoid sparing effect of newer or combination therapies will be an important outcome in future interventional studies.

haematologic parameters have been less systematically investigated.

Deficiency of cytoplasmic UBA1 has been associated with disease severity (eg, death) for the canonical methionine 41 (p.M41) variants (eg, p.M41L, p.M41T and p. M41V) [7]. Co-occurrence of other clonal haematopoiesis (CH)-associated somatic variants, such as those in *DNMT3A* and *TET2*, contributes to disease mortality [8]. *UBA1* variant clones may arise de novo or in succession with other CH clones, with the latter being associated with CH variant allele fractions (VAF) ≥ 10%. Each of these clonal events may impact disease outcomes.

There are currently no approved medications or guidelines for the treatment of VEXAS, but several classes of immunosuppressive and cytotoxic agents may have benefit [9–11]. These include interleukin-6 (IL6) receptor monoclonal antibody antagonists (anti-IL6R) and Janus kinase inhibitors (JAKis) as immunosuppressive medications. Both anti-IL6R and JAKi can reduce IL-6 signalling, whereas JAKis can inhibit a broader range of cytokine signalling, including noninflammatory cytokines (eg, erythropoietin) [12,13]. While these cytokine-directed medications have been proposed to have benefit, glucocorticoids have been the mainstay of therapy for controlling inflammatory symptoms [14,15], but also contribute to infectious risk observed in these patients [16,17]. Other glucocorticoid-associated toxicities have been less rigorously evaluated in the VEXAS patient population.

Giant cell arteritis (GCA) is an inflammatory disease exclusive to later adulthood that is more common than VEXAS [3,18]. Though its pathophysiology is distinct from VEXAS, GCA also presents with clinical manifestations due to systemic inflammation, and chronic immunosuppressive treatment frequently include high-dose glucocorticoids and anti-IL6R [19,20]. As a well-defined and more robustly studied disease state, GCA can serve as a reference population. Given these similarities, comparing disease trajectories in VEXAS to GCA may be useful to delineate the relative burden of disease over time, such as medication-related toxicities and glucocorticoid exposure.

The objective of this study was to assess the trajectory of dysfunctional haematopoiesis, inflammatory burden, and glucocorticoid exposure across the disease time course in patients with VEXAS predominantly treated with cytokine-directed therapies. Here, we present an analysis of such parameters in VEXAS patients with canonical p.M41 variants.

METHODS

Study participants

Consecutive patients from 2012 to 2024 with a genetically confirmed diagnosis of VEXAS syndrome or with GCA meeting the 2022 American College of Rheumatology/ European League Against Rheumatism classification criteria [21] were selected within an ongoing prospective observational cohort study at the National Institutes of Health (NIH; Protocol 14-AR-0200). Each participant provided written informed consent to participate, and an institutional review board approved the study. Patients were excluded to remove small sample size populations with large confounding effects and homogenise demographics of the comparator condition.

Clinical assessments and definitions

For patients with VEXAS, somatic variants were assessed as previously described [8] in either peripheral blood or bone marrow using commercially available tests. Clinical assessment was systematically performed by a team of rheumatologists and haematologists. Laboratory data and body weights were aggregated from referral records as well prospectively collected data at NIH. Daily glucocorticoid dose was aggregated from medical records of outside rheumatologists, hospital discharge summaries or NIH visits. Data regarding patients with GCA was only collected from visits at the NIH.

VEXAS patients were subgrouped using somatic variants in *UBA1* and CH with ≥ 10% VAF given CH with VAF ≥ 10% clones are expected to predate *UBA1* mutant clones [8].

Time of symptom onset was defined as the first day of the month in which patients first developed either symptoms consistent with VEXAS or GCA. Transfusion dependence was defined as requiring ≥ 4 units of any blood product over an 8-week period [22]. Event-free survival was defined as time from symptom onset to either transfusion dependence, haematopoietic stem cell (HSC) transplant or death. VEXAS-related disease manifestations were assessed at prospective NIH visits. Clinician assessment of clinical remission and changes in clinical symptom activity were assessed as previously reported using the French VEXAS (FRENVEX) definition of complete response (composite of clinical symptoms, glucocorticoid dose and C-reactive protein [CRP] levels) and VEXAS Current Activity Form (VEXASCAF, 0-11 score based on active symptoms within the last 28 days) disease activity measure, respectively [10,23,24]. Patient reported outcome of patient global assessment (PtGA) of disease severity was recorded on a visual analogue scale of 'how severe is your disease within the last 28 days?' (0 indicates least severe, 10 indicates most severe).

As time of disease onset may be subject to recall bias, laboratory values were assessed either continuously from symptom onset or in 3 time intervals, including 0 to 3 years (early), 3 to 6 years (mid), and 6 to 10 years (late). These specific time intervals were chosen based on event distribution in the survival curves.

Statistical analysis

All statistical analysis was performed using R statistical software (version 4.3.1) [25]. Survival analysis was performed using Cox-proportional hazard or Fine–Gray competing risk models with the *survival* and *tidycmprisk* packages [26,27]. Hazard ratios are reported for univariate analysis using prespecified grouping of patient characteristics as linear predictors. For multivariate analyses, a full model with all variables and a model with only significant predictors from the univariate analysis were assessed.

To assess laboratory values, generalised linear mixture models (GLMM) or generalised additive mixture models (GAMMs) were used to estimate means (95% confidence interval [CI]) with *lme4* [28] or *mgcv* [29] packages, respectively. Prespecified grouping (eg, GCA vs VEXAS, GCA vs Variant Groups), time intervals, and the interaction between grouping and time points were used as fixed effect predictors to assess changes over time. Data points were restricted to 10 years after symptom onset.

GAMMs were used to assess medication effects with fixed effects of time after symptom onset, time in relation to medication initiation, active medication use, and the interaction between initiation and active use. Data points were restricted for 2 year pre- and postmedication exposure for these models. If multiple agents from the same class were used, only the first exposure was assessed.

For all models involving repeated measures, random effects were included to delineate patient specific random intercepts. Error distributions for models were either binomial, negative binomial or Gaussian distributions depending on dependent variable. $P < .05$ was considered statistically significant for survival and competing risk models. *P* values are not reported for contrast testing in models assessing time varying effects given the high degree multiple hypothesis testing, rather mean difference and 95% CIs are reported, with significance interpreted in the context of all reported findings.

Role of funding source

The funding source did not have any role in in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

RESULTS

Cohort Characteristics and Disease Manifestations

In total, 80 patients with VEXAS and 81 patients with GCA were consecutively assessed at the NIH Clinical Center (Supplementary Fig S1). Nine VEXAS and 57 female GCA patients were excluded. The remaining 71 VEXAS patients had either p.M41L (n = 23, 32.4%), p.M41T (n = 29, 40.8%) or p.M41V (n = 19, 26.8%) mutations. In addition to *UBA1* variants, concomitant CH variants were assessed. $CH^{VAF\geq10\%}$ was predominantly defined by *DNMT3A* variants (Supplementary Table S1). None of the remaining 24 male patients with GCA had a detectable *UBA1* variant in peripheral blood based on error-corrected targeted sequencing.

Patients with VEXAS and GCA shared similar clinical characteristics (Table 1): all male, similar age at symptom onset and similar follow up duration after symptom onset. Transfusion dependence (19.7%), haematopoietic stem cell (HSC) transplantation (19.7%), and death (16.9%) were events that only occurred in the VEXAS patients during the follow-up period.

Table 1
Patient clinical characteristics

	GCA N = 24	VEXAS (p.M41) N = 71
Symptom onset age (years), median (IQR)	69.1 (62.2, 73.6)	62.3 (47.3, 68.0)
Male sex, n (%)	24 (100.0%)	71 (100.0%)
Time to diagnosis[a] (years), median (IQR)	0.1 (0.0, 0.3)	3.4 (2.0, 6.4)
Follow-up time (years), median (IQR)	4.6 (2.5, 7.2)	4.8 (3.2, 8.1)
Transfusion dependence, n (%)	0 (0.0%)	14 (19.7%)
HSC transplant, n (%)	0 (0.0%)	14 (19.7%)
Death, n (%)	0 (0.0%)	12 (16.9%)
Glucocorticoid ever, n (%)	24 (100.0%)	71 (100.0%)
Anti-IL6R or JAKi ever, n (%)	16 (66.7%)	58 (81.7%)
Anti-IL6R ever, n (%)	16 (66.7%)	48 (67.6%)
JAKi ever, n (%)	0 (0.0%)	27 (38.0%)
Hypomethylating agent ever, n (%)	0 (0.0%)	6 (8.5%)

anti-IL6R, anti-IL6 receptor monoclonal antibody antagonist; HSC; haematopoietic stem cell; IQR, interquartile range; JAKi, Janus kinase inhibitor.
[a] Diagnosis for VEXAS defined as genetic testing with the *UBA1* somatic variant.

All patients received glucocorticoids, and a comparable proportion of patients with VEXAS and GCA received treatment with anti-IL6R (n/N [%], tocilizumab 48/71 [67.6%] vs 16/24 [66.7%] and sarilumab 7/71 [9.8%] vs 0/24 [0.0%]). No GCA patients had received JAKi or hypomethylating agents. Regarding JAKi treatment, ruxolitinib (14/71, 19.7%), tofacitinib (12/71, 16.9%), upadacitinib (5/71, 7.0%) and baricitinib (3/71, 4.2%) were used. In VEXAS patients, there was also frequent exposure to immunomodulatory agents (44/71 [62.0%], hydroxychloroquine, colchicine, dapsone or IVIg), small molecule anti-metabolites (48/71 [67.6%], methotrexate, mycophenolate, leflunomide, azathioprine or sulfasalazine) and anti-tumour necrosis factor (TNF) (22/71, 31.0%) and anti-IL1 (13/71, 18.3%) therapies.

Event-free survival in genetically defined subsets of VEXAS

The composite event of transfusion dependence, HSC transplant or death was compared based on the presence of the p.M41 genotype and $CH^{VAF\geq10\%}$ (Supplementary Table S2). In VEXAS patients, p.M41V (hazard ratio [HR] [95% CI]: 12.2 [2.8–52.4]) and $CH^{VAF\geq10\%}$ (HR [95% CI]: 7.4 [1.6–34.0]) were independently associated with increased hazard of the composite events. There was a significant interaction with reduced effect of $CH^{VAF\geq10\%}$ in p.Met41Val subjects that was not observed in other variants. These results were robust to individual components of the composite event. Given that $CH^{VAF\geq10\%}$ may increase risk for events in p.M41L and p.M41T but not for p.M41V, subsequent subanalyses were conducted comparing the following 4 groups of VEXAS patients: p.M41L, p.M41T, p.M41L/T with $CH^{VAF\geq10\%}$, and p.M41V (Supplementary Table S3).

Increased hazard ratios of the composite events were observed for p.M41L/T with $CH^{VAF\geq10\%}$ and p.M41V when compared to p.M41L (Fig 1A, Table 2). Results were similar when adjusting for age at symptom onset, time to *UBA1* variant detection and ever exposure to anti-IL6R or JAKi therapy. There was no meaningful association between anti-IL6R monoclonal

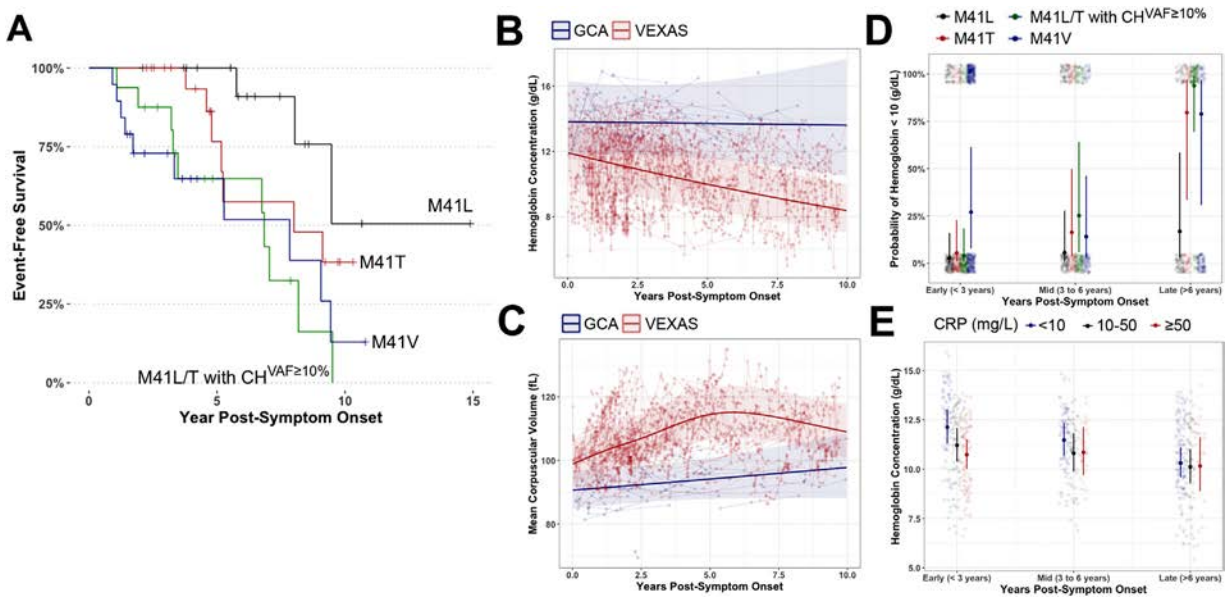


Figure 1. Disease trajectory determined by somatic variants. A, Composite event-free survival (composite outcome of transfusion dependence, HSC transplant or death) Kaplan–Meier curves for genetic groups of patients with VEXAS. Censoring denoted by hash marks. B, Haemoglobin concentration (g/dL). C, Mean corpuscular volume (fL). For B and C, patients with VEXAS (red) and GCA (blue) shown as individual points, and generalised additive mixture model estimated means (95% CI) are presented. D, Generalised linear mixture model (GLMM) estimated mean probability (95% CI) for moderate to severe anaemia (haemoglobin concentration < 10 g/dL) for each genetic group in patients with VEXAS at specified time intervals (early, mid and late). E, Haemoglobin concentration (g/dL) for patients with VEXAS stratified by C-reactive protein (CRP) concentration (mg/L) GLMM estimated means (95% CI). B-E, All transparent marks represent individual data points, all solid points and lines represent means, and 95% CI represented as shaded ribbon or solid error bars.

Table 2
Event-free survival analysis in patients with VEXAS

	Univariate				Multivariate[a]				Multivariate Full[b]			
	HR	95% CI		*P*-value	aHR	95% CI		*P*-value	aHR	95% CI		*P*-value
Genetic Group												
p.M41L	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
p.M41T	2.4	0.6	9.1	.21	1.6	0.4	6.5	.56	1.74	0.43	6.99	.43
p.M41L/T with CH$^{VAF\geq10\%}$	**5.7**	**1.6**	**20.8**	**.01**	**4.6**	**1.2**	**14.8**	**.02**	**4.41**	**1.08**	**18.00**	**.04**
p.M41V	**5.6**	**1.5**	**20.4**	**.01**	**4.6**	**1.2**	**17.7**	**.03**	**4.70**	**1.14**	**19.30**	**.03**
Time to diagnosis (years)[c]	**0.6**	**0.5**	**0.7**	**<.01**	**0.6**	**0.4**	**0.7**	**<.01**	**0.91**	**0.86**	**0.97**	**<.001**
Age at symptom onset (years)[c]	1.0	0.9	1.0	.28	–	–	–	–	**0.92**	**0.87**	**0.97**	**<.01**
Exposure to anti-IL6R ever (yes)	1.3	0.6	2.7	.54	–	–	–	–	0.76	0.32	1.79	.52
Exposure to JAKi ever (yes)	1.2	0.6	2.5	.57	–	–	–	–	1.00	0.42	2.40	.99
NCI Comorbidity Index (0-10)[c]	1.1	0.9	1.3	.45	–	–	–	–	1.14	0.98	1.47	.17

aHR, adjusted hazard ratio, anti-IL6R, anti-IL6 receptor monoclonal antibody antagonist, CI, confidence interval, JAKi, Janus kinase inhibitor, NCI, National Cancer Institute.
Note: Bold values are statistically significant.
[a] Model was adjusted using any confounder(s) that were significant in univariate analysis: time to diagnosis.
[b] Model was adjusted using all confounder that were included in univariate analysis: age at symptom onset, time to diagnosis, exposure to anti-IL6R mAb, exposure to JAKi and NCI Comorbidity Index.
[c] Per 1 unit of exposure (eg, change in 1 year increase or 1 unit increase in 0-10 scale).

antibodies (mAb) or JAKi treatment and event-free survival in univariate analysis.

Trajectories of ineffective haematopoiesis in VEXAS

Anaemia was assessed at 1790 unique time points in patients with VEXAS compared to GCA (Fig 1B). Mean haemoglobin concentration was significantly lower at all time intervals in VEXAS compared to GCA and decreased over time only in VEXAS. Mean corpuscular volume (MCV) was significantly increased in VEXAS with a nonlinear trajectory compared to GCA (Fig 1C). The probabilities (95% CI) of moderate to severe anaemia (haemoglobin concentration < 10 g/dL) across all VEXAS patients were 5.7% (2.4, 13.0%), 13.8% (6.0, 28.2%), and 80.5% (60.0, 92.0%) for early, mid and late time intervals, respectively. The probabilities (95% CI) of macrocytosis (MCV > 100 fL) across all VEXAS patients were 78.2% (63.1, 88.3%), 97.4% (94.2, 98.9%), and 95.8% (90.8, 98.1%) for early, mid and late time intervals, respectively. The disease duration adjusted odds of moderate to severe anaemia was significantly higher in patients with p.M41V (OR [95% CI]: 13.4 [1.1, 157.5]) compared to those with p.M41L. The probability of moderate to severe anaemia was most notably increased for patients with p.M41V at the early time interval with a probability (95% CI) of 27.0% (7.5, 61.4%) (Fig 1D).

Mean values (95% CI) were also estimated for components of the complete blood count across VEXAS genetic groups at time intervals (Supplementary Table S4). Platelet counts displayed

Table 3
First anti-IL6R or JAKi exposure in VEXAS patients

	Anti-IL6R N = 48	JAKi N = 27
Time to initiation, years (IQR)	2.8 (1.8, 5.0)	3.2 (1.9, 5.7)
Follow-up time after initiation, days (IQR)	212 (110, 457)	197 (122, 309)
Discontinued during follow-up, n (%)	28 (58.3%)	19 (70.4%)
Reason for discontinuation, n (%)		
Lack of efficacy	18 (37.5%)	14 (51.8%)
Multiple	8 (16.7%)	8 (29.6%)
HSC transplant	4 (8.3%)	2 (7.4%)
Infection	2 (4.2%)	3 (11.1%)
Neutropenia	3 (6.3%)	1 (3.7%)
Death	2 (4.2%)	2 (7.4%)
Anaemia	0 (0.0%)	4 (15.8%)
Injection site reaction	3 (6.3%)	0 (0.0%)
Systemic infusion reaction	2 (4.2%)	0 (0.0%)
Thrombocytopaenia	1 (2.1%)	1 (3.7%)
Gastrointestinal intolerance	0 (0.0%)	2 (7.4%)
Diverticulitis	1 (2.1%)	0 (0.0%)
Headache	0 (0.0%)	1 (3.7%)

anti-IL6R, anti-IL6 receptor monoclonal antibody antagonist; HSC, haematopoietic stem cell; IQR, interquartile range; JAKi, Janus kinase inhibitor.

progressive declines in each group to varying degrees. Across time points, lower average lymphocyte and monocyte counts were observed in VEXAS compared to GCA, although p.M41L showed relative preservation of these lineages. p.M41V notably had low monocyte counts across all time intervals. Due to a lower number of repeated measures, peripheral lymphocyte subsets were assessed across all time points (Supplementary Table S5).

Effects of systemic inflammation on haematopoiesis are time dependent

Acute phase response was estimated using C-reactive protein (CRP) concentration (Supplementary Table S6). p.M41V demonstrated the highest levels of CRP at the early and mid-time intervals. All groups apart from p.M41T showed reduction in CRP values over time to varying degrees.

To relate systemic inflammation to haematopoiesis, blood counts were assessed as a function of CRP and time in VEXAS (Fig 1E). Most notably, the effect of elevated CRP levels on erythropoiesis diminished over time in VEXAS. However, the effects of elevated CRP levels on myeloid lineages were stable or potentially expanded over time (Supplementary Fig S2). Lymphocyte and platelet effects were less robust and only observed in individuals with the highest CRP concentrations.

To determine whether intervention by immunosuppression with inhibition of suspected key cytokines effects haematopoiesis, blood counts were assessed in relationship to initiation of anti-IL6R or JAKi treatment (Table 3). The median time to initiation (IQR) was 2.8 (1.8, 5.0) and 3.2 (1.9, 5.7) years after symptom onset for anti-IL6R and JAKi treatment, respectively. The median duration of follow up after therapy initiation (IQR) was 212 (110, 457) and 197 (122, 309) days for anti-IL-6R and JAKi treatment, respectively. Discontinuation of anti-IL6R therapy occurred in 28 out of 48 patients (58.3%) and was due to lack of efficacy (18/48, 37.5%), multiple aetiologies (8/48, 16.7%), HSC transplant (4/48, 8.3%), injection site reactions (3/48, 6.3%), neutropenia (3/48, 6.3%), infection (2/48, 4.2%), systemic infusion reaction (2/48, 4.2%), death (2/48, 4.2%), diverticulitis (1/48, 2.1%) and thrombocytopenia (1/48, 2.1%). Discontinuation of JAKi therapy occurred in 19/27 patients (70.4%) and was due to lack of efficacy (14/27, 51.8%), multiple aetiologies (8/27, 29.6%), anaemia (4/27, 14.8%), infection (3/27, 11.1%), gastrointestinal intolerance (2/27, 7.4%), HSC transplant (2/27, 7.4%), death (2/27, 7.4%), neutropenia (1/27, 3.7%), thrombocytopenia (1/27, 3.7%) and headache (1/27, 3.7%).

Mean CRP was reduced after initiation of anti-IL6R but did not normalise in all individuals, whereas JAKis were not observed to lower CRP (Fig 2A, B). There were baseline differences in estimated mean CRP levels (95% CI) between patients initiating anti-IL6R and JAKi treatment: 56.9 (29.0, 111.7) versus 23.4 (11.2, 48.9) mg/L, respectively. Estimated mean (95% CI) CRP 1 year after anti-IL6R initiation was 9.7 (4.8, 19.8) mg/L in those continuing anti-IL6R compared to 57.3 (27.8, 118.3) mg/L in those who had discontinued, which was similar to CRP levels prior to initiation of treatment. Estimated mean (95% CI) CRP levels 1 year after JAKis were 22.5 (9.5, 53.6) mg/L in those continuing JAKis compared to 18.1 (8.8, 37.3) mg/L in those who had discontinued.

Haemoglobin concentration increased with exposure to anti-IL6R (Fig 2C), although the subsequent rate of decline was similar to patients who discontinued therapy. Estimated mean (95% CI) haemoglobin concentrations at 1 year after anti-IL6R were 12.4 (10.9, 14.3) g/dL in those continuing anti-IL6R compared to 11.8 (10.3, 13.4) mg/L in those who had discontinued. The changes in neutrophil, monocyte, lymphocyte and platelet counts post-exposure to anti-IL6R were more modest (Supplementary Fig S3A-D). In contrast, JAKi use was associated with lower estimate mean haemoglobin concentration (95% CI) at 1 year post exposure 10.5 (9.1, 12.5) g/dL compared to those who discontinued JAKi treatment at 11.8 (10.2, 13.5) g/dL (Fig 2D). Changes in other haematopoietic cell counts after JAKi exposure appeared transient, and differences over time were small (Supplementary Fig S3E-H).

Glucocorticoid exposure and metabolic toxicity in VEXAS

Daily glucocorticoid exposure was studied at 910 unique clinical encounters (Table 3). There were no significant differences between VEXAS and GCA at the early time interval (estimated mean difference [95% CI]: 4.1 [-2.4, 10.5] mg/day). Whereas, at later time intervals, VEXAS patients had significantly higher glucocorticoid exposure (estimated mean difference [95% CI]: mid 16.3 [12.1, 20.6], late 11.2 [1.9, 20.5] mg/day). Notably, subjects with p.M41V had the highest exposure to glucocorticoids, particularly at the early (estimated mean [95% CI]: 27.5 [22.1, 34.1] mg/day) and mid (estimated mean [95% CI]: 28.5 [21.5, 37.8] mg/day) time intervals (Table 4).

Patients continuing anti-IL6R or JAKi treatment for 1 year minimally differed in the trajectory of glucocorticoid exposure compared those who discontinued therapy. The estimated mean (95% CI) prednisone equivalent daily doses for patients continuing anti-IL6R 1 year after initiation were 13.1 (9.3, 18.6) versus 16.2 (11.2, 23.5) mg/day in those who discontinued with a mean difference (95% CI) of −3.4 (−7.4, 0.6) mg/day. The estimated mean (95% CI) prednisone equivalent daily dose for patients continuing JAKi treatment 1 year was 10.4 (5.7, 18.9) versus 10.8 (6.8, 17.1) mg/day in those who discontinued.

