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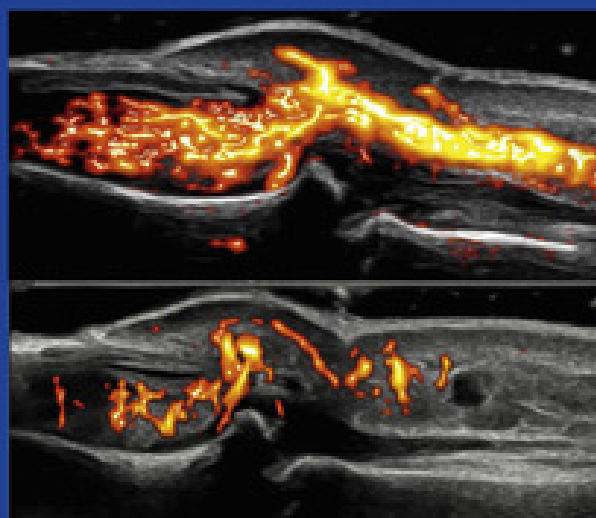
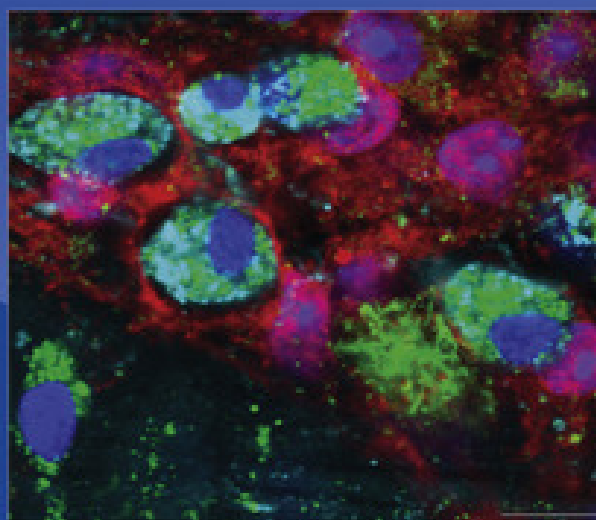
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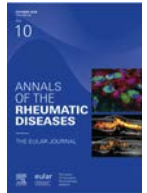
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Editorial

Air pollution and rheumatoid arthritis disease activity: linking pathogenesis and progression

Inhalants such as smoking and pollutants have been linked to rheumatoid arthritis (RA) risk, particularly for those with the shared epitope and elevated risk of seropositive RA [1]. This helped form the mucosal origins hypothesis of RA pathogenesis wherein immune tolerance loss and autoantibody production occur at mucosa such as airways and alveoli—after environmental triggers such as smoking—years before joint symptoms develop [2]. This also might in part explain risk for pulmonary manifestations of RA, such as interstitial lung disease (ILD) and bronchiectasis [3,4]. Most of the research in RA has focused on linking inhalants to pathogenesis of RA [5–7], with less focused on progression outcomes such as disease activity [8,9]. Smoking has been shown to be a risk factor for higher disease activity in RA [10]. However, less is known about the relationship between air pollution and RA disease activity.

In this issue of the *Annals of the Rheumatic Diseases*, Jung and colleagues investigate air pollution and RA disease activity among 12,583 outpatient visits from 1070 patients with RA at a tertiary medical centre in South Korea [11]. They estimated 6 air pollutants (gases: SO₂, NO₂, O₃, CO; particulate matter size ≤ 10 μm [PM₁₀], or ≤ 2.5 μm [PM_{2.5}]) using geocoding. They linked these levels to RA flares and several disease activity measures that included disease activity scale of 28 joints with C-reactive protein (DAS28-CRP), clinical disease activity index (CDAI), tender joint count of 28 joints (TJC28), and swollen joint count of 28 joints (SJC28). The investigators found that PM_{2.5} was most consistently associated with higher RA disease activity. For example, each standard deviation elevation of PM_{2.5} was associated with adjusted odds ratio for flare of 1.104 (95% confidence interval 1.009–1.207) [11]. The association with DAS28-CRP was most pronounced among nonsmokers and women. They also performed a case-crossover study, where air pollution levels from consecutive visits were compared within each person to further minimise potential confounders. There was a lag effect of higher air pollutants resulting in higher RA flare risk about 12 to 20 days later [11]. Of note, specific gas pollutants and PM₁₀ were not consistently associated with RA disease activity.