When restricting assessments to 148 study visits at the NIH Clinical Center (Table 5) [23,24], patients with VEXAS were taking ≤ 10 mg daily prednisone equivalents during 36 (24.3%) of these study visits and achieved the FRENVEX definition of complete response at only 4 (2.7%) of the study visits. VEXASCAF assessment demonstrated ongoing disease activity with few

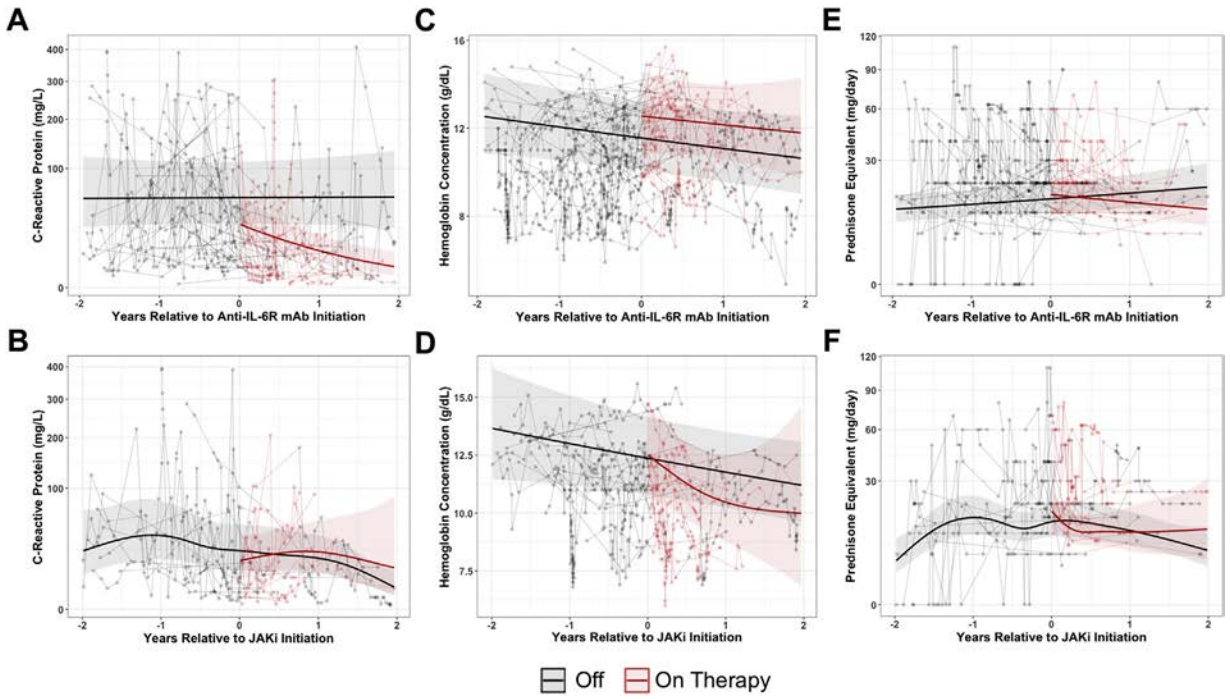


Figure 2. Effects of anti-IL-6R and JAKi therapy on systemic inflammation, anaemia and steroid exposure. A-B, For patient with VEXAS, C-reactive protein concentration (mg/L) shown as individual points and generalised additive mixture model (GAMM) mean estimates (95% CI) for time points in relation to exposure to anti-IL6R (A) or JAKi (B) therapies. C-D, Haemoglobin concentration (g/dL) shown as individual points and GAMM mean estimates (95% CI) for time points in relation to exposure to anti-IL6R (C) or JAKi (D) therapies. E-F, Prednisone equivalents (mg/day) shown as individual points and GAMM mean estimates (95% CI) for time points in patients with VEXAS with relation to exposure to anti-IL6R (E) or JAKi (F) therapies. All transparent marks represent individual data points. All solid lines represent means, and 95% CIs are represented as shaded ribbons.

Table 4
Glucocorticoid exposure and metabolic toxicity in patients with VEXAS

		Daily Glucocorticoid Dose (mg/day)[a]				Random Blood Glucose (mg/dL)				Body Weight (kg)			
		n	Mean	95% CI		n	Mean	95% CI		n	Mean	95% CI	
GCA	Early	34	17.0	12.3	23.4	41	101.1	89.9	113.8	22	79.3	72.0	87.2
	Mid	13	5.4	3.2	9.1	27	96.8	84.3	111.2	22	81.6	74.1	89.9
	Late	5	11.0	5.2	23.0	13	97.9	81.4	117.8	7	80.4	70.2	92.0
p.M41L	Early	45	23.3	16.5	32.8	109	105.7	92.0	121.4	35	85.8	77.3	95.1
	Mid	50	25.8	19.0	34.9	48	122.9	107.2	140.8	47	89.9	81.8	98.8
	Late	59	21.8	16.2	29.5	55	117.3	100.8	136.4	50	94.4	85.8	103.9
p.M41T	Early	128	15.2	12.3	18.8	92	101.2	90.9	112.8	126	83.6	77.6	90.0
	Mid	88	16.4	13.0	20.6	80	106.7	95.2	119.6	92	87.9	81.4	94.9
	Late	64	20.0	15.0	26.6	63	110.1	97.3	124.5	50	83.8	77.2	90.9
p.M41L/T with $CH^{VAF\geq10\%}$	Early	61	20.3	15.1	27.2	98	124.8	110.8	140.5	70	90.6	83.1	98.9
	Mid	57	19.8	15.1	25.9	92	121.3	107.9	136.3	64	91.2	83.8	99.2
	Late	40	25.8	18.3	36.5	77	137.2	118.9	158.3	112	90.1	82.2	98.8
p.M41V	Early	171	27.5	22.1	34.1	223	127.6	114.9	141.6	362	87.0	80.6	93.8
	Mid	60	28.5	21.5	37.8	71	138.1	121.8	156.7	48	87.5	80.5	95.2
	Late	35	19.5	13.5	28.2	107	130.7	112.9	151.3	16	87.1	78.9	96.1

[a] Measured in prednisone equivalents.

differences over time when assessed throughout the disease course. PtGA reporting of disease severity was increased in the overall VEXAS population with disease duration.

Metabolic toxicity was assessed by several metrics including weight, random blood glucose, triglyceride and HDL measurements (Table 4, Supplementary Table S7). At early time points, there were differences favouring a more disrupted metabolic state in VEXAS compared to GCA. Additionally, within VEXAS groups there was general trend of values increasing in direction that favoured a worse dysmetabolic state which was not observed in GCA. Significant renal or hepatic injury was not observed in the cohort (data not shown).

DISCUSSION

This study details the single centre experience of patients with VEXAS who were directly and systematically evaluated by a multidisciplinary team. Patients with VEXAS syndrome and canonical p.M41 variants exhibit a spectrum of inflammatory states, dysfunctional haematopoiesis and mortality based on specific variants in *UBA1* and presence of other somatic haematopoietic clonal populations. Patients with VEXAS have high exposure to glucocorticoids over long periods of time, rarely achieve clinical remission without sustained moderate to high-dose glucocorticoid administration and are prone to a range of

Table 5
VEXAS disease activity stratified by group and time

		N	PtGA 28 day Median (IQR)	VEXASCAF[a] Median (IQR)	CR[b]	No Active Symptoms	CRP ≤ 10 mg/L	GC ≤ 10 mg/day
p.M41L	Early	8	0.0 (0.0, 0.0)	0.0 (0.0, 1.0)	0 (0%)	2 (25.0%)	2 (25.0%)	0 (0%)
	Mid	10	7.0 (6.0, 7.5)	2.0 (1.0, 2.0)	0 (0%)	2 (20.0%)	8 (80.0%)	1 (10.0%)
	Late	14	7.0 (4.0, 8.5)	0.5 (0.0, 3.0)	1 (7.1%)	8 (57.1%)	8 (57.1%)	2 (14.3%)
p.M41T	Early	13	5.0 (2.3, 6.0)	1.0 (1.0, 3.0)	1 (7.7%)	8 (61.5%)	8 (61.5%)	4 (30.8%)
	Mid	16	4.0 (3.0, 6.0)	2.0 (1.0, 4.0)	1 (6.3%)	4 (25.0%)	9 (56.3%)	9 (56.3%)
	Late	17	4.0 (3.0, 5.0)	1.0 (1.0, 3.0)	0 (0%)	1 (5.9%)	8 (47.1%)	12 (70.6%)
p.M41L/T with $CH^{VAF\geq10\%}$	Early	8	6.0 (5.0, 6.0)	1.5 (1.0, 3.3)	0 (0%)	2 (25.0%)	4 (50.0%)	1 (12.5%)
	Mid	8	7.0 (6.3, 9.3)	2.5 (1.0, 3.3)	1 (12.5%)	2 (25.0%)	8 (100%)	3 (37.5%)
	Late	16	3.5 (2.0, 6.0)	1.0 (0.0, 2.5)	0 (0%)	9 (56.3%)	10 (62.5%)	2 (12.5%)
p.M41V	Early	16	3.0 (0.0, 7.5)	2.0 (1.0, 3.0)	0 (0%)	4 (25.0%)	9 (56.3%)	0 (0%)
	Mid	10	5.0 (4.0, 7.0)	1.5 (1.0, 2.0)	0 (0%)	5 (50.0%)	8 (80.0%)	2 (20.0%)
	Late	12	3.5 (1.3, 6.5)	2.5 (0.0, 4.0)	0 (0%)	4 (33.3%)	10 (83.3%)	0 (0%)
VEXAS Overall	Early	45	3.0 (0.0, 6.0)	1.0 (1.0, 3.0)	1 (2.2%)	16 (35.6%)	23 (51.1%)	5 (11.1%)
	Mid	44	6.0 (4.0, 7.0)	2.0 (1.0, 3.0)	2 (4.3%)	13 (28.3%)	35 (76.1%)	15 (32.6%)
	Late	59	5.0 (2.3, 6.8)	1.0 (0.0, 3.0)	1 (1.7%)	22 (37.3%)	36 (61.0%)	16 (27.1%)

CR, complete response, CRP, C-reactive protein; GC, prednisone equivalent daily glucocorticoid dose; IQR, interquartile range; PtGA, Patient Global Assessment.
[a] As defined by Kirino et al. [23].
[b] As defined by Hadjadj et al. [24].

glucocorticoid-associated toxicities. Use of anti-IL6R or JAKi therapies, the most frequently employed nonglucocorticoid immunosuppressive therapies in VEXAS, did not clearly demonstrate a haematologic disease-modifying effect nor did these medications result in substantial glucocorticoid reduction.

This study, consistent with prior reports, demonstrates patients with the p.M41V variant have a worse overall prognosis compared to patients with the p.M41L variant [5,7]. Increased probability of moderate to severe anaemia was observed earlier after symptom onset in p.M41V. These patients also had features suggesting a more toxic effect of the *UBA1* variant on other cell lines including lower monocyte counts across all time points and more progressive lymphopenia, whereas these cell lineages were relatively preserved in patients with p.M41L variants. Worse clinical outcomes may be due to increased deficiency in cytoplasmic UBA1, which contributes to haematologic toxicity and increased inflammation in these patients [30,31]. Accordingly, incremental enzymatic inhibition of UBA1 in myeloid cells with p.M41L mutations leads to further cytotoxicity *in vitro* [32] and suggests a narrow window of tolerance to this mutation with p.M41V abutting the level of tolerance to UBA1 deficiency.

Concomitant CH mutations independently predict mortality in VEXAS [8]. The current study demonstrates an additional novel interaction effect between $CH^{VAF\geq10\%}$ status and the *UBA1* genotype. The presence of $CH^{VAF\geq10\%}$ clones modified outcomes for patients with p.M41L or p.M41T, conferring increased risk for transfusion dependence, HSC transplant or death. Such an effect was not observed with p.M41V mutations. Larger populations with VEXAS and typical CH will be required to further dissect these complex interactions.

Most VEXAS patients who received anti-IL6R mAbs showed a significant reduction in CRP levels. However, active symptoms persisted in a proportion of patients, and few patients reached current definitions of medication response despite combination immunosuppression, a finding consistent with other recent cohort studies [23]. Of interest, a similar reduction in CRP levels was not observed in individuals treated with JAKis, although there were lower average baseline CRP levels compared to those initiating anti-IL6R, possibly secondary to patients with higher acute phase reactants being biased towards receiving anti-IL6R rather than JAKi therapy. Given the known side effects of specific cytopenias and adverse events of both medication classes, it is likely there are other selection biases that potentially contribute to the observed findings. Exact conclusions about efficacy regarding these therapies in comparison with each other cannot be inferred given these likely confounding effects.

As disease duration increased, haematologic differences dissociated from systemic inflammation. Concordantly, anti-IL6R therapy led to an increase in haemoglobin levels without changing the overall trajectory of progressive anaemia. JAKis, which have been effective in some forms of marrow failure [33], appeared to potentially worsen anaemia in VEXAS patients. This effect may be due to the frequent use of ATP-competitive, non-selective JAKis, that cause a reduction in JAK2 signalling and increase inhibition of off target kinases [34]. The lack of significant glucocorticoid sparing and modification of dysfunctional haematopoiesis with either class of medication strongly implies the need for more effective therapies in VEXAS.

Glucocorticoid exposure across time in patients with VEXAS was associated with increased metabolic derangement, including hyperglycaemia and dyslipidaemia. Prior studies have shown increased infectious risk in patients with VEXAS, and opportunistic infections have been observed [16,17]. Glucocorticoid toxicity likely contributes significantly to patient morbidity, as cumulative dose-related effects have been observed in aged populations with inflammatory conditions, such as GCA [35,36]. Future clinical trials should assess steroid-sparing effect, and achievement of clinical remission on ≤ 10 mg daily prednisone will be an important clinical outcome measure.

Although it was not our purpose to define diagnostics, we did observe some striking differences in haematologic parameters between the GCA and VEXAS patients. Macrocytic anaemia has been a defining feature of VEXAS [37,38]. While macrocytosis was observed in most patients with VEXAS, approximately 1 in 4 patients have an MCV < 100 fL early in the course of the disease. In contrast monocytopenia and specific lymphopenias, such as B-cell and natural killer cell (NK)-cell lymphopenia, may be present earlier in the disease. Diagnostic algorithms that include MCV have been proposed [15,39], but it will be important to validate these approaches across a broad range of parameters, disease duration and against other haematologic measurements.

While this study improves upon existing literature by assessing disease features over long periods of time, there are several potential limitations. While we were not able to demonstrate benefit with regards to haematologic trajectory or glucocorticoid sparing, further exploration is required to determine whether these medications affect other important outcomes, such as specific symptom burden, hospitalisations and quality of life. Some of the results are derived from retrospective assessments, which may introduce multiple areas of bias. However, several designs of the analysis attempt to address these issues. With regards to recall bias and identifying symptom onset, patients were assessed using objective measures (eg, complete blood counts) over several large intervals of time, which reaffirmed findings of increased severity in patients with p.M41L/T with $CH^{VAF\geq10\%}$ and p.M41V in survival analyses. Data were captured from outside records as well as prospectively at NIH Clinical Center visits for VEXAS patients, while data for patients with GCA were only captured from NIH visits, which may introduce assessment bias for both patient populations with missing data. Although this cannot be accounted for completely, the parallel analyses assessing multiple clinical parameters reinforces findings of higher glucocorticoid exposure and metabolic toxicity in VEXAS. Last, survival bias may impact laboratory values at later time intervals.

In summary, patients with VEXAS are at risk for progression to marrow failure, and variant-specific effects along with other clonal haematopoietic events likely play a role in the rapidity of marrow failure. Early after symptom onset, reduced erythroid and thrombocyte output occurs in an inflammatory setting. However, later in disease, dysfunctional haematopoiesis occurs regardless of the inflammatory state. Despite the highly prevalent use of anit-IL6R mAbs, which do not usually suppress red blood cell production, in combination with moderate to high doses of daily glucocorticoids, anaemia is progressive in most VEXAS patients. Newer or alternative combinations of therapies will need to address both inflammatory symptoms and marrow failure in patients with VEXAS. When evaluating therapies in the future, it will be important to account for the clonal landscape of disease as well as disease duration, each of which might influence the probability of achieving important clinical end points.

FUNDING

This study was supported by the NIH Intramural Research Programs of the National Institute of Arthritis and Musculoskeletal and Skin Diseases and the National Heart, Lung, and Blood Institute.

Competing interests

All authors declare they have no competing interests.

CRediT authorship contribution statement

Benjamin A. Turturice: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alice Fike:** Writing – review & editing, Investigation, Data curation. **Bhavisha A. Patel:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Emma M. Groarke:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Fernanda Gutierrez-Rodriguez:** Writing – review & editing, Formal analysis, Data curation. **Karyssa Stonick:** Writing – review & editing, Investigation, Data curation. **Hannah Berrett:** Writing – review & editing, Investigation, Conceptualization. **Shanni Liu:** Writing – review & editing, Project administration, Investigation, Data curation. **Ivana Darden:** Writing – review & editing, Project administration, Investigation, Data curation. **Kaitlin A. Quinn:** Writing – review & editing, Investigation, Data curation. **Neal S. Young:** Writing – review & editing, Project administration, Investigation, Funding acquisition. **Peter C. Grayson:** Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Patient consent for publication

Written consent was obtained from each patient prior to participation.

Ethics approval

This study was reviewed and approved by an institutional review board approved of the National Institutes of Health.

Provenance and peer review

Not commissioned; externally peer reviewed.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.05.021.

Orcid

Peter C. Grayson: http://orcid.org/0000-0002-8269-9438

REFERENCES

[1] Beck DB, Ferrada MA, Sikora KA, Ombrello AK, Collins JC, Pei W, et al. Somatic mutations in UBA1 and severe adult-onset autoinflammatory disease. N Engl J Med 2020;383(27):2628–38.
[2] Collins JC, Magaziner SJ, English M, Hassan B, Chen X, Balanda N, et al. Shared and distinct mechanisms of UBA1 inactivation across different diseases. EMBO J 2024;43(10):1919–46.
[3] Beck DB, Bodian DL, Shah V, Mirshahi UL, Kim J, Ding Y, et al. Estimated prevalence and clinical manifestations of UBA1 variants associated with VEXAS syndrome in a clinical population. JAMA 2023;329(4):318–24.
[4] Tan IJ, Ferrada MA, Ahmad S, Fike A, Quinn KA, Groarke EM, et al. Skin manifestations of VEXAS syndrome and associated genotypes. JAMA Dermatol 2024;160(8):822–9.
[5] Georgin-Lavialle S, Terrier B, Guedon AF, Heiblig M, Comont T, Lazaro E, et al. Further characterization of clinical and laboratory features in VEXAS syndrome: large-scale analysis of a multicentre case series of 116 French patients. Br J Dermatol 2022;186(3):564–74.
[6] Kusne Y, Ghorbanzadeh A, Dulau-Florea A, Shalhoub R, Alcedo PE, Nghiem K, et al. Venous and arterial thrombosis in patients with VEXAS syndrome. Blood 2024;143(21):2190–200.
[7] Ferrada MA, Savic S, Cardona DO, Collins JC, Alessi H, Gutierrez-Rodrigues F, et al. Translation of cytoplasmic UBA1 contributes to VEXAS syndrome pathogenesis. Blood 2022;140(13):1496–506.
[8] Gutierrez-Rodrigues F, Kusne Y, Fernandez J, Lasho T, Shalhoub R, Ma X, et al. Spectrum of clonal hematopoiesis in VEXAS syndrome. Blood 2023;142(3):244–59.
[9] Mekinian A, Zhao LP, Chevret S, Desseaux K, Pascal L, Comont T, et al. A phase II prospective trial of azacitidine in steroid-dependent or refractory systemic autoimmune/inflammatory disorders and VEXAS syndrome associated with MDS and CMML. Leukemia 2022;36(11):2739–42.
[10] Heiblig M, Ferrada MA, Koster MJ, Barba T, Gerfaud-Valentin M, Mekinian A, et al. Ruxolitinib is more effective than other JAK inhibitors to treat VEXAS syndrome: a retrospective multicenter study. Blood 2022;140(8):927–31.

[11] Johansen MM, El Fassi D, Nielsen CTH, Krintel SB, Graudal N, Hansen JW. Treatment experiences with focus on IL-6R inhibition in patients with VEXAS-syndrome and a case of remission with azacytidine treatment. Rheumatology (Oxford) 2025;64(2):826–30.
[12] Choy EH, De Benedetti F, Takeuchi T, Hashizume M, John MR, Kishimoto T. Translating IL-6 biology into effective treatments. Nat Rev Rheumatol 2020;16(6):335–45.
[13] Tanaka Y, Luo Y, O'Shea JJ, Nakayamada S. Janus kinase-targeting therapies in rheumatology: a mechanisms-based approach. Nat Rev Rheumatol 2022;18(3):133–45.
[14] Khitri MY, Guedon AF, Georgin-Lavialle S, Terrier B, Saadoun D, Seguier J, et al. Comparison between idiopathic and VEXAS-relapsing polychondritis: analysis of a French case series of 95 patients. RMD Open 2022;8(2):e002255.
[15] Ferrada MA, Sikora KA, Luo Y, Wells KV, Patel B, Groarke EM, et al. Somatic mutations in UBA1 define a distinct subset of relapsing polychondritis patients With VEXAS. Arthritis Rheumatol 2021;73(10):1886–95.
[16] Czech M, Cuellar-Rodriguez J, Patel BA, Groarke EM, Cowen EW, Turturice B, et al. Opportunistic infections, mortality risk, and prevention strategies in patients with vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic (VEXAS) syndrome. Open Forum Infect Dis 2024;11(7):ofae405.
[17] de Valence B, Delaune M, Nguyen Y, Jachiet V, Heiblig M, Jean A, et al. Serious infections in patients with VEXAS syndrome: data from the French VEXAS registry. Ann Rheum Dis 2024;83(3):372–81.
[18] Li KJ, Semenov D, Turk M, Pope J. A meta-analysis of the epidemiology of giant cell arteritis across time and space. Arthritis Res Ther 2021;23(1):82.
[19] Pugh D, Karabayas M, Basu N, Cid MC, Goel R, Goodyear CS, et al. Large-vessel vasculitis. Nat Rev Dis Primers 2022;7(1):93.
[20] Maz M, Chung SA, Abril A, Langford CA, Gorelik M, Guyatt G, et al. 2021 American College of Rheumatology/Vasculitis Foundation guideline for the management of giant cell arteritis and Takayasu arteritis. Arthritis Rheumatol 2021;73(8):1349–65.
[21] Ponte C, Grayson PC, Robson JC, Suppiah R, Gribbons KB, Judge A, et al. 2022 American College of Rheumatology/EULAR Classification criteria for giant cell arteritis. Arthritis Rheumatol 2022;74(12):1881–9.
[22] Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 2006;108(2):419–25.
[23] Kirino Y, Maeda A, Asano T, Migita K, Hidaka Y, Ida H, et al. Low remission rates and high incidence of adverse events in a prospective VEXAS syndrome registry. Rheumatology (Oxford) 2025;64(6):3872–8.
[24] Hadjadj J, Nguyen Y, Mouloudj D, Bourguiba R, Heiblig M, Aloui H, et al. Efficacy and safety of targeted therapies in VEXAS syndrome: retrospective study from the FRENVEX. Ann Rheum Dis 2024;83(10):1358–67.
[25] Team RC. R: A language and environment for statistical computing. R Foundation for Statistical Computing; 2017. https://www.r-project.org/.
[26] Terry M, Therneau TL, Atkinson Elizabeth, Crowson Cynthia. Survival Analysis. 3.7-0 ed. CRAN2024. https://cran.r-project.org/web/packages/survival/index.html.
[27] Sjoberg DD, Fei T. tidycmprsk: competing risks estimation. https://cran.r-project.org/web/packages/tidycmprsk/index.html.
[28] Bates D, Mächler M, Bolker BM, Walker SC. Fitting linear mixed-effects models using lme4. J Stat Softw 2015;67(1):1–48.
[29] Wood SN. Generalized Additive Models; 2017. https://doi.org/10.1201/9781315370279.
[30] Wu Z, Gao S, Gao Q, Patel BA, Groarke EM, Feng X, et al. Early activation of inflammatory pathways in UBA1-mutated hematopoietic stem and progenitor cells in VEXAS. Cell Rep Med 2023;4(8):101160.
[31] Kosmider O, Posseme C, Temple M, Corneau A, Carbone F, Duroyon E, et al. VEXAS syndrome is characterized by inflammasome activation and monocyte dysregulation. Nat Commun 2024;15(1):910.
[32] Chiaramida A, Obwar SG, Nordstrom AEH, Ericsson M, Saldanha A, Ivanova EV, et al. Sensitivity to targeted UBA1 inhibition in a myeloid cell line model of VEXAS syndrome. Blood Adv 2023;7(24):7445–56.
[33] Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med 2012;366(9):799–807.
[34] Taylor PC, Choy E, Baraliakos X, Szekanecz Z, Xavier RM, Isaacs JD, et al. Differential properties of Janus kinase inhibitors in the treatment of immune-mediated inflammatory diseases. Rheumatology (Oxford) 2024;63(2):298–308.
[35] Gale S, Wilson JC, Chia J, Trinh H, Tuckwell K, Collinson N, et al. Risk associated with cumulative oral glucocorticoid use in patients with giant cell arteritis in real-world databases from the USA and UK. Rheumatol Ther 2018;5(2):327–40.
[36] Scherbacher PJ, Hellmich B, Feng YS, Loffler C. Prospective study of complications and sequelae of glucocorticoid therapy in ANCA-associated vasculitis. RMD Open 2024;10(1):e003956.
[37] Obiorah IE, Patel BA, Groarke EM, Wang W, Trick M, Ombrello AK, et al. Benign and malignant hematologic manifestations in patients with VEXAS syndrome due to somatic mutations in UBA1. Blood Adv 2021;5(16):3203–15.
[38] Gurnari C, Pascale MR, Vitale A, Diral E, Tomelleri A, Galossi E, et al. Diagnostic capabilities, clinical features, and longitudinal UBA1 clonal dynamics of a nationwide VEXAS cohort. Am J Hematol 2024;99(2):254–62.
[39] Maeda A, Tsuchida N, Uchiyama Y, Horita N, Kobayashi S, Kishimoto M, et al. Efficient detection of somatic UBA1 variants and clinical scoring system predicting patients with variants in VEXAS syndrome. Rheumatology (Oxford) 2024;63(8):2056–64.