This is 1 of the largest studies to use robust methods to link air pollutants with RA disease activity. Considering rising levels of air pollutants, these results have significant clinical, biologic, and public health implications. They also further reinforce that

inhalants may have broad implications for risk and progression of RA and perhaps other autoimmune diseases. From a clinical perspective, this may offer avenues to lower the risk of RA flares by avoiding air with poor quality and provide some potential explanation for otherwise idiosyncratic RA flares. The work extends previous studies on the relationship of smoking with RA disease activity [10]. Previous smaller studies also suggested a role for air pollution with RA disease activity [12,13]. From a biologic perspective, there are already ample data that pollutants increase reactive oxygen species and generate cytokines to serve as a pro-inflammatory stimulus [14]. Since patients with RA are susceptible to ILD, bronchiectasis, airways disease, and lung cancer [15], this may be another mechanism that could result in lung damage in RA. Of note, 2 previous studies also found no association of PM₁₀ with RA-related outcomes [16,17], similar to the current study's findings. However, there was an association of PM_{2.5} with tender and swollen joints in autoantibody-positive individuals without RA in a United States cohort [17]. Thus, this further suggests that PM_{2.5} may be particularly important in RA pathogenesis and progression.

Some limitations and future directions should be considered. First, the method of exposure ascertainment of air pollution is on a geographic, rather than personal, level. There may be significant variations in air pollution inhalation based on measurement error, time spent travelling outside of the home, and air purification. Most of these issues may be expected to bias towards the null, so would not explain a positive effect. Other factors may also influence findings. For example, residential greenness was recently associated with bone mineral density [18]. The study was performed at a single tertiary medical centre in Korea. Thus, the findings may not apply to other settings, and more work is needed to replicate these results. Although the dataset was large with granular data, there may be residual confounding particularly to genetic factors. For example, a recent large study found that air pollution was most highly associated with incident RA among those at those with low or medium genetic risk of RA, but not those at high genetic RA risk [7]. However, smoking strongly interacts with the shared epitope to enhance seropositive RA risk [1]. It will be interesting to examine whether some participants with RA at high risk for flares from air pollution based on genetic and autoimmune factors such as serostatus or specific treatments. Finally, interventions are needed to bring these findings to clinic [19,20]. This may include limiting ambient air exposure to high levels of PM_{2.5} and air purification methods. From a public health perspective, this may mean enacting or reinforcing policies to ensure safe levels of inhalants, particularly air pollutants.

In conclusion, this impactful study has linked air pollution, specifically PM_{2.5}, to higher risk of flares and increased RA disease activity. Pollution now links pathogenesis and progression

in RA. These findings emphasise how inhalants such as pollution may drive RA and perhaps other autoimmune processes.

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Competing interests

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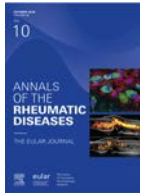
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Viewpoint

Varicella-zoster virus reactivation and cardiovascular risk: implications for patients receiving JAK inhibitors

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ABSTRACT

Varicella-zoster virus (VZV) is a neurotropic herpesvirus responsible for varicella and herpes zoster. Beyond its classical clinical manifestations, recent evidence shows that VZV reactivation may contribute to acute cardiovascular events. Vaccination reduces the incidence of herpes zoster and may confer cardiovascular benefits, although causality remains unproven. To summarise current evidence linking VZV infection to cardiovascular risk, evaluate the impact of VZV vaccination in the general and immune-mediated inflammatory disease (IMiD) populations, and discuss the hypothesis that increased VZV reactivation under Janus kinase inhibitor (JAKi) therapy may partially explain the major adverse cardiovascular event (MACE) signal observed in the ORAL Surveillance trial. Epidemiologic studies show a consistent association between herpes zoster and a transient increase in MACEs. Mechanistic insights support VZV's ability to infect vascular tissues, induce inflammation, destabilise atherosclerotic plaques, and enhance thrombogenicity. Vaccination with the recombinant zoster vaccine (RZV) effectively prevents herpes zoster and is associated with reduced MACEs in large observational cohorts, although randomised trials powered for cardiovascular endpoints are lacking. In IMiD populations, vaccination reduces herpes zoster incidence but has not consistently demonstrated cardiovascular benefit. JAKis, which increase VZV reactivation risk by impairing antiviral immunity, may create episodic vascular insults that contribute to the elevated MACE rates observed with tofacitinib in the ORAL Surveillance trial. VZV reactivation is a plausible and potentially preventable contributor to cardiovascular risk, particularly in older and immunosuppressed individuals. While vaccination shows promise in reducing zoster-associated cardiovascular risk, definitive causal evidence remains lacking. The hypothesised JAKi-VZV-vascular pathway warrants prospective evaluation.

INTRODUCTION

The varicella-zoster virus (VZV) is a highly contagious human herpesvirus responsible for 2 clinically distinct diseases: varicella (chickenpox) and herpes zoster (shingles). As a member of the *Herpesviridae* family, VZV establishes lifelong latency in sensory nerve ganglia following primary infection, which typically occurs in childhood and presents with a generalised vesicular rash, fever, and malaise. Later in life, particularly in older adults or immunocompromised individuals, VZV reactivation can lead

to shingles, characterised by a painful dermatomal rash and neural inflammation [1].

Beyond its cutaneous and neurological manifestations, VZV has demonstrated a significant, previously underappreciated tropism for vascular tissues. This has prompted growing interest in the virus as a potential contributor to acute cardiovascular disease. Understanding the epidemiology and pathophysiology of VZV infection is thus crucial not only for preventing herpes zoster and its complications but also for exploring its broader implications for cardiovascular health [2].

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Janus kinase (JAK)-Signal Transducer and Activator of Transcription (STAT) signalling pathway plays a central role in antiviral immunity, including type I and II interferon pathways. JAK inhibitors (JAKis) attenuate cytotoxic T-cell and natural killer cell responses, lowering host control over viruses, including latent herpesviruses. JAKis have transformed the management of immune-mediated diseases; however, concerns have emerged regarding an excess of major adverse cardiovascular events (MACEs) and thromboembolic events. The biological basis of this signal remains incompletely explained. Clinically, all approved JAKis are associated with an increased risk of herpes zoster activation relative to biologics [3].

The ORAL Surveillance trial revealed higher rates of MACEs with tofacitinib compared with tumour necrosis factor (TNF) inhibitors in patients with rheumatoid arthritis (RA) aged >50 years with additional cardiovascular risk factors [4]. One biologically plausible yet unproven contributor to this cardiovascular risk is augmented VZV reactivation via JAK inhibition.

Given VZV's vascular tropism and its ability to induce endothelial dysfunction, plaque instability, and hypercoagulability, increased viral reactivation in JAKi-treated patients may produce episodic vascular injury.

MECHANISTIC PATHWAYS LINKING VZV INFECTION TO CARDIOVASCULAR INJURY

Several interconnected biological processes likely underlie the observed increase in cardiovascular risk following VZV reactivation, as listed below (Fig 1).

Direct vascular infection (VZV vasculotropism)

VZV infection has a direct vascular effect: the virus can migrate from sensory ganglia along peripheral nerves, infecting endothelial cells and vascular smooth muscle cells. Viral DNA and antigens have been detected in cerebral, coronary, and peripheral arterial walls, confirming the capacity for direct vascular involvement; moreover, anti-VZV immunoglobulin (Ig) G has been detected in cerebrospinal fluid (CSF) and is highly specific for VZV vasculopathy [2,5,6]. VZV-mediated downregulation of programmed death-ligand 1 (PD-L1) and major histocompatibility complex class I in cerebral vascular adventitial fibroblasts facilitates viral immune escape, allowing viral persistence within the arterial wall and consequent chronic vascular inflammation [7].

Vascular inflammation and endothelial dysfunction

Viral invasion induces intense local inflammation, immune cell recruitment, and cytokine release. VZV infection significantly increase proinflammatory and proangiogenic mediators, particularly interleukin (IL)-8, IL-6, and vascular endothelial growth factor (VEGF)-A, with additional cell-type-specific alterations in multiple cytokines and chemokines. These processes impair endothelial nitric oxide production, disrupt vascular tone regulation, and promote oxidative stress [8,9].

Plaque destabilisation and thrombosis

In individuals with atherosclerosis, VZV-induced inflammation may destabilise pre-existing plaques, facilitating rupture and subsequent thrombus formation [10]. The proinflammatory and prothrombotic milieu may precipitate acute coronary syndromes or ischaemic stroke [8].

Hypercoagulability and systemic immune activation

Beyond local vascular injury, VZV-induced systemic inflammation may shift coagulation homeostasis towards a prothrombotic state, potentially sustaining an elevated risk of vascular occlusion even after the infection has resolved [11].

Together, these mechanisms help explain the epidemiologic evidence linking herpes zoster to a transient but clinically meaningful increase in the risk of MACEs.

EVIDENCE FOR AN INCREASED RISK OF MACEs FOLLOWING VZV INFECTION

A substantial body of epidemiologic research indicates that VZV reactivation is associated with an increased heightened risk of MACEs, ischaemic stroke, and myocardial infarction.

Recent evidence continues to support the association between herpes zoster infection and cerebrovascular events. A 2024 systematic review and meta-analysis including over 5.5 million participants confirmed a significantly increased risk of stroke following herpes zoster infection [12]. This was confirmed by numerous studies consistently reporting an increased risk of stroke following VZV infection, particularly during the first weeks after onset. Although the risk starts increasing soon after infection, as early as 1 to 4 weeks, and peaks within the first 3 months, several studies indicate that it remains elevated in the first year after infection [13–17]. Notably, in patients with RA, Calabrese et al [18] confirmed an increased risk of stroke soon after VZV reactivation, showing an incidence rate of 1.24 (95% CI, 1.05–1.49)/100 patient-years within the first 3 months postzoster. In another large population study in RA patients, herpes zoster infection was associated with an increased risk of stroke (adjusted hazard ratio [HR], 1.27), rising to an HR of 1.54 in those with neurological complications and reaching a 2.3-fold higher risk within 90 days after zoster onset [19].