Ann Rheum Dis 85 (2026) 379–387

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Crystal arthropathies

Ultrasound-detected crystal depositions and clinical flares dissolve during successful urate-lowering therapy: 5-year follow-up results from the treat-to-target NOR-Gout study

Hilde Berner Hammer[1,2,*], Lars Karoliussen[1], Lene Terslev[3,4], Espen A. Haavardsholm[1,2], Till Uhlig[1,2]

[1] *Center for Treatment of Rheumatic and Musculoskeletal Diseases (REMEDY), Diakonhjemmet Hospital, Oslo, Norway*
[2] *Faculty of Medicine, University of Oslo, Oslo, Norway*
[3] *Rigshospitalet, Centre for Rheumatology and Spine Diseases, Glostrup, Copenhagen, Denmark*
[4] *Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark*

ARTICLE INFO

Article history:
Received 4 June 2025
Received in revised form 22 August 2025
Accepted 27 October 2025

ABSTRACT

Objectives: The aims of this 5-year study were to explore the maintenance of low serum uric acid (sUA) during treat-to-target urate-lowering therapy (T2T-ULT) and whether long-term low sUA results in continuous decrease of ultrasound-detected crystal depositions, complete dissolution of depositions, and reduction of flares.

Methods: Patients with a recent gout flare were included, initiated T2T-ULT, and were followed for 5 years (outpatient clinic year 1, general practitioner years 2-5) with visits at baseline, 1, 2, and 5 years. Each visit included assessment of sUA, ultrasound examination for crystal depositions (double contour, tophi, and aggregates) in bilateral hands, elbows, knees, ankles, feet, and number of flares the previous year. Calculations included independent or paired samples *t*-tests, analysis of variance (ANOVA), and linear regression analyses.

Results: Of the 209 patients included at baseline (mean age 56.4 years, disease duration 7.9 years), 163 patients were examined at 5 years. At the 5-year visit, sUA was reduced from 500 (baseline) to 337 μmol/L ($P < .001$), 71.2% had sUA <360 μmol/L, all ultrasound depositions were reduced ($P < .001$), full dissolution of double contour (83.4%) and tophi (63.2%) (where examining only first metatarsophalangeal joints identified most of these patients), and only 16% had flares the previous year all of whom had higher sUA and depositions ($P = .035$-$.006$).

Conclusions: Five years of T2T-ULT resulted in most patients achieving sUA target, reduction of crystal depositions, and dissolution of double contour and tophi in most patients. Only a few patients experienced flares, and they had higher levels of sUA and crystal depositions, supporting the importance of keeping sUA low.

*Correspondence to Dr. Hilde Berner Hammer, Department of Rheumatology, Diakonhjemmet Hospital, Vinderen, Oslo, Norway.
E-mail address: hbham@online.no (H.B. Hammer).
Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.10.029

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Applying a treat-to-target urate-lowering therapy (ULT) in the NOR-Gout (Gout in Norway) study resulted in 85.5% of the patients achieving serum uric acid (sUA) levels <360 μmol/L after 1 year as well as a significant reduction in all ultrasound assessments of crystal depositions (double contour, tophi, and aggregates).

WHAT THIS STUDY ADDS

- Supporting longitudinal studies in gout, the 5-year follow-up on ULT in the NOR-Gout study found 71.2% of the patients maintaining the target sUA after 4 years of follow-up by their general practitioner, a continuously decrease of crystal depositions resulting in a high percentage of patients with no detectable depositions (double contour dissolved in 83.4% and tophi in 63.2%), where examination of only the first metatarsophalangeal (MTP1) joints identified almost all of these patients. A few patients still experienced flare (16%), and they had the highest levels of sUA and crystal depositions.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- After a hospital-based first year of treat-to-target ULT followed by regular follow-up by general practitioners, the highly positive first year outcome remained after 5 years, indicating that initial investment in information, dose adjustments to achieve sUA target, and information about the degree of crystal depositions seen by ultrasound seem to result in high compliance in patients with gout. In addition, at the 5-year examination, only assessing the MTP1 joints was sufficient to identify most patients with full resolution of double contour and tophi, thus increasing the feasibility of ultrasound in long-term follow-up.

INTRODUCTION

Gout is an inflammatory joint disease with increasing prevalence and characterised by painful flares [1–3]. Long-standing hyperuricaemia may lead to the formation and deposition of monosodium urate (MSU) crystals in joints, tendons, and soft tissues, causing flares leading to severe pain, bone erosions, and disability [4,5].

Urate-lowering therapy (ULT) is recommended to reduce the crystal burden of gout, and treatment should be initiated shortly after diagnosis [6]. In treat-to-target (T2T) recommendations of gout [7], dissolution of crystals and prevention of flares are fundamental, and patient education, ensuring adherence to medications, and monitoring of serum uric acid (sUA) levels are of major importance. In addition, the American College of Rheumatology (ACR) guidelines for the management of gout from 2020 recommend initiation of ULT for all patients with tophaceous gout, radiographic damage due to gout, or frequent gout flares [8]. Despite established and clear treatment recommendations, and easily available and effective sUA-lowering medication, gout is often poorly managed [9–11]. However, adherence to the recommendations seem to be increasing in recent time [12].

Ultrasound may be used to detect MSU crystal depositions in gout, and the Outcome Measures in Rheumatology (OMERACT) ultrasound working group has defined the elementary lesions as double contour (crystal deposition on the surface of cartilage), tophi, and aggregates [13–15]. The ACR/European League Against Rheumatism (EULAR) has included imaging in the classification criteria for gout. The criteria define typical clinical findings as well as MSU crystal depositions detected by ultrasound or dual-energy computed tomography (DECT) to be diagnostic [7]. When microscopically crystal identification is not possible, it is recommended that any atypical presentation should be investigated by imaging, in particular with ultrasound to seek features suggestive of MSU crystal deposition (double contour sign and tophi) [8].

We have performed a longitudinal study of patients with gout starting on ULT (the NOR-Gout [Gout in Norway] study), where a T2T approach was applied. Patients were examined by ultrasound applying the OMERACT semiquantitative scoring system [16]. We have previously described that the crystal depositions after 12 months were significantly reduced for all the 3 elementary lesions [17]. In addition, after 2 years follow-up, there was a continuous decrease of sUA as well as depositions measured by DECT [18,19].

We lack long-term follow-up studies of primary gout, especially with imaging assessments. Important questions are whether a treatment target of low sUA can be maintained over years, whether crystal depositions continue to decrease with low sUA, how frequently complete dissolution of ultrasound depositions is seen, and whether reduction of crystal depositions over years translates into reduced flares. The present study is a 5-year follow-up in the NOR-Gout study exploring these questions.

METHODS

The NOR-Gout study is a prospective, single-centre, observational study with consecutive inclusion of patients with primary gout confirmed by arthrocentesis, subsequent microscopy-confirmed MSU crystals, and no known renal disease (estimated glomerular filtration rate [eGFR] >45 ml/min/1.73 m^2). The 1- and 2-year results have previously been published [17,19]. The study included patients after a gout flare within the last month as well as having increased serum sUA levels (>360 μmol/L or >6 mg/dL) (ACTRN12618001372279) requesting initiation of or increasing dose of ULT. Patients gave written informed consent according to the Declaration of Helsinki.

The study used ULT in a T2T approach in the first year, aiming to achieve sUA levels of <360 μmol/L (<6 mg/dL) (or <300 μmol/L [<5 mg/dL] if clinical tophi were present). After the first year, the patients were followed by their general practitioners with a request to continue and if necessary, intensify medication to maintain the target sUA level. Follow-up examinations were performed at 2 and 5 years at the outpatient department, and we report the follow-up results with focus on the 5-year outcomes.

Clinical and laboratory assessments

The patients were assessed by a trained study nurse and a rheumatologist at baseline and after 3, 6, and 12 months [17] as well as after 2 and 5 years. All visits included clinical examinations for tophi (the study nurse noted the location of the largest tophus), ultrasound examinations (performed by the rheumatologist) as well as laboratory examinations (sUA, C-reactive protein [CRP] mg/L, erythrocyte sedimentation rate [ESR] mm/h, creatinine [μmol/L], and eGFR [ml/min/1.73 m^2]). Patients not already using ULT initiated allopurinol 100 mg once daily with a monthly increase in dosage if needed to obtain sUA target of

<360 μmol/L (or <300 μmol/L if clinical tophi were present) and with maximum allowed allopurinol dosage of 900 mg. If a patient had intolerance for allopurinol or did not reach the sUA target despite the highest allowed dose, the treatment was changed to febuxostat with a starting dose of 40 mg once daily and with monthly increases in dosage of 40 mg as needed to obtain the sUA target (with maximum allowed febuxostat dosage of 120 mg). If tolerated, all patients were given prophylaxis for gout flares for the first 3-6 months after initiation of ULT, and flares were treated at the discretion of the physician.

At all visits, the patients were asked by the study nurse about the numbers of flare(s) between the visits, except at the 5-year follow-up, where they were asked about the number of flare(s) the previous year.

Ultrasound semiquantitative scoring

The ultrasound examinations were performed by either of 2 rheumatologists (HBH or LK) using a General Electric E9 or E10 machine with a 6 to 15 MHz probe, using grey scale for assessing MSU crystal depositions. The OMERACT definitions for the elementary lesions were applied for double contour, tophi, and aggregates [13]. We used the OMERACT semiquantitative scoring system with score 0 = none, score 1 = possible, score 2 = certain, and score 3 = major deposition of each of the elementary lesions [16]. All examinations included bilateral assessments for double contour, tophi, and aggregates in the following joints: wrist (radiocarpal, midcarpal, radioulnar), metacarpophalangeal2, talonavicular, and first metatarsophalangeal (MTP1) joints. In addition, double contour at the cartilage surface was assessed at the distal femur condyle with maximally flexed knee as well as at the lateral and medial cartilage of talus (flexed knee and the foot resting flat on the bench). The following tendons and insertion of tendons were examined for tophi and aggregates: triceps, quadriceps, patellar (proximal and distal), and Achilles tendons as previously described [17].

In addition, all patients were at baseline examined by ultrasound for the presence of a large tophus (either clinically visible or the largest tophus detected by ultrasound in the regions of elbow, hand, knee, ankle, and foot and being accessible for measurements), which was defined as the ultrasound index tophus. The size of this tophus was followed throughout the study measuring by ultrasound, the largest length, width, and depth in millimetres.

Prior to initiation of the study, the 2 sonographers performing all the ultrasound assessments agreed on the semiquantitative scoring system both on images and in patients with gout. In addition, they performed a reliability assessment on ultrasound images, including similar number of each of the elementary lesions and including all the different scores, resulting in a very high reliability [17].

Statistics

Mean (SD) or median (IQR) were applied as appropriate. For each visit, sum scores of double contour, tophi, and aggregates were calculated separately, as well as the calculation of total sum scores of the 3 elementary lesions. Differences between groups were explored by use of an independent samples *t*-test. Error bar plots visualised findings at the 5-year visit.

Changes between 2 time points in a group were explored by paired samples *t*-test, and analysis of variance (ANOVA) was used for exploring changes over more than 2 time points. To explore the impact of higher sUA levels, patients were divided into 4 different groups dependent on sUA: ≤300, 301 to 360, 360 to 420, or >420 μmol/L. The levels of ultrasound depositions in these different sUA groups were examined by 1-way ANOVA at the 1-, 2-, and 5-year visits. One-way ANOVA explored the association between experiencing at least 1 flare the year before each visit and either of the 4 group levels of sUA. Linear regression explored the association between flares (dependent variable) and each of the elementary lesions, presence of clinical or ultrasound-detected tophus, sUA, or dose of ULT (controlling for age, gender, disease duration, creatinine, and eGFR) calculated for the 1, 2-, and 5-year visits.

Tertiles were created to distinguish increasing levels of the ultrasound sum scores for the 3 elementary lesions at baseline defining minor, moderate, or major levels by use of the following ranges: 0 to 2, 3 to 5, or 6 to 18 for double contour; 0 to 3, 4 to 6, or 7 to 42 for tophi; and 0 to 6, 7 to 10 or 11 to 30 for aggregates, respectively. The same ranges were used during follow-up to explore changes in percentages of patients having minor, moderate, or major sum scores of the elementary lesions.

The volume of the ultrasound index tophus was estimated as the product of length, width, and depth, and changes during follow-up of the volume of the index tophus were explored by paired samples *t*-test. The Wilcoxon test was used to explore changes in the presence of clinical tophi.

The statistical calculations were performed using SPSS V.29, and $P < .05$ was defined as significant.

Patient and public involvement

A patient research partner with gout participated in the project and took part in the discussion of research questions and outcome measures during the first year of the study. Further, the patient research partner evaluated the burden of study participation and supported how patients were recruited.

RESULTS

Patients and laboratory variables

Of the 211 patients included in the NOR-Gout study at baseline, 209 were assessed by ultrasound and included in the present study (mean [SD] age 56.4 [13.8] years and the time since first flare [disease duration] was 7.9 [7.7] years, 95.2% men). The numbers of patients were n = 184 at 1 year, n = 172 at 2 years, and n = 163 at 5 years. Except for higher levels of sum score aggregates in the noncompleters (score 11.4 vs 8.7, $P = .019$), the sum scores of double contour and tophi as well as sUA were not different at baseline between the patients completing or not completing the study.

The sUA levels decreased from 500 (77) μmol/L at the time of inclusion to 337 (83) μmol/L at 5-year follow-up ($P < .001$) (Table 1). At the 1-year visit the target of sUA <360 μmol/L was reached by 85.5% of the patients, but this percentage decreased to 71.2 during the next 4 years of follow-up by their general practitioner (Table 1).

There was a decrease in CRP and ESR from baseline to 5 years ($P = .01$ and .001, respectively) and no significant change in serum creatinine or eGFR from baseline to 5-year examination (Table 1).

Changes from baseline of crystal depositions assessed by ultrasound

Significant reductions were found in the sum scores for double contour, tophi, and aggregates between each of the visits

Table 1
Mean (SD) or percentages of laboratory variables, flare, and drug use as well as mean (SD) and median (range) of sum scores of ultrasound elementary lesions during follow-up

	Baseline (n = 209)	1 y (n = 184)	2 y (n = 172)	5 y (n = 163)
Laboratory variables				
Serum uric acid (μmol/L)	500 (77)	312 (49)	325 (70)	337 (83)
Patients with sUA ≤360 μmol/L (%)	0	85.5	78.6	71.2
Patients with sUA ≤420 μmol/L (%)	12.3	97.3	92.5	84.7
CRP (mg/L)	7 (14)	4 (7)	4 (7)	3 (5)
ESR (mm/h)	15 (14)	10 (10)	11 (10)	10 (10)
Creatinine (μmol/L)	96.3 (18.4)	94.7 (19.4)	93.9 (21.1)	95.3 (26.7)
eGFR (ml/min/1.73 m^2)	78.0 (18.8)	78.7 (19.1)	79.0 (19.9)	78.4 (21.5)
Ultrasound findings				
Double contour (sum scores)	4.3 (3.5)	1.2 (2.2)	0.7 (1.4)	0.4 (1.4)
(mean [SD] and median [range])	3 (0-18)	0 (0-13)	0 (0-9)	0 (0-9)
Tophi (sum scores)	6.5 (6.5)	3.8 (5.2)	2.4 (3.7)	1.2 (3.0)
(mean [SD] and median [range])	5 (0-42)	2 (0-31)	1 (0-27)	0 (0-33)
Aggregates (sum scores)	9.3 (5.6)	6.7 (5.1)	5.5 (4.7)	3.4 (3.5)
(mean [SD] and median [range])	8 (0-30)	5 (0-29)	4 (0-27)	2 (0-17)
Double contour, tophi, and aggregates (total sum scores)	20.0 (13.9)	11.7 (11.3)	8.6 (8.8)	5.1 (6.9)
(mean [SD] and median [range])	16 (1-72)	9 (0-67)	6 (0-49)	3 (0-59)
Flare				
Patients with flare (%)	100	81.0	24.7	16.0
Drug use				
Allopurinol (%)	14.9	87.4	84.1	81.0
Mean (SD) dose of allopurinol (mg)	152 (109)	289 (120)	285 (118)	262 (111)
Febuxostat (%)	0	12.6	15.3	14.1
Mean (SD) dose of febuxostat (mg)	0	59 (24)	60 (20)	57 (20)

CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; sUA, serum uric acid.

from baseline to 5 years ($P < .001$). Table 1 describes the sum scores of ultrasound depositions during follow-up, and Figure 1 illustrates this continuous decrease. Aggregates were found to have a lower speed of dissolution than double contour and tophi, but during follow-up, there was a gradual decrease in the level of the scores.

The percentage of patients with different levels of ultrasound-detected crystal depositions, defined as minor, moderate, or major levels during follow-up, is visualised in Figure 2. The figure illustrates the large increase from baseline to 5 years follow-up in the percentage of patients having minor depositions of the 3 elementary lesions (double contour: change from baseline to 1 year and from 1 year to 2 years, both $P < .001$, tophi: change from baseline to 1 year and from 1 year to 2 years, both $P < .001$, from 2 years to 5 years, $P = .016$, aggregates: changes between all the time points $P < .001$).

Differences in ultrasound depositions dependent on sUA levels

There were no differences in any of the ultrasound elementary lesions at 1 or 2 years when stratifying by sUA levels higher or lower than 360 μmol/L, but patients with sUA >360 μmol/L had at 5 years higher sum scores of both double contour and tophi ($P < .001$ and $P = .002$, respectively) than patients with sUA <360 μmol/L.

From baseline to 1 year, both patients achieving or not achieving sUA target of 360 μmol/L had a significant decrease in double contour, tophi, and aggregates scores (all $P < .001$) [17]. For patients with sUA <360 μmol/L at all visits, significant reduction was found for all elementary lesions in the 1 to 2 and 2 to 5 years intervals ($P < .001$). However, for patients having >360 μmol/L at the 2- and 5-year visits, significant decreases in the ultrasound lesions sum scores were only found for tophi between year 1 and 2 ($P = .006$).

During follow-up, the number of patients with sUA >420 μmol/L increased from 5 patients (2.7%) at 1 year, 13 patients (7.5%) at 2 years, to 25 patients (15.3%) at 5 years. There were no significant differences between sum scores of double contour, tophi, or aggregates in patients with sUA ≤420 μmol/L or >420 μmol/L at the 1- or 2-year visits. However, at 5 years, compared with patients having sUA ≤420 μmol/L, those with sUA >420 μmol/L had higher mean (SD) sum scores of double contour (1.6 [2.5] vs 0.2 [0.9] [$P = .009$]), tophi (3.4 [6.5] vs 0.8 [1.5] [$P = .05$]), and aggregates (5.8 [4.0] vs 3.0 [3.2] [$P = .002$]). Exploring differences in ultrasound depositions between groups with increasing sUA levels showed no significant differences at the 1-year visit, but at the 2-years visit, there were significantly higher levels of double contour scores with increasing sUA ($P = .039$), and at the 5-year visit, there were significantly higher scores of double contour, tophi, and aggregates with increasing sUA ($P \leq .002$). This is illustrated in Figure 3 showing patients with sUA >420 μmol/L to have a higher total sum score of the 3 elementary lesions at the 5-year visit ($P < .001$).

Changes in clinical and ultrasound-detected tophi during follow-up

At baseline, 16.6% of patients had at least one clinically subcutaneously detected tophus, which was decreased to 11.3% at 1 year, 9.2% at 2 years, and 5.8% at 5 years (baseline to 5 years, $P < .001$). The largest clinical tophus was primarily located at the elbow, MTP1, or fingers (in decreasing frequency). Patients with, vs without, a clinical tophus had higher sum score aggregates ($P < .05$) at the 5-year follow-up.

The volume of the ultrasound defined index tophi decreased from baseline to 5 years follow-up ($P = .002$), with median mm^3 (IQR) of 440 (189-1740) at baseline (n = 51), 216 (55-808) at 1 year (n = 33), 123 (44-410) at 2 years (n = 26), and

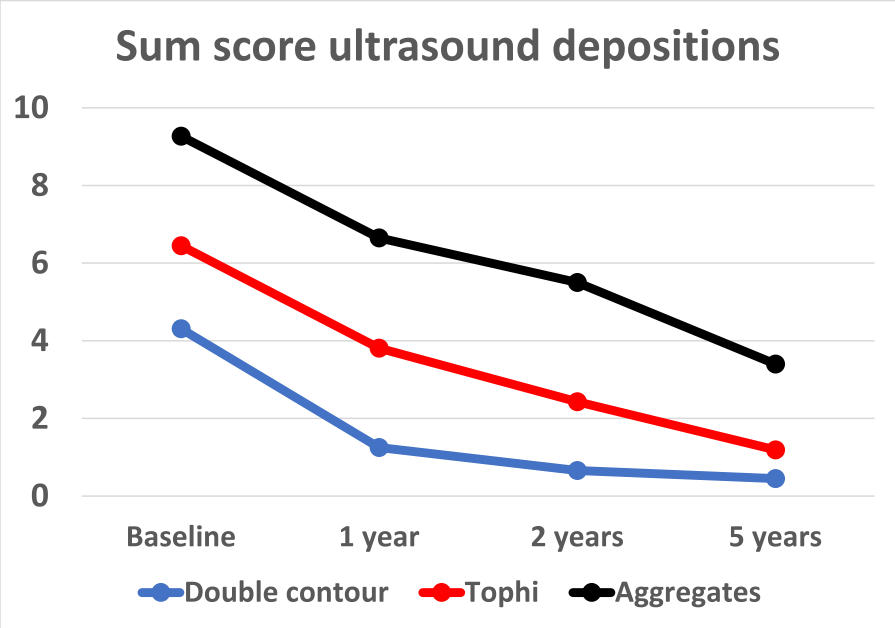


Figure 1. Ultrasound depositions (mean sum scores) of double contour, tophi, and aggregates during 5 years.

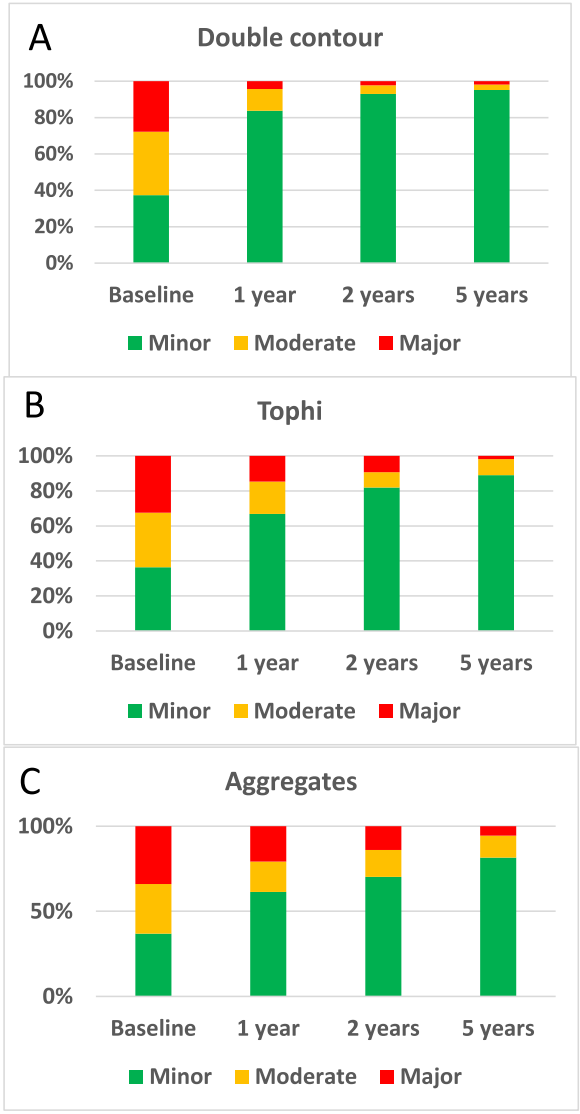


Figure 2. Ultrasound sum scores of double contour (A), tophi (B), and aggregates (C) divided into tertiles from each of the baseline results, using the same ranges at all time points during follow-up (groups defined as minor, moderate, and major levels).

40 (18-140) at 5 years (n = 11). The reduction in the number of patients with index tophi was caused by the disappearance of tophi. Patients still having an index tophus compared to those with no index tophus had at 5 years higher sum scores for all the ultrasound elementary lesions ($P < .05$).

Percentage of patients with no ultrasound depositions

At baseline, all patients had ultrasound-detected crystal depositions. However, during follow-up, increasing percentage of patients achieved complete dissolution of the depositions, with the highest absence of crystal deposition seen for the double contour (83.4%) (Table 2). Table 2 also shows the percentages of different subgroups of joints and tendons included in the calculations. When exploring if a reduced number of joints was possible for detecting patients with full dissolution, we found at the 5-year visit that examining only MTP1 bilaterally gave almost similar percentages of patients with full dissolution of double contour and tophi as compared to examining the total number of joints/tendons.