Several studies have also reported that herpes zoster infection is associated with an increased risk of myocardial infarction following VZV reactivation. Overall, there is a significantly positive association between VZV and myocardial infarction, with a temporal association similar to that observed for stroke, demonstrating an early (1 week) elevated risk that persist after months/years [20]. In a large cohort analysis including over 2.3 million person-years of follow-up, a history of VZV was independently associated with an increased risk of coronary heart disease up to 12 years after the infectious episode [21].

Although VZV is the most clinically relevant viral reactivation during JAKi treatment, other latent herpesviruses, including herpes simplex virus, Epstein-Barr virus, and cytomegalovirus, may also contribute to cardiovascular risk through chronic vascular inflammation. Impaired antiviral control during JAKi therapy may facilitate viral reactivation and promote atherothrombotic processes. Nevertheless, routine antiviral prophylaxis is not currently recommended due to limited direct evidence [22]. Beyond neurotropic herpesviruses, respiratory infections such as influenza and SARS-CoV-2 are established triggers of MACEs, and in patients receiving JAKis, infection-related endothelial injury may interact with JAKi-associated prothrombotic changes, potentially increasing vascular risk [23].

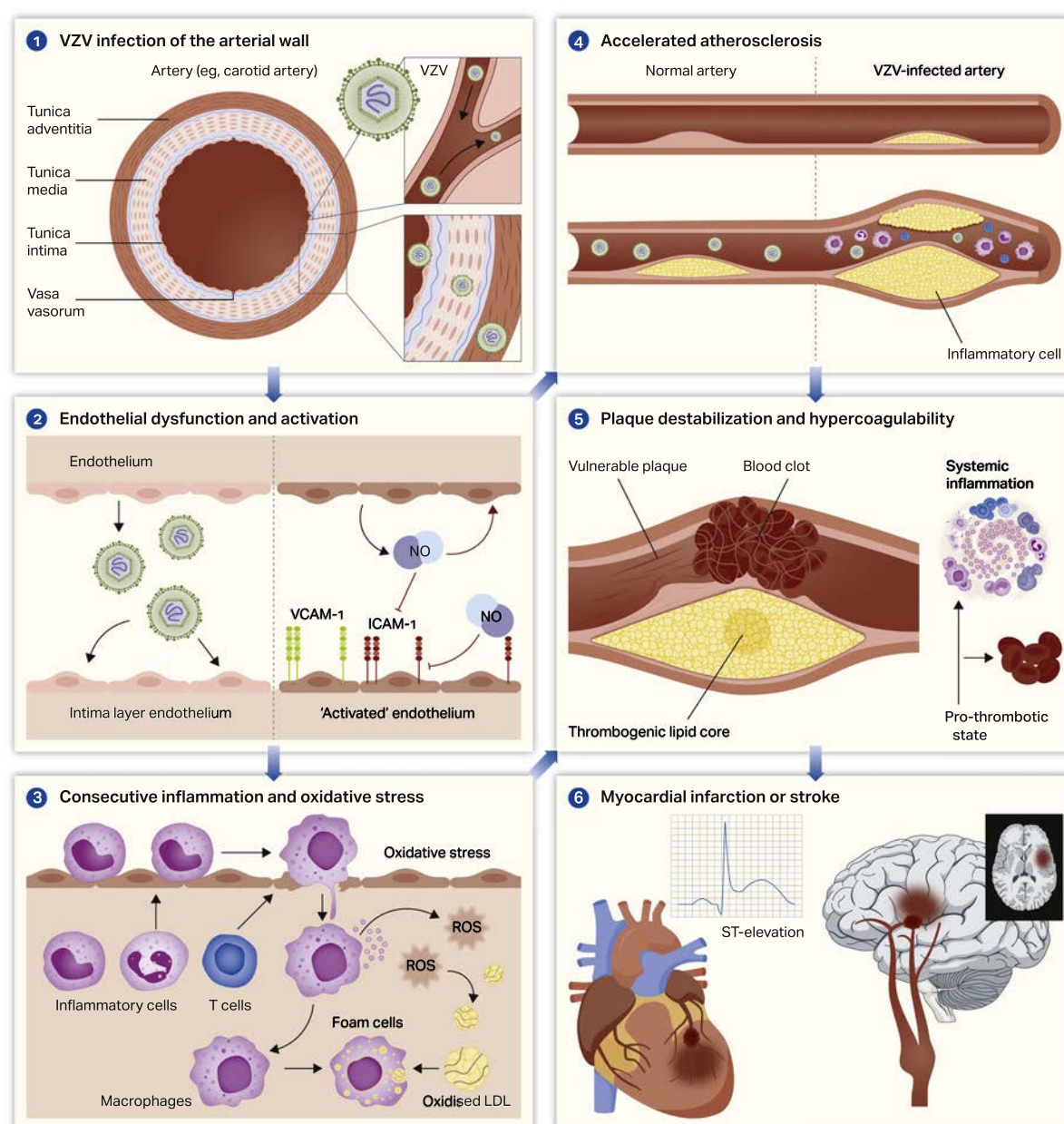


Figure 1. The mechanism linking varicella-zoster virus (VZV) infection to cerebrovascular and cardiovascular events. 1, VZV establishes latency in ganglia and reactivates, spreading transactionally to infect the arterial adventitia and media via the vasa vasorum. 2, Viral infection triggers endothelial cell activation, increasing the expression of adhesion molecules and impairing normal vasodilator and antithrombotic functions. 3, Recruitment of inflammatory cells releases proinflammatory cytokines and reactive oxygen species (ROS), leading to local inflammation and oxidative modification of low-density lipoprotein (LDL) cholesterol (oxLDL). 4, Chronic inflammation and lipid accumulation accelerate the development of atherosclerotic plaques within the infected arterial segment. 5, Inflammation weakens the plaque's fibrous cap, leading to rupture. This, combined with a virus-induced prothrombotic state, triggers blood clot formation. 6, Coronary or cerebral occlusion leading to myocardial infarction or stroke. ICAM-1, intercellular adhesion molecule-1; NO, nitric oxide; VCAM-1, vascular cell adhesion molecule-1.

EFFECTS OF VZV VACCINATION ON HERPES ZOSTER PREVENTION AND CARDIOVASCULAR RISK REDUCTION IN THE GENERAL POPULATION

The advent of effective VZV vaccines, particularly the adjuvanted recombinant zoster vaccine (RZV), has transformed the prevention of herpes zoster. Both the live attenuated vaccine (ZVL) and RZV have been associated with reduced cardiovascular events, though RZV's stronger immunogenicity and widespread adoption of RZV make it the preferred option.

Beyond preventing zoster itself, emerging data suggest that vaccination may also reduce cardiovascular risk. Several

observational studies suggest that vaccination against VZV may be associated with a reduced risk of MACEs, including myocardial infarction and stroke. Large population-based cohort studies reported that vaccination against herpes zoster was associated with a significantly lower long-term risk of cardiovascular disease, including coronary heart disease and stroke [24,25]. Similarly, recent systematic reviews and meta-analyses have confirmed that zoster vaccination may reduce the incidence of cardiovascular events, potentially by preventing VZV reactivation and associated vascular inflammation [12,26,27]. The results of a real-world study, presented at the 2025 European Society of Cardiology Congress, involving a large cohort of patients with atherosclerotic cardiovascular disease showed