Flares during follow-up

As shown in Table 1, the percentage of patients experiencing flares during follow-up was clearly reduced from affecting all patients at baseline (inclusion criteria) to 16% at the 5-year visit. The number (percentage) of patients still using colchicine was 6 (3.3%), 3 (1.7%), and 1 (0.6%) at 12, 24, and 60 months, respectively.

At the 5-year visit, patients with flare had higher mean (SD) sUA levels compared to patients with no flare (n = 26, sUA = 394 [110] μmol/L vs n = 137, sUA = 326 [72] μmol/L) ($P = .005$), and patients with sUA >420 µmol/L, had most flares ($P = .011$). In addition, the patients experiencing flares at 5 years had higher levels of ultrasound crystal depositions (double contour [$P = .007$], tophi [$P = .035$] and aggregates [$P = .006$]) (Table 3). The regression analyses showed flares at 5 years to be associated with sUA levels ($P < .001$), the sum scores of each of the elementary ultrasound lesions ($P = .008$ - <.001), as well as ultrasound-detected presence of index tophus ($P < .001$), while there were no associations with dosage of ULT nor presence of clinical tophus.

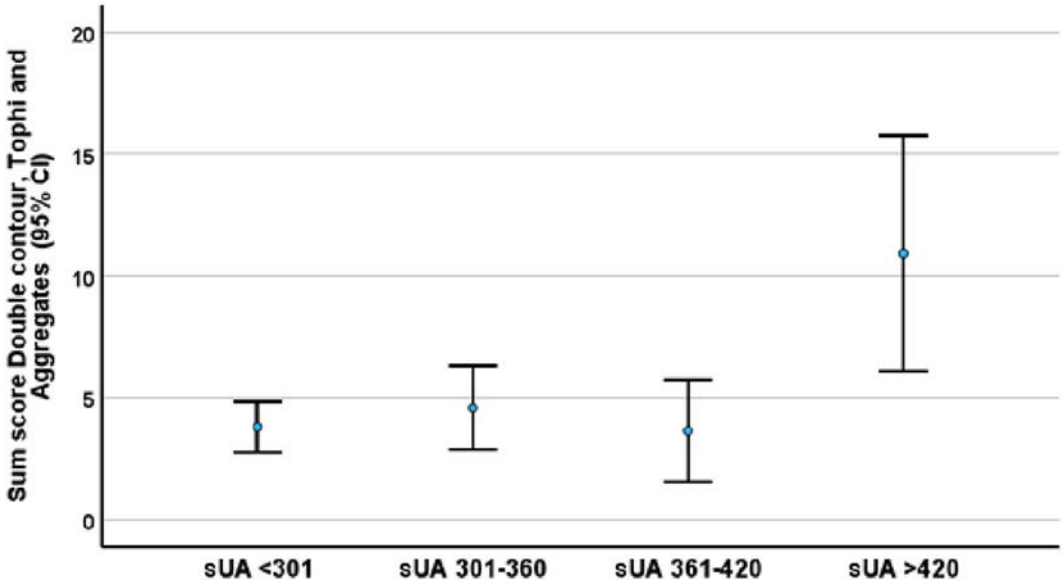


Figure 3. Sum scores at 5-year follow-up of double contour, tophi, and aggregates divided into groups by levels of serum uric acid (sUA).

DISCUSSION

To the best of our knowledge, this is the first large long-term follow-up study on patients with gout treated to a sUA target and assessed for ultrasound depositions and flares. sUA decreased during an extensive T2T approach, and flares became markedly less frequent after year 1. These improvements were mirrored by a continuous decrease of all the ultrasound elementary lesions during the 5-year study. A high percentage of patients achieved full dissolution of crystal depositions after 5 years, and they could be identified by only including MTP1 assessments. At the 5-year visit, patients with the highest sUA levels were found to have the highest levels of depositions as well as experiencing more flares.

In the first year, all patients were monitored at the outpatient clinic, and the patients were thereafter followed by their general practitioner. The percentage of patients maintaining the sUA target decreased during the last 4 years of follow-up. However, the percentage of patients still at the sUA target was much higher than previously described in studies assessing sUA in patients in usual care by the general practitioner, where low percentages of patients with gout achieved the sUA target (19% after 6 months, 30% or 44% after 2 years) [20–22]. Thus, patient information given with the objective to increase understanding of the disease to enhance adherence to medication in the first year as well as informing patients about reductions of ultrasound depositions may all have improved their knowledge as well as their motivation for adherence to therapy. An initial T2T of sUA may therefore be an important investment for improved management and outcome for patients with gout. The importance of sUA control is supported by a recent study finding a progressive increase in hospitalisations, mortality rates, and cost in patients with gout, highlighting the need for better healthcare strategies [23].

The comprehensive ultrasound examination in this study included areas known to be predilection sites for crystal depositions. At baseline, all patients had depositions, but after 5 years, most of the patients had neither double contour nor tophi, indicating that these elementary lesions may dissolve during ULT. However, the percentage of patients still having aggregates was much higher. This could be caused by a transition of tophi into

Table 2
Percentages of patients with no detectable crystal depositions in all examined joints/tendons or in different subgroups of sites during follow-up

	Baseline n = 209	1 y n = 184	2 y n = 172	5 y n = 163
Sum score zero of all joints and tendons				
Sum score double contour	7.2%	55.4%	71.1%	83.4%
Sum score tophi	6.7%	19.9%	32.9%	63.2%
Sum score aggregates	1.0%	4.3%	7.5%	23.9%
Sum score zero of bilateral knees with patellar tendons, ankles with Achilles, and MTP1 joints				
Sum score double contour	7.2%	55.4%	71.7%	84.0%
Sum score tophi	9.1%	26.3%	39.9%	66.9%
Sum score aggregates	2.9%	7%	11.0%	31.3%
Sum score zero of bilateral knees, ankles, and MTP1 joints				
Sum score double contour	7.2%	63.4%	71.7%	84.0%
Sum score tophi	21.5%	35.5%	47.4%	69.9%
Sum score aggregates	17.2%	23.1%	27.7%	46.6%
Sum score zero of bilateral MTP1 joints				
Sum score double contour	20.7%	63.2%	76.9%	85.9%
Sum score tophi	22.0%	36.0%	47.4%	69.9%
Sum score aggregates	18.2%	24.2%	27.7%	46.6%

MTP1, first metatarsophalangeal.

Table 3
Ultrasound depositions during follow-up in patients with no flare vs flare during the previous year

	Year 1		Year 2		Year 5	
	No flare (n = 36)	Flare (n = 148)	No flare (n = 127)	Flare (n = 45)	No flare (n = 136)	Flare (n = 26)
Sum score double contour	0.8 (1.2)	1.4 (2.3)*	0.6 (1.3)	0.9 (1.7)	0.2 (0.6)	1.8 (2.8)**
Sum score tophi	2.0 (3.4)	4.2 (5.4)*	2.2 (3.2)	3.2 (4.9)	0.7 (1.5)	3.6 (6.5)*
Sum score aggregates	5.0 (4.4)	7.1 (5.2)*	5.2 (4.7)	6.4 (4.6)	3.1 (3.2)	5.6 (4.1)**
Sum score double contour, tophi, and aggregates	7.8 (8.5)	12.7 (11.8)*	7.9 (8.1)	10.5 (10.2)	4.0 (4.6)	11.0 (12.2)**

Mean (SD) of sum scores of ultrasound depositions.
Differences between groups:
* $P < .05$.
** $P < .01$.

aggregates when the tophi are dissolved, and those aggregates may need even longer time to disappear. We found the reduction in size and presence of index tophi to correspond to the reduced ultrasound depositions. This is supported by a DECT study describing volumetric reduction of tophi after ULT [24].

The feasibility of ultrasound examinations is an important issue. During the study, we found the percentage of patients without any double contour or tophi to be gradually more similar for the different subsets of joints/tendons included in the calculations. At the 5-year visit, performing an ultrasound assessment of only the MTP1 joints would have given almost similar percentage of patients with no double contour or tophi as if all joints and tendons had been examined. Thus, after long-standing treatment with ULT, it may be sufficient to examine the MTP1 joints to explore the presence of ultrasound depositions. If this is supported by future studies, it would make ultrasound highly feasible for long-term follow-up of patients with gout.

At the 5-year visit, there was a marked reduction in patients experiencing flares, and patients experiencing flares compared to patients with no flares had higher sum score levels for all the 3 elementary lesions. This is supported by previous studies on flares being associated with the presence of higher levels of ultrasound depositions [25–28]. Patients with flares at 5 years were also found to have the highest sUA levels, supported by other studies [29]. Thus, an important aim for physicians following patients with gout is to continuously assure the patients that low sUA levels seem to abolish the production of depositions and thus eliminate the potential for a gout flare.

The patients were treated with allopurinol or febuxostat. Accumulating evidence suggests that hyperuricaemia is a pathological factor in the development and progression of chronic kidney disease. The potential benefit afforded by the control of sUA is controversial [23], but recent studies found both drugs to positively affect kidney function [23,24]. No patients with renal impairment were included in our study. However, our results showed that there was no change in creatinine or eGFR over the 5 years, and this supports ULT to be safe for long-term treatment.

Despite extensive research, it is still unknown how the pathological crystallisation of MSU occurs, but a recent study suggests a distinctive biosignature crystallisation of biotic MSU crystals with a slow growth over long periods of time (possibly years) in patients with hyperuricaemic gout and suggesting that there is a time window to treat potential patients with gout before a critical amount of MSU has slowly formed to trigger a gout flare [30]. A pilot study has previously shown MSU-verified ultrasound-detected depositions in asymptomatic persons with hyperuricaemia [31], and this important topic will be further explored in an ongoing longitudinal study of participants with asymptomatic hyperuricaemia being examined for ultrasound evidence of MSU crystal deposition [32]. Thus, in the future, we may find ultrasound examination to be useful for diagnosing asymptomatic gout and thus be able to start treatment with ULT at an early stage.

A strength of the present study is the high number of patients followed for 5 years with clinical and laboratory assessments as well as comprehensive ultrasound assessment performed on high-end machines by experienced rheumatologists with a high reliability for scoring lesions, since a high level of experience is important when scoring crystal depositions by ultrasound. Another strength is that 78% of the patients attended the 5-year visit, giving valuable information about the results of long-term ULT treatment including 4 years of follow-up by their general practitioner. The study has some limitations: it is a single-centre study, and the results may therefore not be generalised; it was not a randomised study, so that causalities cannot be explored. In addition, it may be a limitation that the ultrasound scoring was performed in real time since it may be a bias that the sonographer was not blinded to the sequence in time, and it is a limitation that the recording of flares was over the previous year, which is subject to recall bias.

As a conclusion, in this first large 5-year follow-up study of patients with gout with T2T ULT, during the first year, and follow-up by the general practitioners the next 4 years, we found a major decrease in sUA, in ultrasound-detected crystal depositions, and in the number of patients with flares. In addition, it seems that after 5 years of ULT, examining only MTP1 joints for assessing double contour and tophi may be sufficient to detect patients with full dissolution of these depositions, which could improve the feasibility of ultrasound in follow-up of patients with gout. Patients reporting flares at the 5-year visit had the highest levels of sUA and crystal depositions. Thus, our findings support that the major goal in the treatment of patients with gout is a low sUA to avoid crystal depositions and thus eliminate flares.

Competing interests

HBH reports personal fees from AbbVie, Lilly, UCB, and Novartis, outside the submitted work. LK has nothing to disclose. LT reports personal fees from Novartis, Roche, BMS, and Pfizer outside the submitted work. EAH reports personal fees from Novartis, Janssen-Cilag, and AbbVie outside the submitted work. TU reports personal fees from Galapagos, Pfizer, and UCB, outside the submitted work.

Acknowledgements

We thank research secretary Mona Thorkildsen for organisation and research nurses Gina Stenberg, Anita Reinhard, Heidi Lunøe, and Ingerid Müller who contributed as trained study nurses in the follow-up of the first year with a T2T approach.

We also thank the patient representatives Espen Reksten and Ole Petter Synnestvedt.

Some of the present 5-year results have been communicated in 2024 as oral presentations at the European League Against Rheumatism (EULAR) congress (EULAR 2024 OP0197 Hammer et al) and American College of Rheumatology (ACR) congress (ACR 2024 Abstract Number: 2560; Hammer et al).

Contributors

HBH has made a substantial contributions to the conception and design of the work; the acquisition of data, the analysis, interpretation of data for the work; drafted the manuscript as well as revising it critically for important intellectual content; given a final approval of the version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. LK, LT, EAH, and TU have given substantial contributions to the design of the manuscript as well as the interpretation of data for the work; revised the manuscript critically for important intellectual content; given a final approval of the version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding

There was no external funding, and the study was performed as employees at National Advisory Unit on Rehabilitation in Rheumatology (NKRR) and Department of Rheumatology, Diakonhjemmet Hospital, Oslo, Norway.

Patient consent for publication

Not required.

Ethics approval

The study was approved by the Norwegian Regional Committee for Medical and Health Research Ethics South East (reference number 2015/990).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Deidentified participant data are available on reasonable request.

Orcid

Hilde Berner Hammer: http://orcid.org/0000-0001-7317-8991

Till Uhlig: http://orcid.org/0000-0002-6881-9552

REFERENCES

[1] Kuo CF, Grainge MJ, Mallen C, Zhang W, Doherty M. Rising burden of gout in the UK but continuing suboptimal management: a nationwide population study. Ann Rheum Dis 2015;74(4):661–7.

[2] Zhu Y, Pandya BJ, Choi HK. Prevalence of gout and hyperuricemia in the US general population: the National Health and Nutrition Examination Survey 2007-2008. Arthritis Rheum 2011;63(10):3136–41.

[3] Dehlin M, Drivelegka P, Sigurdardottir V, Svärd A, Jacobsson LT. Incidence and prevalence of gout in Western Sweden. Arthritis Res Ther 2016;18:164.

[4] Khanna PP, Nuki G, Bardin T, Tausche AK, Forsythe A, Goren A, et al. Tophi and frequent gout flares are associated with impairments to quality of life, productivity, and increased healthcare resource use: results from a cross-sectional survey. Health Qual Life Outcomes 2012;10:117.

[5] Scire CA, Manara M, Cimmino MA, Govoni M, Salaffi F, Punzi L, et al. Gout impacts on function and health-related quality of life beyond associated risk factors and medical conditions: results from the KING observational study of the Italian Society for Rheumatology (SIR). Arthritis Res Ther 2013;15(5):R101.

[6] Richette P, Doherty M, Pascual E, Barskova V, Becce F, Castañeda-Sanabria J, et al. 2016 updated EULAR evidence-based recommendations for the management of gout. Ann Rheum Dis 2017;76(1):29–42.

[7] Kiltz U, Smolen J, Bardin T, Cohen Solal A, Dalbeth N, Doherty M, et al. Treat-to-target (T2T) recommendations for gout. Ann Rheum Dis 2017;76(4):632–8.

[8] FitzGerald JD, Dalbeth N, Mikuls T, Brignardello-Petersen R, Guyatt G, Abeles AM, et al. 2020 American College of Rheumatology Guideline for the management of gout. Arthritis Rheumatol 2020;72(6):879–95.

[9] Kuo CF, Grainge MJ, Mallen C, Zhang W, Doherty M. Eligibility for and prescription of urate-lowering treatment in patients with incident gout in England. JAMA 2014;312(24):2684–6.

[10] Doherty M, Jansen TL, Nuki G, Pascual E, Perez-Ruiz F, Punzi L, et al. Gout: why is this curable disease so seldom cured? Ann Rheum Dis 2012;71(11):1765–70.

[11] Abhishek A, Doherty M. Education and non-pharmacological approaches for gout. Rheumatology (Oxford) 2018;57(suppl 1):i51–8.

[12] Sautner J, Sautner T. Compliance of primary care providers with gout treatment recommendations-lessons to learn: results of a nationwide survey. Front Med (Lausanne) 2020;7:244.

[13] Gutierrez M, Schmidt WA, Thiele RG, Keen HI, Kaeley GS, Naredo E, et al. International consensus for ultrasound lesions in gout: results of Delphi process and web-reliability exercise. Rheumatology (Oxford) 2015;54(10):1797–805.

[14] Terslev L, Gutierrez M, Christensen R, Balint PV, Bruyn GA, Delle Sedie A, et al. Assessing elementary lesions in gout by ultrasound: results of an OMERACT patient-based agreement and reliability exercise. J Rheumatol 2015;42(11):2149–54.

[15] Terslev L, Gutierrez M, Schmidt WA, Keen HI, Filippucci E, Kane D, et al. Ultrasound as an outcome measure in gout. A validation process by the OMERACT ultrasound working group. J Rheumatol 2015;42(11):2177–81.

[16] Christiansen SN, Filippou G, Scirè CA, Balint PV, Bruyn GA, Dalbeth N, et al. Consensus-based semi-quantitative ultrasound scoring system for gout lesions: results of an OMERACT Delphi process and web-reliability exercise. Semin Arthritis Rheum 2021;51(3):644–9.

[17] Hammer HB, Karoliussen L, Terslev L, Haavardsholm EA, Kvien TK, Uhlig T. Ultrasound shows rapid reduction of crystal depositions during a treat-to-target approach in gout patients: 12-month results from the NOR-Gout study. Ann Rheum Dis 2020;79(11):1500–5.

[18] Uhlig T, Karoliussen LF, Sexton J, Borgen T, Haavardsholm EA, Kvien TK, et al. 12-month results from the real-life observational treat-to-target and tight-control therapy NOR-Gout study: achievements of the urate target levels and predictors of obtaining this target. RMD Open 2021;7(1):e001628.

[19] Uhlig T, Eskild T, Karoliussen LF, Sexton J, Kvien TK, Haavardsholm EA, et al. Two-year reduction of dual-energy CT urate depositions during a treat-to-target strategy in gout in the NOR-Gout longitudinal study. Rheumatology (Oxford) 2022;61(SI) SI81-5.

[20] Doherty M, Jenkins W, Richardson H, Sarmanova A, Abhishek A, Ashton D, et al. Efficacy and cost-effectiveness of nurse-led care involving education and engagement of patients and a treat-to-target urate-lowering strategy versus usual care for gout: a randomised controlled trial. Lancet 2018;392(10156):1403–12.

[21] Audet T, Picard-Turcot MA, Robindaine J, Carrier N, Dagenais P. Improving gout care in a Canadian Academic Medical Centre through a multidisciplinary, nurse-led protocol. J Rheumatol 2025;52(3):285–9.

[22] Rasmussen C, Larsen JW, Christensen HM, Larsen MB, Thomsen AM, Leishmann T, et al. Optimising gout treatment: insights from a nurse-led cohort study. RMD Open 2024;10(2):e004179.

[23] Benavent D, Peiteado D, Martinez-Huedo MÁ, Hernandez-Hurtado M, Balsa A, de Miguel E. Healthcare-related impact of gout in hospitalized patients in Spain. Sci Rep 2021;11(1):13287.

[24] Chui CSK, Choi AKY, Lam MMY, Kwan TH, Li OC, Leng Y, et al. Volumetric reduction and dissolution prediction of monosodium urate crystal during urate-lowering therapy - a study using dual-energy computed tomography. Mod Rheumatol 2021;31(4):875–84.

[25] Cipolletta E, Abhishek A, Di Battista J, Grassi W, Filippucci E. Ultrasonography in the prediction of gout flares: a 12-month prospective observational study. Rheumatology (Oxford) 2023;62(3):1108–16.
[26] Wang J, Shao Q. An observational study of ultrasound semiquantitative scoring for predicting the risk of gout flare. Z Rheumatol 2024;83(suppl 3):321–8.
[27] Han L, Li R, Dalbeth N, Liu M, Yu Q, Jiang C, et al. The value of musculoskeletal ultrasound in predicting gout flares in index joints: a prospective cohort study of people with gout starting urate-lowering therapy. Semin Arthritis Rheum 2024;67:152418.
[28] Yang S, Lin X, Gao Y, Liang N, Han Y, Sun H, et al. The association between gout flares and monosodium urate burden assessed using musculoskeletal ultrasound in patients with gout. Ther Adv Musculoskelet Dis 2024; 16:1759720X241240837.
[29] Tedeschi SK, Hayashi K, Zhang Y, Choi H, Solomon DH. Identifying the association between serum urate levels and gout flares in patients taking urate-lowering therapy: a post hoc cohort analysis of the CARES trial with consideration of dropout. Ann Rheum Dis 2024;83(10):1375–80.
[30] Rodriguez-Navarro C, Elert K, Ibañez-Velasco A, Monasterio-Guillot L, Andres M, Sivera F, et al. Unraveling the pathological biomineralization of monosodium urate crystals in gout patients. Commun Biol 2024;7 (1):828.
[31] De Miguel E, Puig JG, Castillo C, Peiteado D, Torres RJ, Martín-Mola E. Diagnosis of gout in patients with asymptomatic hyperuricaemia: a pilot ultrasound study. Ann Rheum Dis 2012;71(1):157–8.
[32] Stewart S, Gamble G, Taylor W, Su I, Merriman T, Mihov B, et al. Development of gout in people with asymptomatic hyperuricemia: study protocol for a 5-year prospective cohort. BMJ Open 2024;14(11):e090415.

Ann Rheum Dis 85 (2026) 388–390

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Letter

Autoreactive B cells in extremes of rheumatoid arthritis disease phenotypes

Rheumatoid arthritis (RA) is a chronic disease hallmarked by autoimmunity against citrullinated proteins. The processes governing disease onset and chronicity are incompletely understood. The anti-citrullinated protein antibody (ACPA) response is characterised by somatically hypermutated immunoglobulin G (IgG) and ACPA-expressing B cells circulating as proliferating (~30% Ki-67$^+$) memory B cells (MBCs) that express costimulatory molecules (and activation markers) CD80 and CD86 and low levels of CD32 (the inhibitory Fcγ-receptor IIb) [1]. Patients may also harbour substantial numbers of ACPA-expressing plasmablasts [2]. Collectively, these characteristics indicate that ACPA-IgG responses emerge from T cell-driven, germinal centre-derived B cell stimulation that is active in established disease [3].

RA is often preceded by an 'at-risk' phase in which inflammatory joint pain and ACPA are present, but arthritis is absent. In up to 60% to 70% of cases, this phase of clinically suspect arthralgia (CSA) converts to RA quickly (<1 year). However, 20% to 30% of CSA individuals do not convert despite the presence of ACPA [4]. Previously, we observed that ACPA-expressing MBCs in long-term CSA nonconverters (>2 years) are less active than in patients with established disease [1]. Specifically, the frequency of CD80$^+$ MBCs (CD20$^+$CD27$^+$) and CD20$^-$CD27^{++} B cells (plasmablasts) were lower in CSA nonconverters. These findings suggest that the degree of ACPA-expressing MBC activation could signal or determine progression to RA. Here, we substantiated this notion in CSA patients followed prospectively. Additionally, we studied another extreme phenotype of RA, sustained disease-modifying antirheumatic drug-free remission (SDFR). SDFR is achieved by only 15% to 20% of ACPA-positive patients, as ACPA-positivity strongly associates with disease relapse upon treatment discontinuation [5]. Hence, both CSA nonconversion and SDFR are phenotypes that may help to identify immunological parameters determining onset and chronicity of ACPA-positive RA.

Peripheral blood was collected from 13 CSA patients, 13 patients in SDFR (median SDFR-time 6.8 years) and 10 patients with established RA (Supplementary Methods). ACPA-expressing B cells were detectable in patients of all groups (Fig 1 A). The frequency of ACPA-expressing B cells tended to be lower in CSA and SDFR compared to RA patients using methotrexate (of which an effect cannot be excluded). ACPA-expressing MBCs consistently dominated the response (Fig 1 B-D). ACPA-IgG levels were comparable in CSA and RA and lower in SDFR (Supplementary Fig S1A). ACPA levels and ACPA-expressing MBC frequency correlated on the group level (R = 0.65; Supplementary Fig S1B).

After 1 year, 6 CSA patients had progressed to RA (converters). At baseline, we noted significant differences in the expression of CD80 by ACPA-expressing MBCs between future converters and nonconverters (Fig 1 F). Converters showed CD80 expression comparable to that observed in RA (96% positive), whereas the expression in nonconverters was lower (60%). This follows our previous (retrospective) observation in CSA long-term nonconverters [1]. We also observed lower expression of Ki-67 in nonconverters (Fig 1 E), fewer circulating B cells with a plasmablast phenotype (Fig 1 D), and lower ACPA-IgG levels (Supplementary Fig S1A). In addition, low expression of CD32 was a feature of RA and CSA converters but not of CSA nonconverters (Fig 1 H). CD86 expression appeared comparable (Fig 1 G). SDFR patients showed a profile comparable to that of CSA nonconverters (Fig 1). CD80 expression by ACPA-expressing MBCs was low in SDFR patients, as were the frequencies of ACPA-expressing MBCs and plasmablasts (Fig 1 B,D).