that vaccination against Herpes Zoster was associated with a lower risk of MACEs and secondary cardiovascular outcomes, including new-onset pulmonary embolism or deep vein thrombosis, heart failure, and atrial fibrillation or flutter over the course of 1 year [28].

Growing evidence suggests that VZV vaccination is associated with a reduced long-term risk of dementia and mild cognitive impairment, supporting a role for VZV-related cerebrovascular injury in cognitive decline; VZV can infect cerebral vessels, causing vasculopathy, chronic ischaemic damage, blood-brain barrier disruption, and persistent neuroinflammation [29]. Recurrent viral reactivation may therefore contribute to stroke, cerebral small-vessel disease, and vascular dementia, while chronic neuroinflammatory responses may further exacerbate neurodegenerative processes. By preventing both herpes zoster and subclinical viral reactivation, vaccination may reduce infectious vasculopathy and its downstream cognitive consequences.

Although most data have been collected from observational studies and specific randomised clinical trials are needed, the consistency and biological plausibility of the findings suggest that VZV vaccination may confer indirect cardiovascular benefits beyond the prevention of herpes zoster.

EFFECTS OF VZV VACCINATION IN PATIENTS WITH IMMUNE-MEDIATED INFLAMMATORY DISEASES

Patients with immune-mediated inflammatory diseases (IMIDs), including RA, spondyloarthritis (SpA), psoriatic arthritis (PsA), and inflammatory bowel disease, have an increased baseline risk of herpes zoster due to both the underlying disease and immunosuppressive therapies. RZV is effective and generally safe in these populations, including in those receiving biologics or JAKis [30,31].

Nonetheless, evidence regarding the cardiovascular benefits of VZV vaccination in IMID populations remains limited. In a large American study of patients with RA, SpA, and PsA, Curtis et al [32] reported a vaccine effectiveness of about 50% but found no significant reduction in MACEs among those vaccinated. In another cohort study of patients with IMIDs receiving JAKis, RZV was not associated with a significant reduction in MACEs, although a decrease in all-cause mortality was observed [33]. Thus, although vaccination is advisable to prevent herpes zoster, its cardioprotective role in IMIDs is still to be proven, and targeted randomised clinical trials should therefore be designed to assess the cardioprotective effect of herpes zoster vaccination in these high-risk populations.

LINKING JAK INHIBITION, VZV REACTIVATION, AND CARDIOVASCULAR RISK

A potential, still speculative hypothesis is that augmented VZV reactivation during JAK inhibition may contribute to cardiovascular risk; however, formal analyses to date have not demonstrated a clear association between clinically apparent herpes zoster events and venous thromboembolism (VTE), underscoring the need for further evaluation of clinical trial datasets and large real-world cohorts. Although difficult to establish definitively, it remains possible that the cumulative impact of clinically overt or subclinical VZV reactivation could contribute to cardiovascular risk observed in JAKi-treated patients, including those enrolled in the ORAL Surveillance

study. Prospective studies including viral surveillance, endothelial biomarkers assessment, and adjudicated cardiovascular endpoints are warranted to formally test this mechanistic model.

In conclusion, VZV is increasingly recognised as a contributor to vascular injury and cardiovascular events. The evidence linking VZV reactivation to an increased risk of MACEs is substantial and biologically plausible. Vaccination against herpes zoster is highly effective in preventing zoster and may also reduce cardiovascular events in the general population, though causality remains to be proven.

In IMID populations, vaccination effectively reduces zoster incidence, but current evidence does not demonstrate a clear reduction in MACEs, particularly among patients treated with JAKis. The hypothesis that VZV reactivation contributes to the increased MACE risk observed with tofacitinib in the ORAL Surveillance trial is compelling and warrants further mechanistic and clinical investigation.

Preventing VZV reactivation, including via widespread use of RZV, remains a promising strategy for reducing infectious and possibly cardiovascular morbidity, especially among older adults and immunosuppressed patients.

CRedit authorship contribution statement

Fabio Cacciapaglia: Writing – review & editing, Writing – original draft, Conceptualization. **Massimo Gadina:** Writing – review & editing, Writing – original draft, Conceptualization. **Francesca Romana Spinelli:** Writing – review & editing, Writing – original draft, Conceptualization.

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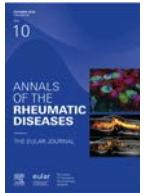
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Recommendation

2026 EULAR updated standardised operating procedures and methods for the development of recommendations or points to consider for the diagnosis and management of people with rheumatic and musculoskeletal diseases

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ABSTRACT

Recommendations and points to consider (PtCs) are important tools to improve the quality-of-care for people living with rheumatic and musculoskeletal diseases (RMDs). An important requirement of the European Alliance of Associations for Rheumatology (EULAR) recommendations and PtCs is that they resonate with clinical experience and are practical, easy to understand, and actionable. At the same time, they should commit to the 3 pillars of evidence-based medicine (best available research evidence, clinical expertise, and patient values and preferences) and to good clinical practice and they should include a responsible level of

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cost-consciousness. The target audience for recommendations and PtCs consists of people living with RMDs, health professionals in rheumatology (HPRs), rheumatologists, RMD researchers, and national and international policy- and decision-makers worldwide. This article describes the structure and key principles of the EULAR standard operating procedures (SOPs) to develop recommendations and PtCs. For the most recent updates to the SOP, the ‘living document’ that is gradually adapted over time, can be found on the EULAR website and should be consulted. The main steps in developing recommendations and PtCs include convening an appropriate list of experts and patient-research partners (PRPs), performing a literature review, then performing a process to develop consensus. The SOP ensure EULAR recommendations and PtCs continue to reflect scientific rigour, feasibility, and value for health professionals, people living with RMDs, and policymakers.

INTRODUCTION

Evidence-based recommendations are essential to promote high-quality, equitable, and cost-effective care for people with rheumatic and musculoskeletal diseases (RMDs). The aim of the recommendations is to aggregate scientific evidence, expertise from clinicians and other health professionals in rheumatology (HPRs), and preferences from both people living with RMDs and clinicians. They should be developed according to the framework of evidence-based medicine (EBM) [1] and produce practical guidance that can be applied internationally. The European Alliance of Associations for Rheumatology (EULAR) is an organisation of people living with RMDs, HPRs, and rheumatologists. Recommendations produced by EULAR provide support to colleagues and improve the lives of people living with RMDs across the world.

An important goal of recommendations is to raise standards of care by reducing variation in clinical practice across clinics, hospitals, and different countries. This ensures that people living with RMDs receive the best possible care, regardless of personal socio-economic situation, or the country they live in. The voice of people living with RMDs has a fundamental role in developing recommendations. In addition, recommendations may help direct research priorities, drive policy decisions about the reimbursement of drugs and other treatments and help to define quality indicators (QIs). This is why EULAR increasingly focuses on the implementation process once recommendations have been developed.

Within EULAR, the Quality-of-Care (QoC) Committee is responsible for the standardised processes leading to the development of recommendations and points to consider (PtCs) (referred to collectively as ‘recommendations’). EULAR published its first standard operating procedures (SOPs) in 2004, with an update in 2014 [2,3]. Subsequently, EULAR adapted these procedures based on feedback received over the years from the EULAR community. The most recent version of EULAR’s SOP for recommendations is a ‘living document’ hosted on the EULAR website [4]. The document is regularly adapted to reflect changing views on what EULAR should provide for in guidance for people living with RMDs and health care professionals, and to reflect changes in methodological expertise in guideline development. The ‘living document’ is vital reading material for individuals developing EULAR recommendations.