Although based on relatively low numbers, these data show that the degree of ACPA-expressing MBC activation is associated with disease phenotype. In fact, the activity of ACPA-expressing MBCs may predict imminent RA onset and possibly identify patients in immunological remission capable of reaching SDFR. The profile observed in RA (high CD80, low CD32, abundant plasmablasts) is reminiscent of T cell-driven, germinal centre-derived B cell responses [6], while the phenotype observed in SFDR/nonconverting CSA suggests that certain disease features may be absent that otherwise (chronically) stimulate ACPA-expressing B cells. Identifying these features will be an important step towards understanding how disease chronicity develops.

https://doi.org/10.1016/j.ard.2025.05.006
Received 28 November 2024; Revised 18 February 2025; Accepted 7 May 2025

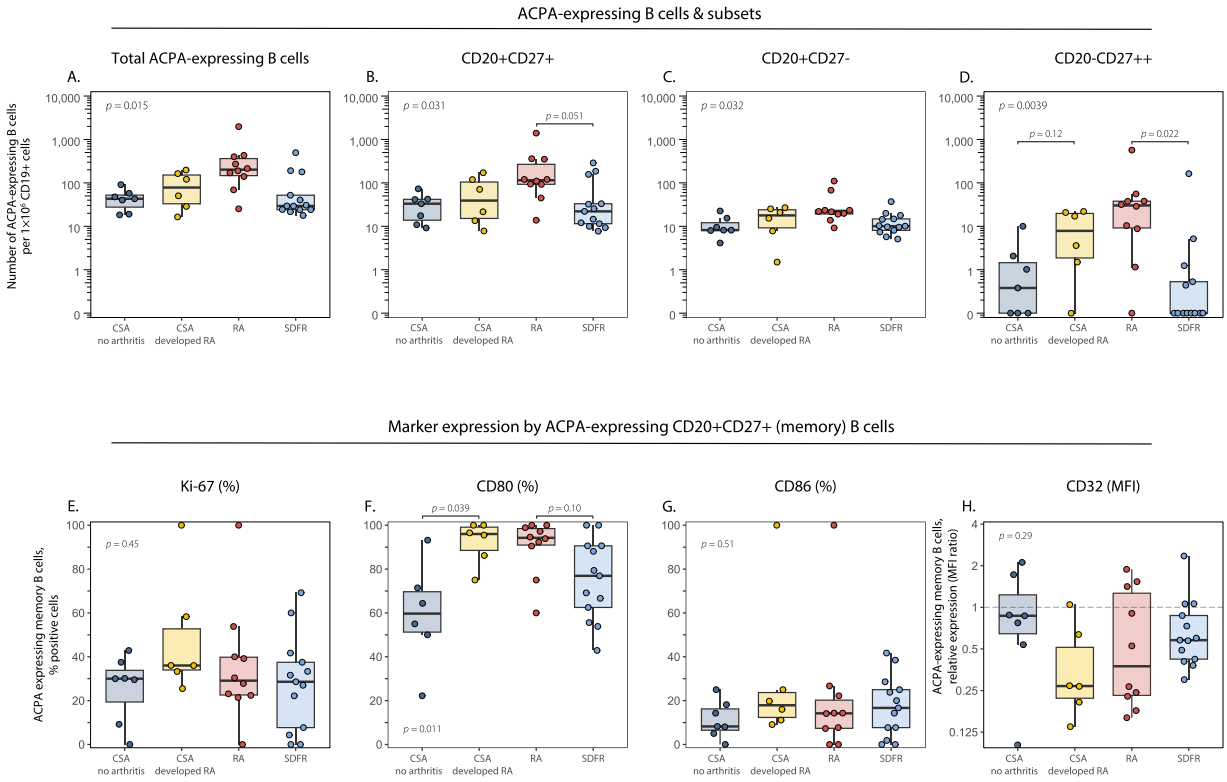


Figure 1. (A) Identification of CD19$^+$ ACPA-expressing B cells in CSA nonconverters (n = 7), CSA future converters (n = 6), RA patients (n = 10), and RA patients in SDFR (n = 13). All CSA samples were collected at CSA baseline. ACPA-expressing B cells were divided into subsets based on CD20 and CD27 expression: (B) CD20$^+$CD27$^+$ cells were considered MBCs, (C) CD20$^+$CD27$^-$ cells were mainly naive B cells, and (D) CD20$^-$CD27^{++} cells were plasmablasts. (E-H) Expression of Ki-67 (E), CD80 (F), CD86 (G), and CD32 (H) by ACPA-expressing MBCs in CSA nonconverters vs CSA future converters, and in RA vs SDFR. (Panel F depicts 6 CSA nonconverters instead of 7 due to a technical problem with the CD80 staining at this individual time point). Panels display boxplots with individual datapoints; each datapoint represents 1 patient. Groups were compared with Kruskal–Wallis test (*P* values in upper left or bottom left corner) followed by post hoc analysis with multiple testing correction to compare specific groups, if applicable. ACPA, anti-citrullinated protein antibody; CSA, clinically suspect arthralgia; MFI, mean fluorescence intensity; RA, rheumatoid arthritis; SDFR, sustained disease-modifying antirheumatic drug-free remission.

Competing interests

All authors declare they have no competing interests.

Contributors

NJB, HK, AHM, REMT, HUS: concept and design of the study. NJB, MV, SN: collection of patient samples. NJB, SN: data collection and interpretation. REMT, HUS: supervision of the work. NJB, REMT, HUS: main draft of the manuscript. All authors read and approved the current manuscript.

Funding

The authors acknowledge funding from the Dutch Arthritis Foundation (grants 17-1-402 [to REMT], 15-2-402 [to HUS], and 18-1-205 [to HUS]), the IMI funded project RTCure (777357), and the Health ~ Holland Life Sciences & Health sector programs Target to B! (LSHM18055-5 GF) and Immune-HealthSeed (LSHM22042- SGF). REMT is the recipient of a European Research Council Advanced grant (AdG2019-884796). HUS is the recipient of a NWO-ZonMW VIDI grant (project 09150172010067).

Patient consent for publication

Not applicable.

Ethics approval

The ethical review board of the LUMC gave permission to conduct the study (protocol P17.151). All donors gave written informed consent.

Provenance and peer review

Not commissioned; externally peer reviewed.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.05.006.

Orcid

Nienke J. Blomberg: http://orcid.org/0009-0008-0287-6395
Hendy Kristyanto: http://orcid.org/0000-0002-8022-6637

Sam Neppelenbroek: http://orcid.org/0000-0002-9582-2837
Annette H.M. van der Helm-van Mil: http://orcid.org/0000-0001-8572-1437
René E.M. Toes: http://orcid.org/0000-0002-9618-6414
Hans Ulrich Scherer: http://orcid.org/0000-0002-5700-5617

REFERENCES

[1] Kristyanto H, Blomberg NJ, Slot LM, van der Voort EIH, Kerkman PF, Bakker A, et al. Persistently activated, proliferative memory autoreactive B cells promote inflammation in rheumatoid arthritis. Sci Transl Med 2020;12(570): eaaz5327.
[2] Reijm S, Kwekkeboom JC, Blomberg NJ, Suurmond J, van der Woude D, Toes RE, et al. Autoreactive B cells in rheumatoid arthritis include mainly activated CXCR3+ memory B cells and plasmablasts. JCI Insight 2023;8 (20):e172006.
[3] Scherer HU, van der Woude D, Toes REM. From risk to chronicity: evolution of autoreactive B cell and antibody responses in rheumatoid arthritis. Nat Rev Rheumatol 2022;18(7):371–83.
[4] Ten Brinck RM, van Steenbergen HW, van Delft MAM, Verheul MK, Toes REM, Trouw LA, et al. The risk of individual autoantibodies, autoantibody combinations and levels for arthritis development in clinically suspect arthralgia. Rheumatology (Oxford) 2017;56(12):2145–53.
[5] Matthijssen XME, Niemantsverdriet E, Huizinga TWJ, van der Helm-van Mil AHM. Enhanced treatment strategies and distinct disease outcomes among autoantibody-positive and -negative rheumatoid arthritis patients over 25 years: a longitudinal cohort study in the Netherlands. PLoS Med 2020;17 (9):e1003296.
[6] Ellebedy AH, Jackson KJ, Kissick HT, Nakaya HI, Davis CW, Roskin KM, et al. Defining antigen-specific plasmablast and memory B cell subsets in human blood after viral infection or vaccination. Nat Immunol 2016;17(10):1226–34.

Nienke J. Blomberg *, Hendy Kristyanto, Marloes Verstappen, Sam Neppelenbroek, Annette H.M. van der Helm-van Mil, René E.M. Toes, Hans Ulrich Scherer
Department of Rheumatology, Leiden University Medical Center, Leiden, Netherlands

***Correspondence to** Nienke J. Blomberg.
E-mail address: n.j.blomberg@lumc.nl (N.J. Blomberg).

Ann Rheum Dis 85 (2026) 391–392

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Letter

Belimumab induces early and sustained resolution of lupus arthritis

Belimumab, an antibody against B lymphocyte stimulator/B cell activating factor (BLys/BAFF), was approved as the first biological therapy for systemic lupus erythematosus (SLE) in 2011 [1,2]. The overall therapeutic efficacy of belimumab is well established, including reduction in SLE disease activity scores, reduction in glucocorticoid doses, prevention of SLE flares, and increased rates of patients in lupus low disease activity state (LLDAS) and Definition of Remission in SLE (DORIS) remission [3–5]. In clinical routine, however, the primary goal is usually the resolution of ongoing SLE activity in a specific organ system. Lupus arthritis is one of the most common inflammatory manifestations of SLE and is associated with pain, impaired function, and irreversible damage [6].

We investigated the resolution of lupus arthritis in the cumulated data of 5 large randomised controlled trials (RCTs) of belimumab, ie, BLISS-52 (NCT00424476), BLISS-76 (NCT00410384), BLISS-NEA (NCT01345253), BLISS-SC (NCT01484496), and EMBRACE (NCT01632241), kindly provided by GlaxoSmithKline based on a Data Sharing Agreement of November 6, 2019. These datasets included 2913 patients treated either with an approved dose of belimumab (n = 1769) or placebo (n = 1144).

Active lupus arthritis was defined as a musculoskeletal British Isles Lupus Assessment Group index (BILAG) A, B, or C, given that joint count assessments were not performed. The BILAG categories were analysed at baseline and at 12, 24, and 52 weeks. The primary analysis of this study was on sustained resolution of arthritis, defined as a BILAG D score, from the time of resolution to the 52-week visit. Differences between the curves of sustained resolution of the index items were analysed by log-rank test.

In the belimumab and placebo groups, the patients were 94.7% and 94.0% female, respectively, and at baseline were aged 36.7 ± 11.4 (mean ± SD) and 37.4 ± 12.0 years, with a disease duration of 6.4 ± 6.3 and 6.6 ± 6.5 years and body mass index of 25.7 ± 6.3 and 25.6 ± 6.1 kg/m^2, respectively. At baseline, in all 5 trials together, 88% of the patients were on glucocorticoids and 52% were on conventional immunosuppressants (primarily azathioprine, mycophenolate, or methotrexate). We analysed 2383 patients (81.8%) with active lupus arthritis at baseline; of those, 1427 received belimumab and 956 received placebo.

For an overview of potential changes, BILAG A, B, C, and D scores were plotted for belimumab and placebo at baseline and weeks 12, 24, and 52 (Fig A). Changes were seen from week 12 onwards, and the analysis suggested differences between belimumab and placebo.

Therefore, we next analysed persistent resolution of lupus arthritis, observing it in 55.9% (797/1427) patients under belimumab plus standard therapy versus 47.8% (457/956) patients under placebo plus standard therapy ($P < .0001$). Notably, separation between belimumab and placebo was apparent from week 12 onwards but began at week 8 (Fig B).

This post-hoc analysis of data from 5 large belimumab RCTs found a rapid effect of belimumab on lupus arthritis, with sustained resolution of the manifestations documented in more than half of the patients. Importantly, our investigation revealed a somewhat unexpectedly early differentiation between belimumab and placebo.

These post-hoc analyses were not a part of the original analysis plan of the RCTs. Although the hypotheses were independently tested in this dataset, the results of the statistical tests are

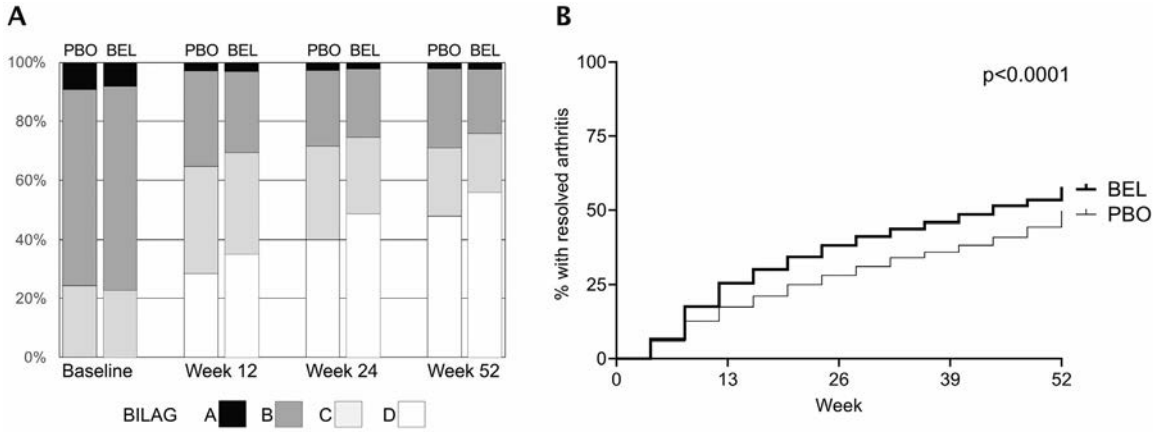


Figure. Arthritis analysed by changes in musculoskeletal BILAG (A) and by persistent resolution of arthritis (B). BEL, belimumab; BILAG, British Isles Lupus Assessment Group index; PBO, placebo.

https://doi.org/10.1016/j.ard.2025.08.016
Received 10 June 2025; Revised 12 August 2025; Accepted 13 August 2025

therefore not comparable with those performed on predefined endpoints addressing the research questions in the original studies. Nevertheless, all patients with the respective organ manifestations in the 5 RCTs were included, the data stem from double-blind placebo-controlled trials, and the numbers of participants are large, contributing to the robustness of the findings.

These data suggests that many patients with lupus arthritis may be expected to show improvement in joint symptoms as early as 8 weeks after initiation of belimumab therapy.

Competing interests

Nicolai Leuchten reports a relationship with GlaxoSmithKline Inc that includes: consulting or advisory, speaking and lecture fees, and travel reimbursement. Nicolai Leuchten reports a relationship with AstraZeneca Pharmaceuticals LP that includes: consulting or advisory, speaking and lecture fees, and travel reimbursement. Nicolai Leuchten reports a relationship with AbbVie Inc that includes: consulting or advisory, speaking and lecture fees, and travel reimbursement. Nicolai Leuchten reports a relationship with Chugai Pharmaceutical Co Ltd that includes: consulting or advisory. Martin Aringer reports a relationship with GlaxoSmithKline Inc that includes: consulting or advisory, speaking and lecture fees, and travel reimbursement. Martin Aringer reports a relationship with Hoffmann-La Roche Limited that includes: speaking and lecture fees and travel reimbursement. Martin Aringer reports a relationship with AstraZeneca Pharmaceuticals LP that includes: consulting or advisory, speaking and lecture fees, and travel reimbursement. Ioannis Parodis reports a relationship with AstraZeneca Pharmaceuticals LP that includes: consulting or advisory, funding grants, and speaking and lecture fees. Ioannis Parodis reports a relationship with GlaxoSmithKline Inc that includes: consulting or advisory, funding grants, and speaking and lecture fees. Ioannis Parodis reports a relationship with Hoffmann-La Roche Limited that includes: consulting or advisory, funding grants, and speaking and lecture fees. Ioannis Parodis reports a relationship with Aurinia Pharmaceuticals Inc that includes: consulting or advisory, funding grants, and speaking and lecture fees. Ioannis Parodis reports a relationship with Bristol-Myers Squibb Company that includes: consulting or advisory, funding grants, and speaking and lecture fees. Ioannis Parodis reports a relationship with Otsuka Pharmaceutical Co Ltd that includes: consulting or advisory, funding grants, and speaking and lecture fees. Ioannis Parodis reports a relationship with Novartis Pharmaceuticals Corporation that includes: consulting or advisory, funding grants, and speaking and lecture fees. The other author declares no competing interests.

CRediT authorship contribution statement

Nicolai Leuchten: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Ioannis Parodis:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Ralph Brinks:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Martin Aringer:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization.

Acknowledgements

The authors would like to thank all patients with SLE who participated in the trials.

Funding

IP is supported by grants from the Swedish Rheumatism Association (R-995882), King Gustaf V's 80-year Foundation (FAI-2023-1055), Swedish Society of Medicine (SLS-974449), Nyckelfonden (OLL-1000881), Professor Nanna Svartz Foundation (2021-00436), Ulla and Roland Gustafsson Foundation (2024-43), Region Stockholm (FoUI-1004114), and Karolinska Institutet.

Patient consent for publication

Not applicable.

Ethics approval

Was obtained as part of the original RCTs.

Provenance and peer review

Not commissioned; externally peer reviewed.

Orcid

Nicolai Leuchten: http://orcid.org/0000-0002-9348-5259

REFERENCES

[1] Furie R, Petri M, Zamani O, Cervera R, Wallace DJ, Tegzová D, et al. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. Arthritis Rheum 2011 Dec;63(12):3918–30.

[2] Navarra SV, Guzmán RM, Gallacher AE, Hall S, Levy RA, Jimenez RE, et al. Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. Lancet 2011 Feb 26;377(9767):721–31.

[3] Parodis I, Lindblom J, Levy RA, Zen M, Cetrez N, Gomez A, et al. Attainment of remission and low disease activity after treatment with belimumab in patients with systemic lupus erythematosus: a post-hoc analysis of pooled data from five randomised clinical trials. Lancet Rheumatol 2024 Nov;6(11):e751–61.

[4] van Vollenhoven RF, Petri M, Wallace DJ, Roth DA, Molta CT, Hammer AE, et al. Cumulative corticosteroid dose over fifty-two weeks in patients with systemic lupus erythematosus: pooled analyses from the phase III belimumab trials. Arthritis Rheumatol 2016 Sep;68(9):2184–92.

[5] Ginzler EM, Wallace DJ, Merrill JT, Furie RA, Stohl W, Chatham WW, et al. Disease control and safety of belimumab plus standard therapy over 7 years in patients with systemic lupus erythematosus. J Rheumatol 2014 Feb;41(2):300–9.

[6] Tsokos GC. The immunology of systemic lupus erythematosus. Nat Immunol 2024 Aug;25(8):1332–43.

Nicolai Leuchten [1,*], Ioannis Parodis [2,3], Ralph Brinks [4], Martin Aringer [1]

[1] *Division of Rheumatology, Department of Medicine III, University Center for Autoimmune and Rheumatic Entities (UCARE), University Medical Center and Faculty of Medicine TU Dresden, Dresden, Germany*

[2] *Division of Rheumatology, Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital, and Center for Molecular Medicine (CMM), Stockholm, Sweden*

[3] *Department of Rheumatology, Faculty of Medicine and Health, Örebro University, Örebro, Sweden*

[4] *Department for Medical Biometry and Epidemiology, Faculty of Health, University Witten, Herdecke, Germany*

*Correspondence to Dr Nicolai Leuchten, Department of Medicine III, Carus Medical Center, Dresden, Germany.
E-mail address: nicolai.leuchten@uniklinikum-dresden.de (N. Leuchten).

Ann Rheum Dis 85 (2026) 393–395

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Letter

The TFIID complex is a new autoantibody target in systemic sclerosis

Systemic sclerosis (SSc) is characterised by systemic fibrosis, vascular dysfunction, and the presence of antinuclear autoantibodies. The major SSc-associated antinuclear antibodies include anticentromere, antitopoisomerase I, anti−RNA polymerase III and antifibrillarin autoantibodies [1]. These autoantibodies are generally mutually exclusive and define clinically relevant subgroups.

In up to 10% of persons with SSc, the target antigens of the antinuclear autoantibodies are unknown. Using an unbiased immunoprecipitation-mass spectrometry (IP-MS) approach, we recently identified novel and rare autoantigens in SSc, including telomere-associated and telomerase-associated proteins and components of the spliceosome [2,3]. Using IP-MS, in this letter, we describe another new target antigen in SSc.

We examined a database of IP-MS identifications of 106 persons with SSc from 2 Belgian academic hospitals (Ghent University Hospital [n = 59] and University Hospitals Leuven [n = 47]) without autoantibody specificity as tested by commercial assays, previously described [2], for potential novel autoantibody targets associated with transcription. TATA-binding protein (TBP)-associated factors (TAFs) were precipitated by sera of 4 persons (2 from Ghent and 2 from Leuven; confirmed in a second experiment (Supplementary Table S1)). Together with TBP, TAFs form the 3-lobed transcription factor IID complex (TFIID) (Fig C). The TFIID complex recognises core promoter sequences on DNA. The binding of TFIID is the first step of the assembly of the preinitiation complex, which is needed to position RNA polymerase II onto the correct DNA sequence to start transcription [4].

To identify additional cases, we tested samples from individuals with SSc with unidentified autoantibodies from 3 extra hospitals (Cliniques Universitaires Saint-Luc, Belgium [n = 45], Chris Hani Baragwanath Hospital, South Africa [n = 28], and Centre Hospitalier Universitaire d'Angers, France [n = 13]) by IP-MS. The analysis was done using a quadrupole time-of-flight mass spectrometer (Supplementary Methods). Two additional cases precipitating TFIID subunits were identified (Supplementary Table S2). Figure A and Supplementary Table S3 summarise the results from a confirmatory experiment including all 6 anti-TFIID−positive cases and 6 controls (3 healthy controls and 3 topoisomerase I antibody-positive disease controls). In the 6 index cases, different subunits of the TFIID complex were identified in the immunoprecipitate: TAF2, TAF4, TAF4B, TAF5, TAF6, TAF8, TAF9, TAF9B, TAF10, TAF11, TAF12, and TAF13. TBP was identified in 2 of the 6 of the cases. In controls, only TAF9 and TAF9B were precipitated. In order to confirm the specificity of anti-TIFIID antibodies for SSc, IP-MS was performed in healthy individuals (n = 34) and in patients with idiopathic inflammatory myopathy (IIM, n = 12), Sjögren's disease (n = 12), systemic lupus erythematosus (SLE, n = 36), and antitopoisomerase I−positive, anticentromere-positive, or anti−RNA polymerase III−positive SSc (n = 28). TAF9, TAF9B, and TAF10 were often precipitated in these control samples (Supplementary Table S4).

Of the 6 patients, 5 anti−TFIID antibody−positive patients had interstitial lung disease, and 4 patients had limited cutaneous SSc. None of the patients had scleroderma renal crisis or pulmonary arterial hypertension. The HEp-2 indirect immunofluorescence patterns of the patients were nuclear speckled (n = 4), nuclear speckled/homogeneous (n = 1), and nuclear speckled/nucleolar (n = 1). Additional clinical features are shown in Figure B and Supplementary Table S5.

Autoantibodies against TBP have been described in up to 24% of persons with systemic sclerosis, overlap syndromes, and systemic lupus erythematosus [6]. However, in this study, purified recombinant TBP, without the other subunits of the TFIID complex, was used as protein source [6]. Notably, we found TBP in the immunoprecipitate of some anti-TFIID−positive samples. As we did not encounter similar IP-MS results with sera of persons with SLE, IIM and other controls, the anti−TFIID autoantibodies identified in this study are most likely distinct from the nonspecific reactivity identified in the study of Chauhan et al [6]. Similarly, we observed nonspecific precipitation of TAF9, TAF9B, and TAF10 subunits, but precipitation of multiple (other) subunits was restricted to the 6 index patients. The exact epitope of the anti−TFIID autoantibodies in persons with SSc is currently unclear, but it is possible that it is a conformational autoepitope that depends on higher-order protein interactions in the protein complex, which are not easily captured by recombinant protein−based assays.

In conclusion, we describe TFIID as a new target of autoantibodies in SSc, thereby expanding the spectrum of SSc-associated autoantigens related to transcription. In the future, international multicentric IP-MS studies are needed to characterise and further delineate potential clinical associations of anti-TFIID autoantibodies, in parallel to other new and rare autoantibodies in SSc.

J-BV and BM shared first authorship.