This manuscript, approved by the current EULAR Board, follows more than a decade after the previous update in 2014. It aimed to summarise the structure and key principles of the EULAR SOP that underpin the development process and methodology of EULAR recommendations. For the most recent updates of the SOP and for procedural details, the task force (TF) members and those who consider submitting projects to EULAR’s QoC Committee are advised to have a closer look at EULAR’s website for a thorough reading of the ‘living document’ [4].

PROCEDURAL CONSIDERATIONS

Context

EULAR endorses TFs to develop recommendations for the management of RMDs. Occasionally, proposals involve the (re)definition of international classification criteria for RMDs or projects that may have a high impact on an entire clinical field (a recent example is the concept of ‘prerheumatoid arthritis [RA]’) [5]. Any individual member of EULAR, or a member of a national society under the umbrella of EULAR, can apply for leadership of a TF project (a ‘convenor’). The convenor should have an established academic or clinical record in the topic area. As outlined above, EULAR recommendations should adhere to the EULAR SOP described in the ‘living document’ and work under the supervision of QoC Committee [4].

Occasionally, recommendations are collaborative projects being developed together with the American College of Rheumatology (ACR), and EULAR maintains an EULAR-ACR standing committee to support this purpose [6]. In principle, themes for collaborative initiatives with ACR are consensually solicited and lead to requests for proposals. Proposals for joint EULAR-ACR projects follow a specific route for submission and the processes and call specifications as described in the relevant website [7]. Also, in instances where a topic has a shared interest with another scientific society, projects are initiated jointly with other professional organisations (eg, the Assessment in SpondyloArthritis International Society [8] and the European Respiratory Society [9]); collaboration follows the basic principles and rules of an EULAR TF and is governed by a specific agreement between EULAR and the partner organisation. The agreement specifies details such as the structure of governance, shared responsibilities, data use, authorship, funding, and publication rights.

Potential EULAR QoC projects

Projects should be designed to improve the QoC for people living with RMDs. Projects are often focused on rheumatology’s main treatment areas (for diseases such as rheumatoid arthritis, psoriatic arthritis, systemic sclerosis, and others) but may include rare diseases (such as immunoglobulin [Ig] G4-related disease, autoinflammatory diseases, and others). Projects may also involve an update of existing EULAR recommendations. Projects are most likely to be supported when it is of interest to the international community and likely to be impactful.

There are fixed maximum budgets for EULAR QoC projects, with rules regarding the budget and application (also see the section ‘The TF’).

Development and Implementation of EULAR Recommendations

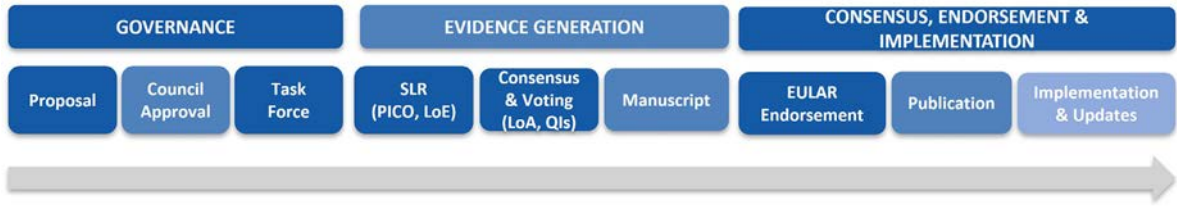


Figure 1. Process for the development of European Alliance of Associations for Rheumatology (EULAR) recommendations at a glance. LoA, levels of agreement; LoE, levels of evidence; PICO, Patient, Intervention, Control, Outcome; QI, quality indicator; SLR, systematic literature review.

The application process

Each project application submitted to EULAR QoC undergoes a standardised evaluation process (Fig 1). The applicants must provide a full proposal, a lay summary, and an abstract summarising current knowledge, the study’s contribution, and its potential impact. The proposal must also outline the planned methodology (eg, systematic literature reviews [SLRs], surveys, or analyses of existing data). The EULAR Council reviews the submitted documents biannually. The evaluation includes 2 phases: (1) the pre-eligibility phase, in which the (anonymised) proposal is scored (0-10, 10 meaning most relevant) by the members of the EULAR Council for its relevance to EULAR’s strategy. Proposals will pass the pre-eligibility check if the mean score of ≥ 7 is reached. (2) The phases of ‘Eligibility and Council evaluation’. Proposals that pass the pre-eligibility phase are evaluated and discussed in the Council for relevance, feasibility, innovation, methodological rigour, and consideration of EULAR’s core values of equality, diversity, and inclusion. The proposals must also provide available data on their planned implementation, such as findings from implementation studies, surveys, or other sources reporting the degree of uptake and use of the previously issued recommendations. Council decisions for the submitted proposal include approval, approval with minor revisions, decline with resubmission, and decline without resubmission.