EDL and XB contributed equally.

https://doi.org/10.1016/j.ard.2025.05.008
Received 30 April 2025; Accepted 11 May 2025

A

Identified TFIID proteins	Abbr.	#1	#2	#3	#4	#5	#6	DC1	DC2	DC3	HC1	HC2	HC3
TFIID subunit 1	TAF1	0	0	0	0	0	0	0	0	0	0	0	0
TFIID subunit 2	TAF2	0	0	61250	0	43086	0	0	0	0	0	0	0
TFIID subunit 3	TAF3	0	0	0	0	0	0	0	0	0	0	0	0
TFIID subunit 4	TAF4	94303	163797	192601	98662	157561	171092	0	0	0	0	0	0
TFIID subunit 4B	TAF4B	0	5153	0	0	0	0	0	0	0	0	0	0
TFIID subunit 5	TAF5	58593	119503	129468	64821	140278	155704	0	0	0	0	0	0
TFIID subunit 6	TAF6	74148	152870	154420	74191	154218	150588	0	0	0	0	0	0
TFIID subunit 7	TAF7	0	0	0	0	0	0	0	0	0	0	0	0
TFIID subunit 8	TAF8	34354	71481	54896	0	82083	80606	0	0	0	0	0	0
TFIID subunit 9	TAF9	132676	315343	220972	146620	202760	192164	41912	49050	53309	58472	49227	70107
TFIID subunit 9B	TAF9B	57704	109858	44499	47549	63538	88554	14154	9997	5405	15176	13411	14068
TFIID subunit 10	TAF10	96158	169525	122595	62200	128845	124886	0	0	0	0	0	0
TFIID subunit 11	TAF11	0	34449	0	0	0	32076	0	0	0	0	0	0
TFIID subunit 12	TAF12	0	0	19998	0	0	0	0	0	0	0	0	0
TFIID subunit 13	TAF13	0	59166	45804	17361	0	0	0	0	0	0	0	0
TATA-box-binding protein	TBP	0	37326	48964	0	0	0	0	0	0	0	0	0
DNA topoisomerase I	SCL70	0	0	0	0	0	0	2077867	2677551	869261	0	0	0

B

Clinical features	#1	#2	#3	#4	#5	#6
Limited cutaneous involvement						
Diffuse cutaneous involvement						
Interstitial lung disease						
Elevated creatine kinase						
Malignancy						

C

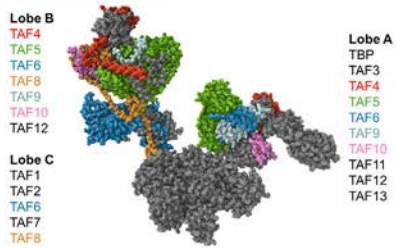


Figure. Anti-TFIID autoantibodies. (A) Hi-3 values of precipitated proteins by sera from 6 anti–TFIID-positive index patients (1-6): 3 antitopoisomerase I–positive SSc disease controls (DC1, DC2, and DC3) and 3 healthy controls (HC1, HC2, and HC3). Hi-3 values represent per protein the sum of the intensity of the top 3 most intense peptides and are an estimate of the relative abundance of the protein in the immunoprecipitate. Confidence colour scores of the protein identifications are shown (green and yellow) (Supplementary Methods). Samples 1 and 2 are from Ghent University Hospital, samples 3 and 4 are from University Hospitals Leuven, sample 5 is from Cliniques Universitaires Saint-Luc, and sample 6 is from Centre Hospitalier Universitaire d'Angers. (B) Clinical features of 6 anti-TFIID–positive individuals with SSc. (C) Structure of TFIID (https://doi.org/10.2210/pdb6MZL/pdb), with colour matching to subunits from (A). Made with Mol* Viewer [5]. DC, disease control; HC, healthy control; SSc, systemic sclerosis; TAF, TATA box–binding protein (TBP)–associated factor; TF, transcription factor; TFIID, transcription factor IID complex.

Competing interests

J-BV reports financial support was provided by Research Foundation Flanders. VS has received research grants, consulting fees, and support from Boehringer-Ingelheim, Janssen-Cilag, Argenx BV, WebMD Global LLC, and BKC Moving Media Makers BV (outside the scope from the submitted work). NM was member of the data safety monitoring board for NIHIR funded Astute Trial (outside the scope from the submitted work). XB was part of a scientific advisory committee for Inova Diagnostics and Thermo Fisher (outside the scope from the submitted work).

Acknowledgements

We thank An Knoops for performing the indirect immunofluorescence assay.

Contributors

J-BV, EDL, and XB designed the study. J-BV, XB, DD, BM, and TDH analysed the data. XB, BM and J-BV drafted the manuscript. J-BV, DD, BM, and TDH performed the IP-MS experiments. J-BV, VS, YP, PDH, JL, DB, CB, EDL, and XB collected patient data and revised the draft manuscript. All authors revised the draft manuscript.

Funding

J-BV held a Research Foundation—Flanders (FWO) SB Fellowship (1S62419N). This study was supported by the Research Fund KU Leuven (C3/20/042) and by FWO-Applied Biomedical research (TBM) (T004821N). VS is a Senior Clinical Investigator of the Research Foundation–Flanders (Belgium) (FWO) [grant number 1802925N].

Patient consent for publication

The samples from Leuven and from Angers were collected as part of a retrospective study using left-over material of samples submitted to the clinical laboratory (secondary use) for which an informed consent waiver was authorized by the Ethical Committee of the University Hospitals Leuven (Belgium) and of CHU Angers (France). Patients were notified of the content and aim of the study. All patients from Ghent university hospital (Belgium), Cliniques Universitaires Saint-Luc (Belgium) and Chris Hani Baragwanath Hospital (South Africa) signed informed consent.

Ethics approval

The study was approved by the local Ethical Committees: S60347/B32220071204, S65989 (University Hospitals Leuven);

B67020084358 (Ghent University Hospital). MTA was in place between University Hospitals Leuven and Cliniques Universitaires Saint-Luc, Centre Hospitalier Universitaire d'Angers, and University of Bath.

Provenance and peer review

Not commissioned; externally peer reviewed.

Patient and public involvement

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

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.05.008.

Orcid

Xavier Bossuyt: http://orcid.org/0000-0002-3977-4172

REFERENCES

[1] Bossuyt X, De Langhe E, Borghi MO, Meroni PL. Understanding and interpreting antinuclear antibody tests in systemic rheumatic diseases. Nat Rev Rheumatol 2020;16:715–26.
[2] Vulsteke J-B, Smith V, Bonroy C, Derua R, Blockmans D, De Haes P, et al. Identification of new telomere- and telomerase-associated autoantigens in systemic sclerosis. J Autoimmun 2023;135:102988.
[3] Vulsteke J-B, Smith V, Bonroy C, Lenaerts JL, Derua R, Wuyts WA, et al. Autoantibodies against the NineTeen complex and U5 RNP in systemic sclerosis. J Autoimmun 2023;139:103056.
[4] Patel AB, Louder RK, Greber BJ, Grünberg S, Luo J, Fang J, et al. Structure of human TFIID and mechanism of TBP loading onto promoter DNA. Science 2018;362:eaau8872. doi: 10.1126/science.aau8872.
[5] Sehnal D, Bittrich S, Deshpande M, Svobodová R, Berka K, Bazgier V, et al. Mol* Viewer: modern web app for 3D visualization and analysis of large biomolecular structures. Nucleic Acids Res 2021;49:W431–7.
[6] Chauhan R, Handa R, Das TP, Pati U. Over-expression of TATA binding protein (TBP) and p53 and autoantibodies to these antigens are features of systemic sclerosis, systemic lupus erythematosus and overlap syndromes. Clin Exp Immunol 2004;136:574–84.

Jean-Baptiste Vulsteke[1,2], Birthe Michiels[2,3], Vanessa Smith[4,5,6,7], Yves Piette[4,5,7,8], Marie Vanthuyne[7,9,10], Carole Lacout[11], Antoine Brochard[11], Mohammed Tikly[12], Jonas De Leeuw[1,2], Doreen Dillaerts[2], Tom Dehaemers[2], Petra De Haes[7,13,14], Jan L Lenaerts[1], Daniel Blockmans[7,15,16], Neil McHugh[17], Sarah Tansley[17], Emeline Vinatier[18], Frédéric Houssiau[7,9], Carolien Bonroy[19,20], Ellen De Langhe[1,7,21], Xavier Bossuyt[2,3,*]

[1] *Rheumatology, University Hospitals Leuven, Leuven, Belgium*
[2] *Clinical and Diagnostic Immunology, Department Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium*
[3] *Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium*
[4] *Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium*
[5] *Rheumatology, Ghent University Hospital, Ghent, Belgium*
[6] *Unit for Molecular Immunology and Inflammation, VIB Inflammation Research Center (IRC), Ghent, Belgium*
[7] *European Reference Network on Rare and Complex Connective Tissue and Musculoskeletal Diseases (ERN ReCONNET)*
[8] *Rheumatology, AZ Sint-Jan Brugge AV, Bruges, Belgium*
[9] *Rheumatology, Cliniques Universitaires Saint-Luc, Brussels, Belgium*
[10] *Rheumatology, CHU Mont Godinne, Yvoir, Belgium*
[11] *Internal Medicine, Immunology, Centre Hospitalier Universitaire d' Angers, Angers, France*
[12] *Division of Rheumatology, Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[13] *Dermatology, University Hospitals Leuven, Leuven, Belgium*
[14] *Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium*
[15] *General Internal Medicine, University Hospitals Leuven, Leuven, Belgium*
[16] *Laboratory for Clinical Infectious and Inflammatory Disorders, KU Leuven, Leuven, Belgium*
[17] *Department of Life Sciences, University of Bath, Bath, UK*
[18] *Laboratoire d'Immunologie et d'Allergologie, Centre Hospitalier Universitaire d'Angers, Angers, France*
[19] *Diagnostic Sciences, Ghent University, Ghent, Belgium*
[20] *Laboratory Medicine, Ghent University Hospital, Ghent, Belgium*
[21] *Skeletal Biology and Engineering Research Center, KU Leuven, Leuven, Belgium*

*__Correspondence to__ Dr. Xavier Bossuyt.
E-mail address: xavier.bossuyt@uzleuven.be (X. Bossuyt).

Ann Rheum Dis 85 (2026) 396–397

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Images in rheumatology

Vasculopathy, embolism, xerostomia, arthritis, and stomachache

Weijin Zhang[1], Songhao Cai[2], Shaoyu Zheng[1], Shijian Hu[1], Kedi Zheng[1], Jianqun Lin[1], Manna Chen[3], Guangzhou Du[4], Marco Matucci-Cerinic[5,6], Daniel E. Furst[7,8,9], Yukai Wang[1,*]

[1] *Department of Rheumatology and Immunology, Shantou Central Hospital, Shantou, China*
[2] *Department of Hematology, Shantou Central Hospital, Shantou, China*
[3] *Department of Clinical Laboratory, Shantou Central Hospital, Shantou, China*
[4] *Department of Radiology, Shantou Central Hospital, Shantou, China*
[5] *Unit of Immunology, Rheumatology, Allergy and Rare diseases (UnIRAR), IRCCS San Raffaele Hospital, Milan, Italy*
[6] *Vita Salute San Raffaele University, Milan, Italy*
[7] *Division of Rheumatology, Department of Medicine, University of California at Los Angeles, LA, USA*
[8] *University of Washington, Seattle, WA, USA*
[9] *Division of Rheumatology, Department of Experimental and Clinical Medicine, Careggi University Hospital, University of Florence, Florence, Italy*

A 65-year-old man was hospitalised with 1 month of a stomachache. Over the past 2 years, he has been diagnosed with Behcet's disease, rheumatoid arthritis, or cutaneous vasculitis at different medical centres based on recurrent fever, oral ulcers, ocular inflammation, rash, arthritis, xerostomia, and bilateral auricular chondritis, as well as elevated acute-phase reactants. His symptoms were alleviated with oral high-dose prednisone of 40 mg/d, methotrexate (15 mg/wk), and hydroxychloroquine (0.2 g twice a day). However, fever and rash recurred during the tapering of prednisone to 30 mg/d. Physical examination revealed erythematous plaques on the neck/thoracic region (Fig, A) and tenderness in the left upper quadrant. Laboratory findings revealed significant systemic inflammation, with elevated biomarkers: C-reactive protein 117 mg/L (normal range [NR] < 8), erythrocyte sedimentation rate 60 mm/h (NR < 20), interleukin (IL)-6 64.16 pg/mL (NR < 5.5), and D-dimer 3210 μg/L (NR < 550). It also demonstrated macrocytic anaemia with a mean corpuscular volume level of 100.9 fL (NR 82-100). Human leukocyte antigen B51, antinuclear antibody, anti-extractable nuclear antigen antibody, and double-stranded DNA were negative. Neutrophilic vasculitis was confirmed by skin biopsy (Fig, B). Computed tomography (CT) angiography revealed thoracoabdominal aortic thrombosis (Fig, C), accompanied by superior mesenteric artery dissection (Fig, D). Contrast-enhanced CT revealed splenic infarction (Fig, E). Bone marrow aspiration showed characteristic cytoplasmic vacuolisation in myeloid precursors without dysplasia (Fig, F). Targeted gene sequencing identified a somatic *UBA1* exon 3 mutation (c.121A>C, p.Met41Leu). A diagnosis of vacuoles, E1 enzyme, X-linked, autoinflammatory, and somatic (VEXAS) syndrome was made. The patient was treated with methylprednisolone 1 mg/kg/d, tocilizumab 8 mg/kg monthly, and anticoagulation therapy. He achieved resolution of symptoms and normalised inflammatory markers.

VEXAS syndrome, an adult-onset autoinflammatory disorder driven by somatic *UBA1* mutations, manifests with cytopenia, myeloid vacuolisation, chondritis, pulmonary involvement, vasculitis, and progression of haematologic malignancies [1]. The striking genotype-phenotype correlation underscores the necessity of integrating molecular diagnostics into classification criteria [2]. Aortic thrombosis has been reported only in isolated cases of VEXAS syndrome, while this case demonstrates catastrophic and multiple vessel complications, including thoracoabdominal aortic thrombosis, superior mesenteric artery dissection, and splenic infarction, expanding the recognised spectrum of vascular phenotypes in VEXAS syndrome. Current management relies on high-dose glucocorticoids, targeted biologics, hypomethylating agents, and allogeneic bone marrow

*Correspondence to Dr Yukai Wang. (Y. Wang).
E-mail address: stzxyywyk@126.com (Y. Wang).
Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.06.003
Received 2 May 2025; Revised 2 June 2025; Accepted 4 June 2025

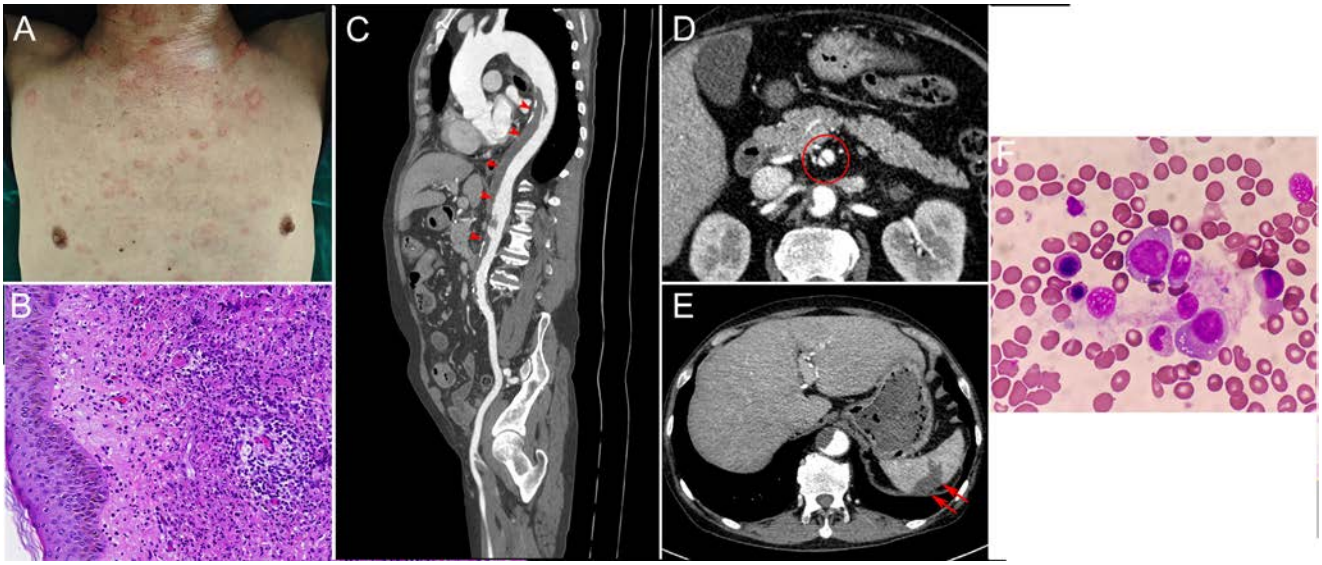


Figure. VEXAS syndrome with vessel complications and inflammatory manifestations. (A) Physical examination revealed erythematous plaques on the neck/thoracic region. (B) Skin biopsy showed inflammatory cell infiltration, predominantly composed of neutrophils, observed throughout the entire dermis and within the superficial subcutaneous adipose septa. (C) Curved planar reformation of aortic computed tomography (CT) angiography demonstrated a long-segment thrombus in the thoracoabdominal aorta (arrowhead). (D) Axial aortic CT angiography image revealed a dissection of the superior mesenteric artery with true and false lumina (circled). (E) Contrast-enhanced axial scan displayed a splenic infarction manifesting as a patchy, wedge-shaped hypoenhancing lesion (long arrow). (F) Bone marrow aspiration showed vacuolisation in myeloid precursors.

transplantation, though evidence remains limited [3]. Considering the patient's hyperinflammatory state, glucocorticoids and targeted biologics were used. IL-6 blockade was prioritised over Janus kinase inhibitors due to thrombotic risk concerns. However, long-term follow-up remains crucial.

Contributors

WZ, SC, and SZ were involved in the management of the patient; SH, KZ, JL, MC, and GD prepared the clinical image and the medical description of the case; WZ drafted and critically revised the article, which was finished by YW; MM-C, DEF, and YW are the guarantors.

Funding

This work was supported by grants from the National Natural Science Foundation of China (number 82271853) and the Zhongnanshan Medical Foundation of Guangdong Province (ZNSXS-20240011).

Competing interests

All authors declare they have no competing interests.

Patient consent for publication

Consent was obtained directly from the patient.

Ethics approval

This case report is exempt from formal ethics review, and written informed consent was obtained from the participant prior to inclusion.

Provenance and peer review

Not commissioned; externally peer reviewed.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.06.003.

Orcid

Weijin Zhang: http://orcid.org/0000-0002-1477-7465
Yukai Wang: http://orcid.org/0000-0003-2468-3208

REFERENCES

[1] Beck DB, Ferrada MA, Sikora KA, Ombrello AK, Collins JC, Pei W, et al. Somatic mutations in UBA1 and severe adult-onset autoinflammatory disease. N Engl J Med 2020;383(27):2628–38. doi: 10.1056/NEJMoa2026834.
[2] Grayson PC, Beck DB, Ferrada MA, Nigrovic PA, Kastner DL. VEXAS syndrome and disease taxonomy in rheumatology. Arthritis Rheumatol 2022;74(11):1733–6. doi: 10.1002/art.42258.
[3] Koster MJ, Lasho TL, Olteanu H, Reichard KK, Mangaonkar A, Warrington KJ, et al. VEXAS syndrome: clinical, hematologic features and a practical approach to diagnosis and management. Am J Hematol 2024;99(2):284–99. doi: 10.1002/ajh.27156.

Ann Rheum Dis 85 (2026) e9–e10

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Correspondence

Correspondence to 'Assessing the performance of AI chatbots in answering patients' common questions about low back pain'

Dear Editor,

We have been vigilantly monitoring the integration of artificial intelligence (AI) within the healthcare sector, with particular interest in the study titled "Evaluating the Performance of AI Chatbots in Responding to Patients' Common Queries on Low Back Pain" [1]. This research harnessed the capabilities of ChatGPT to generate queries pertaining to lifestyle modifications within the management and subsequent evaluation of low back pain (LBP), offering a novel perspective on the role of AI in clinical settings. The study's findings suggest that ChatGPT is adept at engaging in interactive dialogues, disseminating information, addressing inquiries, and providing advice related to lifestyle alterations to assist in the management of LBP symptoms.

However, it is with concern that we note that nearly half (42.1%) of the 1069 recommendations generated from 120 responses contained inaccuracies. Moreover, studies on other medical conditions, including common gastrointestinal disorders, oncology, and radiology, have also identified significant accuracy issues with large language models (LLMs), indicating that a substantial proportion of the responses produced are erroneous or inaccurate [2–4]. These revelations underscore the critical risk of patients receiving and acting upon potentially flawed medical advice [5].

Critics argue that LLMs, such as ChatGPT, may create an illusion of intelligence rather than demonstrating true understanding. These models predict subsequent words or syntactic groups based on previous ones through a process known as "tokenisation," where each token approximates 0.75 words. They are then refined through reinforcement learning with human feedback and sparse training data, prioritising output consistency over depth of understanding [6]. This approach raises concerns about the reliability of AI-generated medical advice and the risk of spreading incorrect information.

Given these concerns, it is crucial to expand our discourse to address the critical issue of clarifying responsibilities for erroneous outcomes in AI-assisted healthcare. Determining who is responsible when things go wrong necessitates a nuanced understanding of the intricate interplay among various stakeholders: the AI developers who craft the underlying algorithms, the healthcare providers who implement AI in clinical settings, and the patients who rely on AI-generated advice. Each party occupies a distinct yet interconnected position within the AI-assisted healthcare ecosystem. Developers bear the responsibility of ensuring the AI's robustness, reliability, and accuracy; healthcare providers must rigorously verify AI responses before communicating them to patients; and patients, in turn, should be fully informed of AI's limitations and capabilities.

However, delineating these responsibilities is not a straightforward endeavour. In order to foster accountability and mitigate risks associated with AI-assisted healthcare, we propose that the following key measures should be implemented:

1. **Establish legal and ethical guidelines:** It is essential to establish clear legal and ethical guidelines to regulate the role of AI in medical decision-making. This includes defining responsibility allocation and protecting patient rights, ensuring that the actions of all parties involved in AI-assisted medical decisions comply with legal and ethical standards.
2. **Clarify responsibility assignment:** The responsibilities of AI tool developers, users, and regulators must be clearly defined. This facilitates the rapid tracking of responsibility and the implementation of corresponding accountability measures in the event of issues, ensuring transparency and fairness in accountability. We propose that while AI developers bear the responsibility for creating accurate and reliable tools, medical institutions must ensure the proper integration and application of these tools in clinical settings. Patients, on the other hand, should be educated on the limitations and potential risks associated with AI-assisted healthcare. The primary responsibility should lie with the medical institutions, as they are in the best position to oversee the safe and effective use of AI in patient care.
3. **Adherence to regulatory frameworks:** It is imperative to adhere to stringent regulatory frameworks to ensure the safety and efficacy of AI applications in healthcare. Compliance with regulatory bodies is not merely a procedural requirement but a fundamental safeguard for patient welfare. Given the dynamic evolution of AI technologies, a flexible yet robust regulatory approach is advocated. This approach should accommodate the iterative advancements in AI while maintaining a vigilant stance on safety. It is proposed that the regulatory guidelines should encompass provisions for ongoing surveillance and periodic updates of AI tools. This

Y.H., H.W., and X.M. contributed equally.

Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2024.12.001

Received 8 December 2024; Accepted 12 December 2024

ensures that while AI tools remain compliant with the highest safety standards, the spirit of innovation is preserved. The objective is to create a regulatory milieu that nurtures both the evolution of AI and the stringent adherence to standards, thereby enhancing patient care and propelling healthcare advancements through AI integration.

4. **Interdisciplinary collaboration:** Encourage collaboration among experts in the fields of medicine, technology, law, and ethics. Through this interdisciplinary cooperation, the development and regulation of AI applications in the medical field can be jointly managed, ensuring that the development of AI technology meets the demands of technological progress and the needs of medical practice.

Competing interests

All authors declare they have no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Patient consent for publication

Not applicable.

Ethics approval

Not applicable.

Provenance and peer review

Not commissioned; externally peer reviewed.

REFERENCES

[1] Scaff SPS, Reis FJJ, Ferreira GE, Jacob MF, Saragiotto BT. Assessing the performance of AI chatbots in answering patients' common questions about low back pain. Ann Rheum Dis 2024 ard-2024-226202. doi: 10.1136/ard-2024-226202.

[2] Kerbage A, Kassab J, El Dahdah J, Burke CA, Achkar JP, Rouphael C. Accuracy of ChatGPT in common gastrointestinal diseases: impact for patients and providers. Clin Gastroenterol Hepatol 2024;22(6):1323–1325.e3. doi: 10.1016/j.cgh.2023.11.008.

[3] Halawani A, Almehmadi SG, Alhubaishy BA, Alnefaie ZA, Hasan MN. Empowering patients: how accurate and readable are large language models in renal cancer education. Front Oncol 2024;14:1457516. doi: 10.3389/fonc.2024.1457516.

[4] Sun SH, Huynh K, Cortes G, Hill R, Tran J, Yeh L, et al. Testing the ability and limitations of ChatGPT to generate differential diagnoses from transcribed radiologic findings. Radiology 2024;313(1):e232346. doi: 10.1148/radiol.232346.

[5] Huo B, Cacciamani GE, Collins GS, McKechnie T, Lee Y, Guyatt G. Reporting standards for the use of large language model-linked chatbots for health advice. Nat Med 2023;29(12):2988. doi: 10.1038/s41591-023-02656-2.

[6] Lansiaux E, Baron MA, Vromant A. Navigating the landscape of medical triage: unveiling the potential and challenges of large language models and beyond. Am J Emerg Med 2024;78:224. doi: 10.1016/j.ajem.2024.02.013.

Yuanbin Huang[2], Hongjin Wang[1,*], Xinmiao Ma[2]
[1] *Department of Cardiovascular Surgery, Longyan First Affiliated Hospital of Fujian Medical University, Longyan, Fujian, China*
[2] *Department of Urology, The Second Hospital of Dalian Medical University, Dalian, Liaoning, China*
*__Correspondence to__ Dr Hongjin Wang.
E-mail address: doctorwhj047@163.com (H. Wang).

Ann Rheum Dis 85 (2026) e11–e12

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Correspondence

Correspondence on: 'EULAR recommendations for the treatment of systemic sclerosis: 2023 update' by Del Galdo et al

We would like to express our thanks to the task force for their efforts in developing the updated European Alliance of Associations for Rheumatology (EULAR) recommendations for systemic sclerosis (SSc) [1]. We are pleased to see the inclusion of treatment algorithms for the first time in the EULAR SSc treatment recommendation sets. The pulmonary arterial hypertension (PAH) treatment algorithm emphasises the importance of upfront combination therapy and prioritises the use of selexipag or riociguat for patients already receiving phosphodiesterase 5 inhibitors (PDE5i) and/or bosentan for digital ulcers (DUs). The latter is very important as it has been overlooked in PAH guidelines [2,3]. Given that connective tissue diseases including SSc account for approximately one-quarter to one-third of PAH patients, there is a clear need for a separate PAH treatment algorithm for this patient population. On the other hand, we have some criticisms about the PAH treatment algorithm in the recommendation set, which have summarized point by point below.

First, according to current PAH treatment guidelines, initial treatment regimen of systemic sclerosis-associated PAH (SSc-PAH) patients in high-risk groups should include intravenous (i.v.) or subcutaneous (s.c.) prostacyclin analogues (PCAs) in addition to upfront combination of endothelin receptor antagonists (ERAs) + PDE5i, which is a missing point in the algorithm [3]. Currently, epoprostenol is the only PAH-specific medication that reduced mortality in a randomised controlled trial and has also been documented to be an effective option in SSc-PAH [4]. It has been reported that, despite having poorer prognosis, patients with SSc-PAH are less frequently treated with i.v./s.c. PCAs, which indicates that management of SSc-PAH is generally inadequate in daily clinical practice [5,6]. To our point of view, this issue should be emphasised within the algorithm.

Second, the main difference in the current SSc-PAH treatment algorithm is the recommendation about adding selexipag or riociguat to the treatment or switching PDE5i to riociquat in patients who are already taking PDE5i and/or bosentan for DUs. For SSc-PAH patients at low/intermediate risk, guidelines recommend initial combination therapy with oral PAH-specific medications. Although evidence showing a clear difference in the efficacy of oral PAH-specific drugs is lacking, the combination of ERAs and PDE5i has been recommended due to its easy use and tolerability [3]. However, in a patient who is only taking a PDE5i for DU, switching PDE5i to riociquat should not be classified as a combination therapy, and this point should be explained, at least using a footnote. Furthermore, the addition of bosentan to sildenafil is not considered as effective in the treatment of PAH and is not recommended by guidelines [3]. This point should also be addressed.

Third, authors preferred to use the terms "WHO (World Health Organisation) class II or ERS (European Respiratory Society) low/intermediate risk" or "WHO class III/IV or ERS intermediate/high risk" for patient follow-up. Classification of patients using WHO functional class (FC) may improve the understanding of rheumatologists who are not familiar with PAH risk stratification. On the other hand, these 2 statements clearly overlap with each other. While the WHO FC is one of the best parameters for evaluating patients during follow-up, it is insufficient alone to determine the patient's risk status for mortality [3]. Certainly, there will be patients with WHO FC II who require treatment escalation. A simple evaluation of a PAH patient should include at least WHO FC, serum natriuretic peptide levels, and 6-minute walk distance [3,7]. Therefore, we believe that use of WHO FC classification is unnecessary in the algorithm, and recommendations should encourage rheumatologists to use multiple parameters to determine the risk status in SSc-PAH.

Fourth, there is no recommendation for the patients with other manifestations of SSc. In patients with both PAH and interstitial lung disease, the use of ERAs (mainly ambrisentan) or riociquat treatment may be harmful [8,9].

Finally, there is no comment about sotatercept in the text or in the algorithm, which could be beneficial in SSc-PAH [10].

In conclusion, we have addressed some points that are important in treatment planning for patients with SSc-PAH according to our point of view. We are aware of that it is difficult, even impossible, to develop recommendations for every situation in a complex systemic disease such as SSc. We once again congratulate the authors for including the first EULAR treatment algorithms for SSc in this recommendation set.

Competing interests

All authors declare they have no competing interests.

Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2024.10.001
Received 24 October 2024; Accepted 26 October 2024

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Patient consent for publication

Not applicable.

Ethics approval

Not applicable

Provenance and peer review

Not commissioned; externally peer reviewed.

REFERENCES

[1] Del Galdo F, Lescoat A, Conaghan PG, Bertoldo E, Čolić J, Santiago T, et al. EULAR recommendations for the treatment of systemic sclerosis: 2023 update. Ann Rheum Dis. Published online October 17, 2024. doi: 10.1136/ard-2024-226430.

[2] Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension: the Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). Eur Heart J 2016;37(1):67–119. doi: 10.1093/eurheartj/ehv317.

[3] Humbert M, Kovacs G, Hoeper MM, Badagliacca R, Berger RMF, Brida M, et al. 2022 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Respir J 2023;61(1):2200879. doi: 10.1183/13993003.00879-2022.

[4] Badesch DB, Tapson VF, McGoon MD, Brundage BH, Rubin LJ, Wigley FM, et al. Continuous intravenous epoprostenol for pulmonary hypertension due to the scleroderma spectrum of disease. A randomized, controlled trial. Ann Intern Med 2000;132(6):425–34. doi: 10.7326/0003-4819-132-6-200003210-00002.

[5] Condliffe R, Kiely DG, Peacock AJ, Corris PA, Gibbs JS, Vrapi F, et al. Connective tissue disease-associated pulmonary arterial hypertension in the modern treatment era. Am J Respir Crit Care Med 2009;179(2):151–7. doi: 10.1164/rccm.200806-953OC.

[6] Sarı A, Satış H, Ayan G, Küçükşahin O, Kalyoncu U, Fidancı AA, et al. Survival in systemic sclerosis associated pulmonary arterial hypertension in the current treatment era-results from a nationwide study. Clin Rheumatol 2024;43(6):1919–25. doi: 10.1007/s10067-024-06961-0.

[7] Brown Z, Hansen D, Stevens W, Ferdowsi N, Ross L, Quinlivan A, et al. Evaluation of the European Society of Cardiology risk assessment score in incident systemic sclerosis-associated pulmonary arterial hypertension. Arthritis Care Res (Hoboken) 2024;76(7):973–83. doi: 10.1002/acr.25328.

[8] Raghu G, Behr J, Brown KK, Egan JJ, Kawut SM, Flaherty KR, et al. Treatment of idiopathic pulmonary fibrosis with ambrisentan: a parallel, randomized trial. Ann Intern Med 2013;158(9):641–9. doi: 10.7326/0003-4819-158-9-201305070-00003.

[9] Nathan SD, Behr J, Collard HR, Cottin V, Hoeper MM, Martinez FJ, et al. Riociguat for idiopathic interstitial pneumonia-associated pulmonary hypertension (RISE-IIP): a randomised, placebo-controlled phase 2b study. Lancet Respir Med 2019;7(9):780–90. doi: 10.1016/S2213-2600(19)30250-4.

[10] Humbert M, McLaughlin V, Gibbs JSR, Gomberg-Maitland M, Hoeper MM, Preston IR, et al. Sotatercept for the treatment of pulmonary arterial hypertension. N Engl J Med 2021;384(13):1204–15. doi: 10.1056/NEJMoa2024277.

Alper Sari[1,*], Ali Akdogan[2]

[1] *Department of Rheumatology, Etlik City Hospital, Ankara, Turkey*

[2] *Department of Rheumatology, Hacettepe University Faculty of Medicine, Ankara, Turkey*

*__Correspondence to__ Dr Alper Sari, Department of Rheumatology, Etlik City Hospital, Ankara, Turkey.

E-mail address: snalpersari@hotmail.com (A. Sari).

Ann Rheum Dis 85 (2026) e13–e14

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Correspondence

Correspondence on 'Anifrolumab in childhood-onset systemic lupus erythematosus: a promising option after belimumab failure' by Argüelles Balas et al

Dear Editors,

We read with great interest the letter titled 'Anifrolumab in childhood-onset systemic lupus erythematosus: a promising option after belimumab failure' [1]. We would like to support the potential role of anifrolumab as a promising therapeutic option for children affected by systemic lupus erythematosus (SLE), sharing the case of a young boy.

In December 2022, at the age of 8, he presented with severe cutaneous lupus erythematosus, with malar rash, diffuse infiltrative papules, and nonscarring alopecia, and was poorly responsive to topical steroid therapy (Figure 1). Blood tests revealed a high antinuclear antibody titre (1:1280) and positivity for SSA/Ro, Rib-P, and Scl-70 autoantibodies. The interferon (IFN) signature showed high activity. A strong family history of autoimmune diseases was reported, as his maternal grandmother and younger sister were affected by Sjögren's syndrome.

Hydroxychloroquine (200 mg/d), oral prednisone (2 mg/kg/d with progressive tapering), and mycophenolate mofetil (1200 mg/m²/d), initially started, showed a poor clinical response. In March 2024, mycophenolate was discontinued, and ruxolitinib was introduced. However, the patient experienced progressive worsening of cutaneous symptoms, and in September 2024, ruxolitinib was also discontinued, and anifrolumab was administered at a dose of 300 mg/mo.

Remarkably, after 2 infusions, cutaneous symptoms dramatically improved, with complete resolution of the papules, malar rash, and alopecia (Figure 2). To date, he has received 7 infusions, and skin inflammation is still totally controlled, with no new disease flares.

This is a notable case of juvenile-onset SLE with prominent cutaneous involvement, in which multiple therapies failed to achieve disease control. The importance of IFNs in the pathogenesis of SLE is well known; specifically, type I IFNs play a key role in perpetuating the inflammatory loop in the lesional skin by stimulating both the innate and adaptive immune systems [2]. Plasmacytoid dendritic cells, together with neutrophils, monocytes, and keratinocytes in lupus erythematosus cutaneous layers, significantly contribute to the aetiology of lesions by type I IFN release and persistent activation, attraction and expansion of lymphocytes, and B–cell hyperactivity via B-cell activating factor [3].

Despite evidence regarding skin manifestations in interferonopathy diseases, our patient failed to respond to Janus kinase inhibition. Conversely, anifrolumab, specifically targeting IFN signalling, obtained persistent clinical remission with treatment in our patient, suggesting a disease-specific mechanism in cutaneous SLE. It should be considered that anifrolumab may represent a valuable therapeutic strategy for childhood-onset SLE with predominant cutaneous disease, especially with elevated IFN levels, as in our patient. Treatment target therapy by detailed patient stratification might move the use of anifolumab to a precocious step-up approach rather than using this strategy as a rescue treatment in refractory cases.

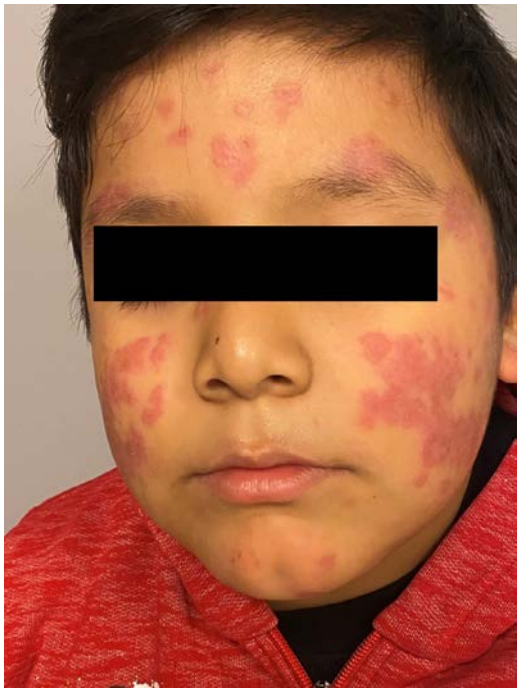


Figure 1. Clinical presentation at the time of cutaneous systemic lupus erythematosus diagnosis.

Laura Gatti and Edoardo Marrani contributed equally to this study.

Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.07.022
Received 24 July 2025; Accepted 25 July 2025

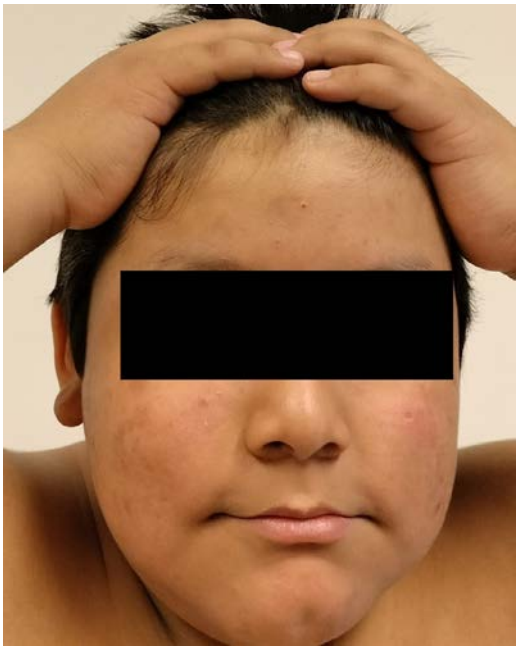


Figure 2. Clinical presentation after 2 doses of anifrolumab.

Funding

No fundings to declare.

Competing interests

The authors declare that they have no competing interests.

Patient consent for publication

Informed consent was obtained from the patient and his caregivers.

Ethics approval

Ethics committee approval was not required for this case report.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Orcid

Laura Gatti: http://orcid.org/0009-0001-4631-3614

References

[1] Argüelles Balas D, Barral Mena E, Calvo-Aranda E. Anifrolumab in childhood-onset systemic lupus erythematosus: a promising option after belimumab failure. Ann Rheum Dis 2025 in press.
[2] Niebel D, de Vos L, Fetter T, Brägelmann C, Wenzel J. Cutaneous lupus erythematosus: an update on pathogenesis and future therapeutic directions. Am J Clin Dermatol 2023 Jul;24(4):521–40. doi: 10.1007/s40257-023-00774-8.
[3] Yamamoto T. Role of neutrophils in cutaneous lupus erythematosus. J Dermatol 2024 Feb;51(2):180–4. doi: 10.1111/1346-8138.17036.

Laura Gatti [1,*], Edoardo Marrani[1], Ilaria Maccora[1,2], Ilaria Pagnini[1], Maria Vincenza Mastrolia[1], Teresa Giani[1], Gabriele Simonini[1,2]

[1] *Pediatric Rheumatology Unit, ERN-ReCONNET Center, Meyer Children's Hospital IRCCS, Florence, Italy*
[2] *Neurofarba department, University of Florence, Florence, Italy*

*__Correspondence to__ Dr Laura Gatti, Pediatric Rheumatology Unit, ERN-ReCONNET Center, Meyer Children's Hospital IRCCS, Florence, Italy.
E-mail address: lauragatti.md@gmail.com (L. Gatti).

Ann Rheum Dis 85 (2026) e15–e16

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Correspondence

Response to correspondence on 'Anifrolumab in childhood-onset systemic lupus erythematosus: a promising option after belimumab failure' by Argüelles Balas et al

We appreciate the correspondence regarding our recently published letter, 'Anifrolumab in childhood-onset systemic lupus erythematosus: a promising option after belimumab failure' [1], and thank the authors for their valuable contribution in highlighting the therapeutic potential of interferon blockade in childhood-onset systemic lupus erythematosus (cSLE).

They describe an 8-year-old boy with cSLE and severe cutaneous involvement, refractory to hydroxychloroquine, corticosteroids, mycophenolate mofetil, and ruxolitinib, who achieved rapid and sustained remission with anifrolumab. Their case shares important similarities with ours: both involved refractory disease and a striking clinical response to interferon inhibition. While interferon activity was directly assessed in their patient, in our case, it was not formally measured but can be reasonably assumed given the clinical phenotype and the well-established pathophysiological role of type I interferons in systemic lupus erythematosus (SLE) [2]. The main difference lies in the clinical context. Our patient, a 13-year-old girl, presented with multiorgan disease including possible renal involvement and received anifrolumab after belimumab failure, achieving complete clinical remission, normalisation of laboratory parameters, and glucocorticoid withdrawal. In contrast, the case described in the correspondence was dominated by severe cutaneous manifestations after multiple therapeutic failures.

Although the correspondence raises the possibility of an earlier "step-up" use of anifrolumab in patients with high interferon level, this strategy is already supported in adults by the 2023 European Alliance of Associations for Rheumatology (EULAR) recommendations, which recognise both belimumab and anifrolumab as valid options for treatment escalation without requiring prior failure of conventional immunosuppressants [3]. In paediatrics, however, additional evidence is still needed to clearly define its role. As cSLE is a chronic disease that often begins in childhood or adolescence, shares the same pathogenic mechanisms as adult-onset SLE, and frequently presents with greater severity and earlier organ involvement [4], adapting and consistently implementing EULAR-based strategies in paediatric practice is essential to improve long-term outcomes.

It should also be emphasised that, unlike belimumab [5], anifrolumab is not yet formally approved for paediatric use, and current experience in cSLE remains limited to off-label reports such as ours and the present correspondence. Nevertheless, both cases collectively strengthen the rationale for targeted interferon blockade in refractory paediatric SLE and highlight the need for prospective studies to establish its safety and efficacy in this population.

In summary, while the correspondence illustrates the efficacy of anifrolumab in cutaneous-predominant cSLE after multiple therapeutic failures, our letter provided novel evidence of its benefit in multiorgan disease and specifically after belimumab failure. Together, these complementary experiences support precision medicine approaches in cSLE, guided by immunological stratification and the pathogenic role of interferon activity, and underscore the importance of extending adult-based therapeutic strategies to paediatric patients.

Competing interests

All authors declare that they have no competing interests.

Contributors

All authors (DAB, EBM, and ECA) contributed equally to the conception, drafting, and critical revision of this response. All authors approved the final version and agree to be accountable for all aspects of the work.

Funding

No funding was received for this study.

Patient consent for publication

Not required.

Ethics approval

Ethical approval was not required for this study in accordance with local legislation and institutional requirements.

Provenance and peer review

Comissioned; not externally peer reviewed.

Orcid

Delia Argüelles Balas: http://orcid.org/0009-0004-2198-2071

https://doi.org/10.1016/j.ard.2025.08.026
Received 21 August 2025; Accepted 23 August 2025

REFERENCES

[1] Argüelles Balas D, Barral Mena E, Calvo-Aranda E. Anifrolumab in childhood-onset systemic lupus erythematosus: a promising option after belimumab failure. Ann Rheum Dis 2025;84(9):1608–10. doi: 10.1016/j.ard.2025.03.013.
[2] Postal M, Vivaldo JF, Fernandez-Ruiz R, Paredes JL, Appenzeller S, Niewold TB. Type I interferon in the pathogenesis of systemic lupus erythematosus. Curr Opin Immunol 2020;67:87–94. doi: 10.1016/j.coi.2020.10.014.
[3] Fanouriakis A, Kostopoulou M, Andersen J, Aringer M, Arnaud L, Bae SC, et al. EULAR recommendations for the management of systemic lupus erythematosus: 2023 update. Ann Rheum Dis 2024;83(1):15–29. doi: 10.1136/ard-2023-22476.
[4] Mina R, Brunner HI. Update on differences between childhood-onset and adult-onset systemic lupus erythematosus. Arthritis Res Ther 2013;15(4):218. doi: 10.1186/ar4256.
[5] European Medicines Agency [Internet]. Benlysta Summary of product characteristics https://www.ema.europa.eu/en/documents/product-information/benlysta-epar-product-information_en.pdf; 2019 Accessed August 01, 2025.

Delia Argüelles Balas[a,*], Estefanía Barral Mena[b], Enrique Calvo-Aranda[c,d]
[a] *Paediatric Department, Hospital Universitario Infanta Leonor, Madrid, Spain*
[b] *Paediatric Department, Hospital Universitario Infanta Leonor, Madrid, Spain*
[c] *Rheumatology Department, Hospital Universitario Infanta Leonor, Madrid, Spain*
[d] *Department of Medicine, Universidad Complutense de Madrid, Madrid, Spain*

*__Correspondence to__ Dr Delia Argüelles Balas, Paediatric Department, Hospital Universitario Infanta Leonor, Madrid, Spain.
E-mail address: deliarguellesbb@gmail.com (D. Argüelles Balas).

Ann Rheum Dis 85 (2026) e17–e22

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Corrigendum

Corrigendum to 'Compendium of Skin Molecular Signatures Identifies Key Pathologic Features Associated with Fibrosis in Systemic Sclerosis' Ann Rheum Dis. 2019;78:817-825

Su-Jin Moon[1], Jung Min Bae[2], Kyung-Su Park[3], Ilias Tagkopoulos[4,5], Ki-Jo Kim[3,*]

[1] *Division of Rheumatology, Department of Internal Medicine, Yeouido St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea*
[2] *Department of Dermatology, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea*
[3] *Division of Rheumatology, Department of Internal Medicine, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea*
[4] *Department of Computer Science, University of California, Davis, CA, USA*
[5] *The Tagkopoulos Lab, Genome Center, University of California, Davis, CA, USA*

There has been an issue regarding the presence of duplicate samples in the dataset we used [1]. We sincerely apologise for any unintended concerns this may have caused. In fact, we did not identify the duplication of certain samples between the 2 datasets at that time. We re-examined the potential duplication of certain samples between the 2 datasets (GSE45485 and GSE59785) and reanalysed the combined data, excluding the duplicated samples. We adhered to the original analysis protocol, and all analyses were conducted using R (version 4.4.2, R Foundation for Statistical Computing, www.r-project.org). The results remained largely consistent with our previous findings, with no significant differences that could impact the overall conclusions.

Minor differences were as follows:

1. The number of differentially expressed genes (DEGs), enrichment scores, and statistical values varied slightly; however, the key features were consistently reproduced, and statistical significance was maintained.
2. In the analysis using the updated dataset, 1060 DEGs were identified in the diffuse subset of systemic sclerosis (dSSc). Of these, 98% (1048) were consistent with the previously identified DEGs. The key genes of interest were reproduced (Supplementary Fig S4).
3. We used the recently updated Gene Ontology Biological Process (GOBP) database (c5.go.bp.v2024.1. Hs.symbols.gmt, https://www.gsea-msigdb.org/gsea/msigdb/human/collections.jsp#C5) for gene-set enrichment analysis (GSEA). In our previous analysis conducted in 2018, an older version (c5.bp.v7.1. symbols) was used. The updated version provided more refined results, leading to the identification of 674 GOBP terms. GOBP terms unrelated to skin tissues (eg, nervous system, bone, heart, and haematopoiesis) were excluded, as were redundant terms. A normalised enrichment score greater than 1.5 and a false discovery rate of less than .05 were applied to identify significant terms. The major enriched processes were reproduced (see the separate EXCEL file), and the enrichment map is provided in Figure 2B.
4. In the GSEA of the PI3K-AKT signalling pathway, the top 12 leading-edge genes were analysed, with FN1 ranked

DOI of original article: http://dx.doi.org/10.1136/annrheumdis-2018-214778.

*Correspondence to Dr Ki-Jo Kim, Division of Rheumatology, Department of Internal Medicine, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea, Seoul, The Republic of Korea

E-mail address: md21c@catholic.ac.kr (K.-J. Kim).

https://doi.org/10.1016/j.ard.2025.05.001

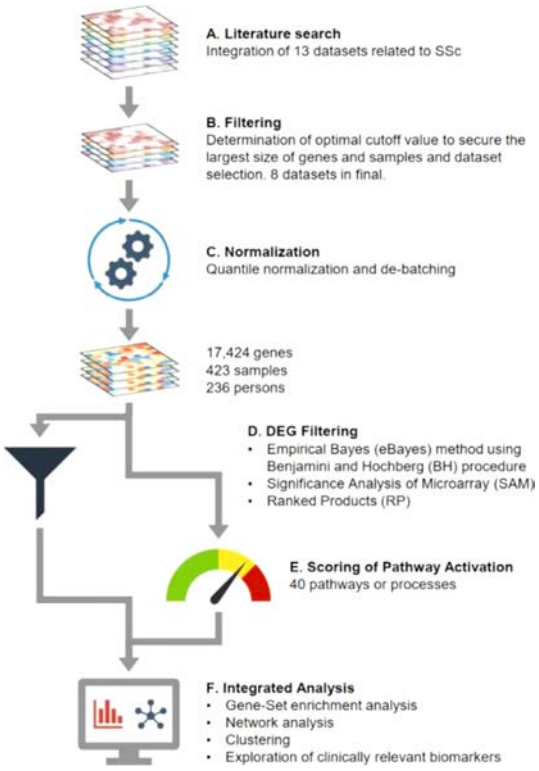


Figure 1. Overview of the data processing steps. (A) A total of 20 studies covering 5009 to 23,684 genes were retrieved from the literature. (B) Eight datasets were selected for integrated analysis, including samples from 175 systemic sclerosis (SSc) patients and 61 normal control (NC) patients, covering 17,424 genes. (C) The merged dataset was normalised using quantile normalisation, and its batch effect was further corrected. (D) Differentially expressed genes (DEGs) of SSc compared with NC were obtained using 3 methods: empirical Bayes (eBayes), significance analysis of microarray (SAM), and rank products (RP). The intersection of 3 DEG sets was designated as significant. (E) Calculation of pathway activation scores using the whole gene expression profiles. (F) A list of strategies for integrated analysis.

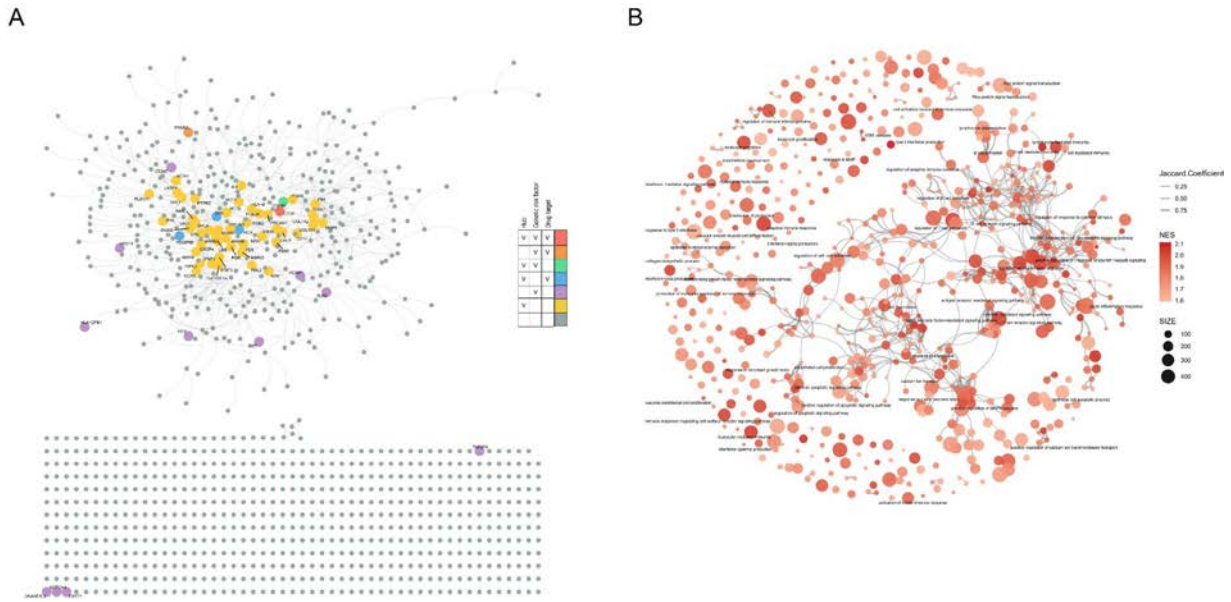


Figure 2. Differentially expressed genes (DEGs) and their functional networks. (A) Protein–protein interaction network of upregulated DEGs. Informative genes are coloured and highlighted in the right-hand table. (B) Gene-set enrichment map for whole gene expression profiles. Nodes represent Gene Ontology-termed gene sets. Their colour intensity and size are proportional to enrichment significance and gene size, respectively. Edge thickness represents the degree of overlap between gene sets, and only edges with a Jaccard similarity coefficient larger than 0.25 were visualised. Key representative terms are labelled. Please refer to the separate data table (EXCEL file) for more detailed information. NES, normalized enrichment score.

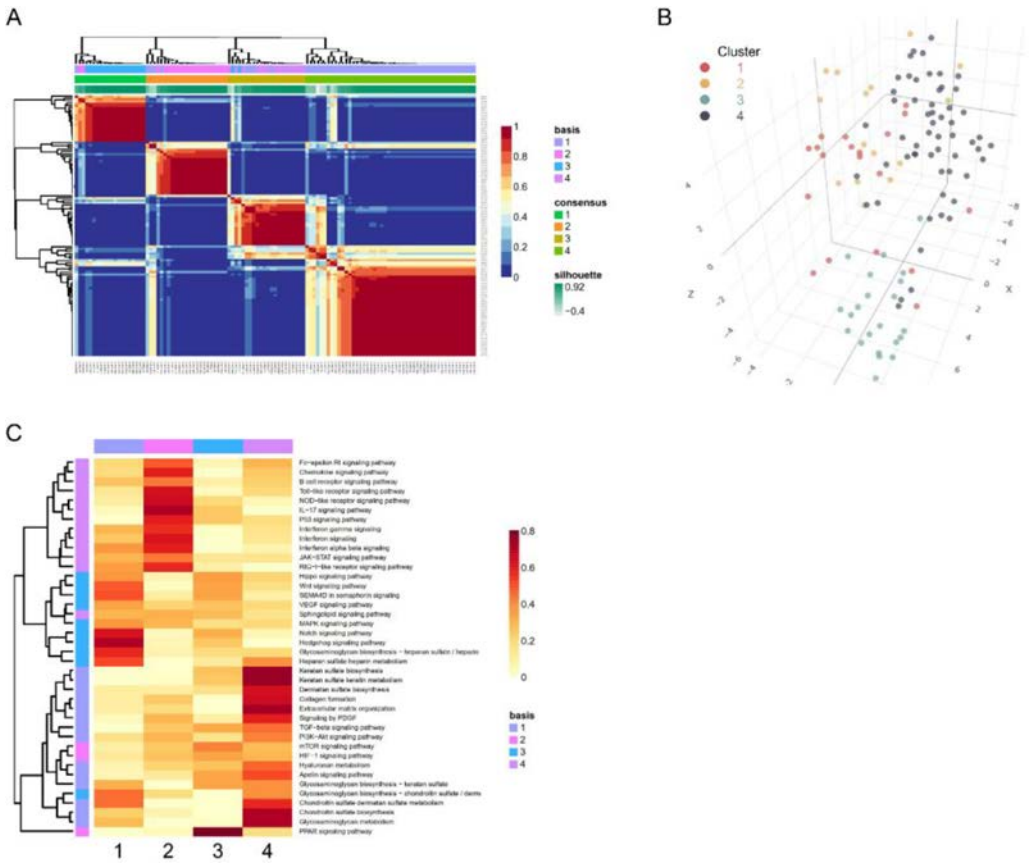


Figure 3. Identification of systemic sclerosis (SSc) subgroups according to pathway-based skin signatures. (A) Reordered consensus matrices on the SSc compendium. The samples were clustered using average linkage and 1-correlation distances. The deep red colour indicates perfect agreement of the solution, while the blue colour indicates no agreement (right-side colour bar). 'Basis' and 'consensus' represent clusters based on the basis and consensus matrices, respectively. The silhouette score is a similarity measure within its own cluster compared with other clusters. (B) t-SNE reduces the dimensions of a multivariate dataset. Each data point is assigned a location in a 3-dimensional map to illustrate potential clusters of neighbouring samples, which contain similar gene expression patterns. (C) Element matrix of pathways or processes by NMF. Heatmap with a red gradient represents the level of fold enrichment for each pathway or process. t-SNE, t-distributed Stochastic Neighbor Embedding; NMF, non-negative matrix factorisation.

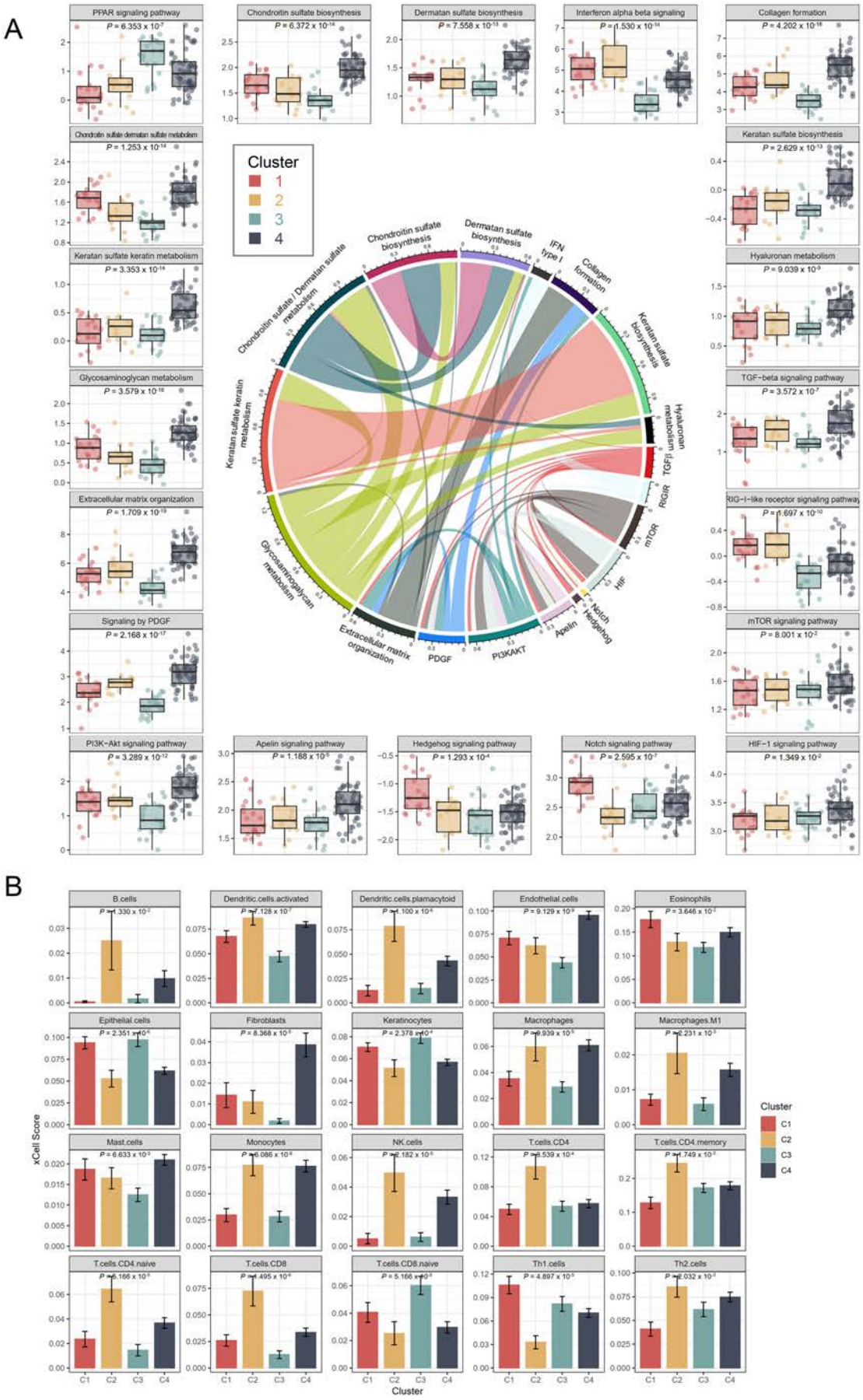


Figure 4. (A) Pathway activation scores according to systemic sclerosis (SSc) subgroups. The chord diagram shows the interrelationship among pathways, and link thickness is proportional to the overlap between 2 pathways. These were calculated using the Jaccard similarity coefficients. Turkey boxplots reveal pathway activation scores among the SSc subgroups, and analysis of variance (ANOVA) was used to analyse the differences among the 4 subgroups. (B) xCell inferred enrichment score of cell types according to the 4 clusters. xCell scores predict relative enrichment for cell types, not the proportions. Statistical significance was assessed using ANOVA. PPAR, peroxisome proliferator-activated receptors; TGF, transforming growth factor;

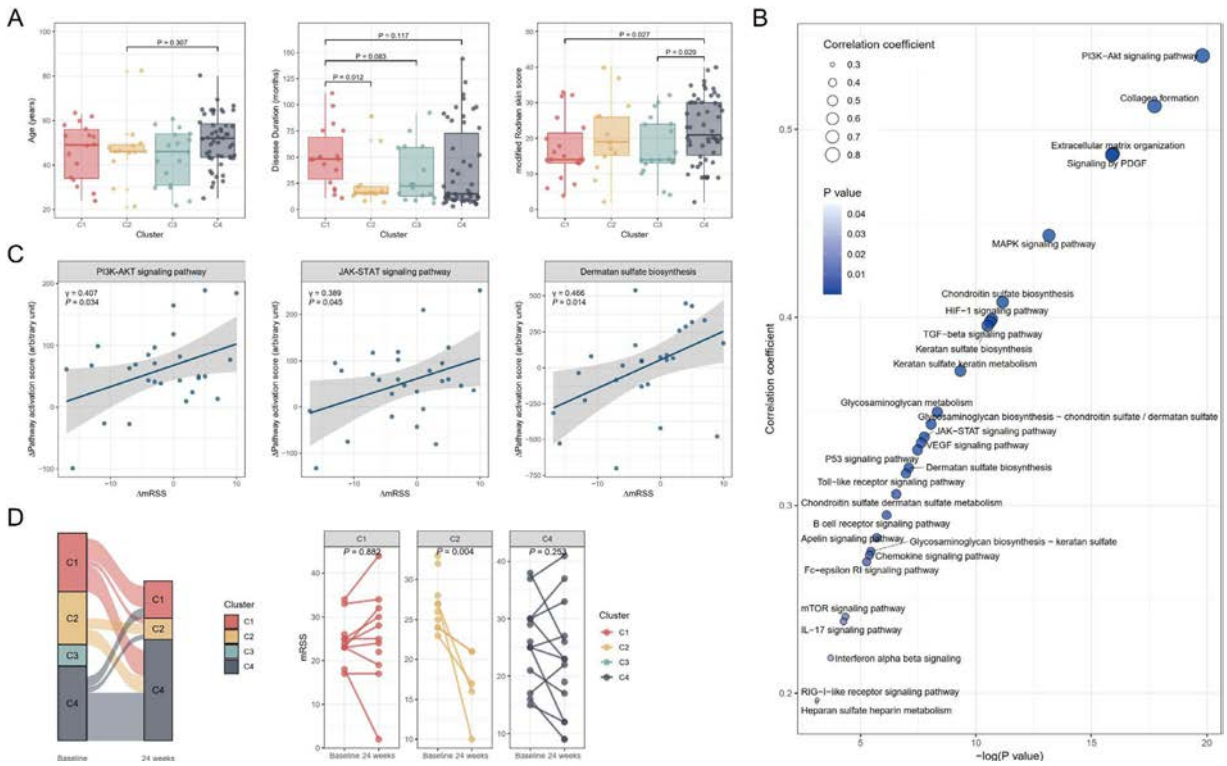


Figure 5. (A) Levels of clinical variables by systemic sclerosis (SSc) subgroup. Comparison between the 2 subgroups was done using the *t*-test. (B) Correlation between the modified Rodnan Skin Score (mRSS) and pathway activation score. Correlation analysis was performed using Pearson's method, and the *P* value on the x-axis was log-scaled for better visualisation. (C) Correlation of variations between mRSS and pathway enrichment scores. Correlation analyses for the 27 paired samples at baseline and 24-week follow-up by subcutaneous tocilizumab therapy in patients with diffuse SSc (GSE106358) were performed using Spearman's method. ΔmRSS was positively correlated with the Δenrichment score of PI3K-Akt ($\gamma = 0.407$, $P = .034$) and JAK-STAT signalling pathways ($\gamma = 0.389$, $P = .045$) and dermatan sulphate biosynthesis ($\gamma = 0.466$, $P = .014$). (D) Evolution of the cluster (left panel) and change in mRSS by clusters (right panel) after 24 weeks. PDGF, platelet-derived growth factor; MAPK, mitogen-activated protein kinase; HIF, hypoxia-inducible factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; IL, interleukin; RIG, retinoid acid-inducible gene.

HIF, hypoxia-inducible factor; RIG-IR, retinoic acid-inducible gene-I-like receptor; CSDS, chondroitin sulfate/Dermatan sulfate; CS, chondroitin sulfate; DS, dermatan sulfate; IFN, interferon; COL, collagen; KS, keratan sulfate; HA, hyaluronan; PDGF, platelet-derived growth factor; GAG, Glycosaminoglyan; NK, natural killer; IL, interleukin; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; mTOR,mammalian target of rapamycin; NOD-like, nucleotide-binding oligomerizationdomain-like; PDGF, platelet-derived growth factor; PI3K, phosphoinositide3-kinases; SEMA4D,semaphorin-4D; STAT, signal transducer and activator of transcription; VEGF, vascular endothelial growth factor.

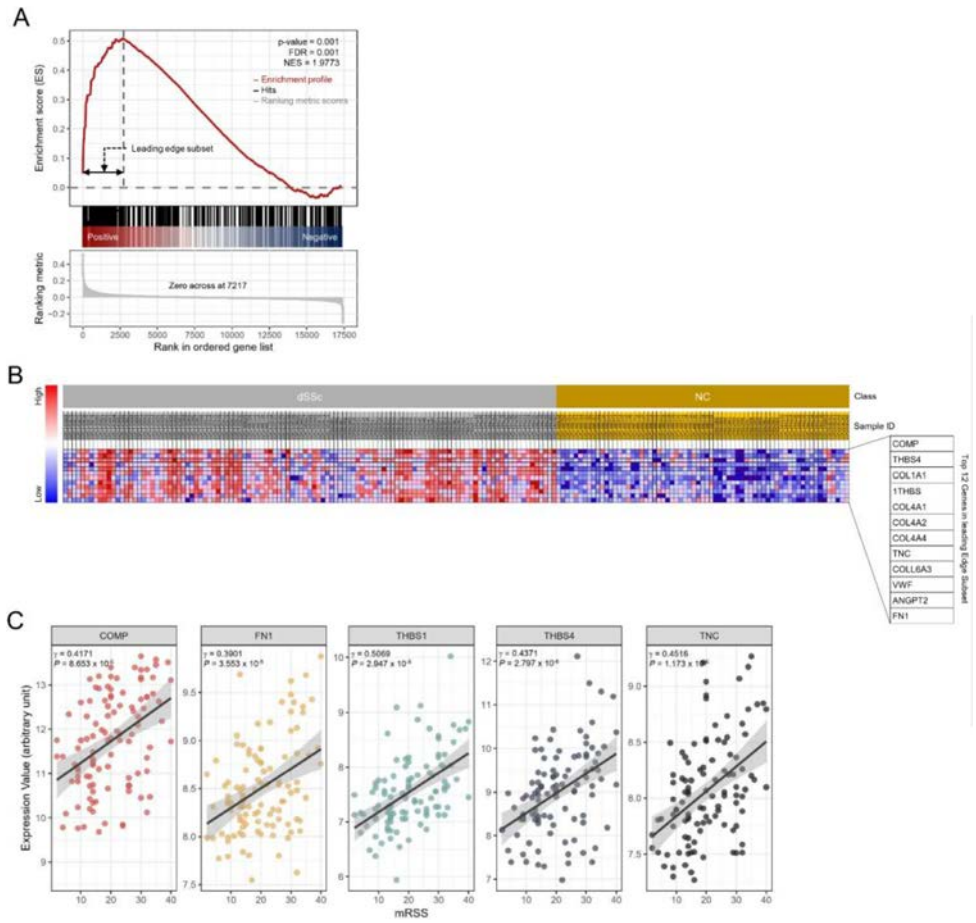


Figure 6. Enrichment of the PI3K-Akt signalling pathway and its leading-edge genes. (A) Enrichment plot generated by gene-set enrichment analysis (GSEA) of ranked gene expression data (left, upregulated [red]; right, downregulated [blue]) by the PI3K-Akt signalling pathway in diffuse systemic sclerosis (dSSc) vs normal control (NC). (B) GSEA-derived heatmap of the top 12 leading-edge genes showing the strongest upregulation in dSSc vs NC based on a signal/noise ratio score. (C) Correlation between the expression values of the leading-edge genes and modified Rodnan Skin Score (mRSS) in patients with dSSc. Correlation analysis was performed using Pearson's correlation as a similarity measure. FDR, false discovery rate. NES, normalized enrichment score.

12th (Fig 6C). In our previous analysis, the top 10 leading-edge genes were explored, and FN1 ranked 10th. In the updated analysis, von Willebrand factor (VWF) and ANGPT12 were identified ahead of FN1; however, the correlation of FN1 with the modified Rodnan Skin Score (mRSS) ($\gamma = 0.3901$, $P = 3.553 \times 10^{-5}$) was significantly stronger than that of VWF and ANGPT12 ($\gamma = 0.260$, $P = .0069$ and $\gamma = 0.334$, $P = .004$, respectively).

We have revised the results using the updated values and have prepared the figures and Supplementary figures accordingly.

Figure 1, Figures 3–5

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2025.05.001.

REFERENCE

[1] Moon S, Bae JM, Park K, Tagkopoulos I, Kim KJ. Compendium of skin molecular signatures identifies key pathological features associated with fibrosis in systemic sclerosis. Ann Rheum Dis 2019;78:817–25.

Ann Rheum Dis 85 (2026) e23–e24

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Corrigendum

Corrigendum to Bimekizumab treatment in biologic DMARD-naïve patients with active psoriatic arthritis: 52-week efficacy and safety results from the phase III, randomised, placebo-controlled, active reference BE OPTIMAL study Ann Rheum Dis. 2023;82:1404-1414

Christopher T. Ritchlin[1,*], Laura C. Coates[2], Iain B. McInnes[3], Philip J. Mease[4], Joseph F. Merola[5,6], Yoshiya Tanaka[7], Akihiko Asahina[8], Laure Gossec[9,10], Alice B. Gottlieb[11], Richard B. Warren[12,13], Barbara Ink[14], Rajan Bajracharya[14], Vishvesh Shende[14], Jason Coarse[15], Robert B.M. Landewé[16,17]

[1] *Allergy, Immunology & Rheumatology Division, University of Rochester Medical School, Rochester, NY, USA*
[2] *Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Diseases, University of Oxford and Oxford Biomedical Research Centre, Oxford University Hospitals NHS Trust, Oxford, UK*
[3] *College of Medical Veterinary and Life Sciences, University of Glasgow, Glasgow, UK*
[4] *Swedish Medical Center and Providence St. Joseph Health and University of Washington, Seattle, WA, USA*
[5] *Department of Dermatology, Harvard Medical School, Brigham and Women's Hospital, Boston, MA, USA*
[6] *Division of Rheumatology, Department of Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston, MA, USA*
[7] *First Department of Internal Medicine, University of Occupational and Environmental Health, Japan, Kitakyushu, Fukuoka, Japan*
[8] *Department of Dermatology, The Jikei University School of Medicine, Tokyo, Japan*
[9] *INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, Sorbonne Université, Paris, France*
[10] *Rheumatology Department, Pitié-Salpêtrière Hospital, AP-HP, Paris, France*
[11] *Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
[12] *Dermatology Centre, Northern Care Alliance NHS Foundation Trust, Manchester, UK*
[13] *NIHR Manchester Biomedical Research Centre, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK*
[14] *UCB Pharma, Slough, UK*
[15] *UCB Pharma, Morrisville, NC, USA*
[16] *Amsterdam Rheumatology & Clinical Immunology Center, Amsterdam, The Netherlands*
[17] *Zuyderland Medical Centre, Heerlen, The Netherlands*

DOI of original article: http://dx.doi.org/10.1136/ard-2023-224431.

*Correspondence to Dr Christopher T Ritchlin, Allergy, Immunology & Rheumatology Division, University of Rochester Medical School, Rochester, NY, USA.
E-mail address: christopher_ritchlin@urmc.rochester.edu (C.T. Ritchlin).

Trial registration: NCT03895203 (ClinicalTrials.gov)
Handling editor Josef S. Smolen.

https://doi.org/10.1016/j.ard.2025.05.013

In this article, the Disease Activity Index for Psoriatic Arthritis (DAPSA) disease states were calculated incorrectly across all treatment arms and timepoints, resulting in patients being wrongly allocated to a worse DAPSA disease state.

This error impacted 2 rows in Table 2. The corrected rows from Table 2 are provided below.

Excerpt from Table 2:

	PBO (Weeks 0–16) n = 281 →	BKZ 160 mg every 4 weeks (Weeks 16–52) n = 281	BKZ 160 mg every 4 weeks (Weeks 0–52) n = 431		Reference arm (ADA 40 mg every 2 weeks; Weeks 0–52) n = 140	
Endpoint	Week 16	Week 52	Week 16	Week 52	Week 16	Week 52
DAPSA state,[§] n (%)						
REM	6 (2.1)	73 (26.0)	84 (19.5)	158 (36.7)	31 (22.1)	49 (35.0)
REM + LDA	54 (19.2)	185 (65.8)	250 (58.0)	279 (64.7)	82 (58.6)	88 (62.9)

Randomised set. Previously reported data through Week 16 included for reference [1]

[§] Patients achieved REM if DAPSA score was ≤4, and REM + LDA if DAPSA score was ≤14.

ADA: adalimumab; BKZ: bimekizumab; DAPSA: Disease Activity Index for Psoriatic Arthritis; LDA: low disease activity; PBO: placebo; REM: remission.

REFERENCE

[1] McInnes IB, Asahina A, Coates LC, Landewé R, Merola JF, Ritchlin CT, et al. Bimekizumab in patients with Psoriatic arthritis, naive to biologic treatment: a randomised, double-blind, placebo-controlled. Lancet 2023;401:25–37.

Ann Rheum Dis 85 (2026) e25

Contents lists available at ScienceDirect

Annals of the Rheumatic Diseases

journal homepage: https://www.sciencedirect.com/journal/annals-of-the-rheumatic-diseases

Corrigendum

Corrigendum to: Statistical review: frequently given comments updated Ann Rheum Dis. 2025;84:660-3

Stian Lydersen

Department of Mental Health, Faculty of Medicine and Health Sciences, Regional Centre for Child and Youth Mental Health and Child Welfare, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

The author regrets that in section 10, 'Adjusting *P* values for multiplicity', the correct reference should be:

'Lydersen S. Adjustment of p values for multiple hypotheses: why, when and how. Ann Rheum Dis. 2024;83(10):1254-5.'

DOI of original article: http://dx.doi.org/10.1016/j.ard.2025.02.007.

E-mail address: stian.lydersen@ntnu.no

https://doi.org/10.1016/j.ard.2025.11.006