The Task force

All EULAR recommendation projects under the supervision of QoC are prepared and performed by TFs consisting of experts in the field. The convenor(s) (there may also be a co-convenor) of an approved project takes the lead, invites an EULAR-approved methodologist to ensure methodological rigour, and appoints 1 or more research fellows to perform the SLR. The research fellow(s) should have experience in the technique of SLRs and are supervised by the methodologist (Table 1). The SLR forms the backbone of each project. A junior methodologist-in-training is appointed by the EULAR QoC to each TF to allow training to become a EULAR-approved methodologist. Then rest of the TF consists of experts. ‘Expert’ in the context of EULAR projects has a broad meaning, including clinicians with scientific expertise in the theme (not limited to rheumatologists), patient-research partners (PRPs), HPRs, RMD epidemiologists, and others. EULAR requires broad representation across EULAR member countries. Once the proposal is approved by the Council (see above), the convenor finalises the TF composition in coordination with the QoC, the Emerging EULAR Network (EMEUNET), HPRs, and People with Arthritis and Rheumatism in Europe (PARE) representatives. Five positions are also filled via an open call announced on the EULAR website and circulated to national

societies; applicants provide a brief statement of expertise and motivation. Each TF may include up to 25 members from at least 5 EULAR member countries, considering balance in gender, discipline, and region. The convenor is responsible for ensuring that all members, including EMEUNET, HPRs, and people living with RMDs, are heard equally. The final membership list is validated by the QoC Chair. After confirmation, the project officially begins, and the EULAR Office provides the approved budget and administrative documentation and supports the organisation of meetings requested by the TF.

METHODOLOGICAL CONSIDERATIONS

An EULAR QoC project by a TF classically follows a predefined format: (1) a planning meeting; (2) an SLR; (3) a consensus meeting with formulation of recommendations and voting; (4) manuscript(s) writing; and (5) obtaining EULAR Council

Table 1
Minimum mandatory elements for the development of the EULAR recommendations

Domain	Minimum mandatory elements
Task force composition	• Inclusion of convenor(s), EULAR-approved methodologist, and fellow(s) • Involvement of PRPs, HPRs, and EMEUNET representatives^a • Representation from ≥ 5 countries • Adherence to EULAR principles of equality and diversity
Evidence synthesis (SLR)	• Conduct of an SLR that forms the basis of the project • Predefined research questions (PICO format) • Use of established methodology (eg, Cochrane) • Assessment of risk-of-bias • Transparent reporting of methods and results
Consensus process and voting	• Structured consensus process led by convenor(s) and methodologist • Formulation of concise, actionable statements • Formal voting on each item
Recommendation/PtC output	• Development of a set of clear and actionable statements (typically 10-15) • Inclusion of overarching principles • Assignment of levels of evidence using OCEBM
Reporting and deliverables	• Reporting of levels of agreement • Main manuscript including methods, results, levels of evidence and agreement, quality indicators, and a research agenda • SLR report

EMEUNET, Emerging EULAR Network; EULAR, European Alliance of Associations for Rheumatology; HPR, health professional in rheumatology; OCEBM, Oxford Centre for Evidence-Based Medicine; PICO, Patient, Intervention, Control, Outcome; PRP, patient-research partner; PtC, point to consider; SLR, systematic literature review.

approval and publication (Fig 1). The methodological steps might be adapted if the TF's aim is different to the preparation of management recommendations or updating existing recommendations, where one meeting is usually conducted.

The people who are responsible for the methodologic integrity and for the conduct of the EULAR TF are the convenor and the methodologist. The convenor is responsible for conceiving the project, preparing the proposal, leading the scientific process, and provides also scientific leadership, organises meetings, oversees the SLRs, supervises budgetary aspects and coordinates manuscript development. The convenor and the methodologist together ensure adherence to all EULAR procedures. The EULAR methodologist also ensures that the work follows high methodological standards, in particular for the SLR, assisting in the formulation of research questions, Patient, Intervention, Control, Outcome (PICO) frameworks, and grading of evidence. A junior methodologist-in-training acts under the supervision of an EULAR-approved methodologist and provides hands-on experience in tasks assigned by the methodologist.

The planning meeting

The first TF meeting, whether live or virtual, serves to plan the entire process, formulate the research questions, and rephrase them in PICO format [10]. This meeting marks the start of the SLR; practical details and timelines are discussed, and a literature search deadline is established.

The SLR

An SLR forms the basis of all TF projects (Table 1). The type of research questions developed in the planning meeting will further guide the SLR strategy and the types of evidence that should be retrieved. An SLR serves primarily to inform the TF to assist with the process of consensus building later on and in creating a final TF product (see below). EULAR generally recommends following Cochrane's Handbook for Systematic Reviews of Interventions [10] for the evaluation of medical interventions, or alternatively principles described by the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) working group [11], but other equivalent methodologies may be acceptable, especially when the project proposal includes topics such as nonmedical interventions or qualitative research questions [12]. Evidence from the SLR will be provided in a table format, including risk-of-bias assessments. "Validated artificial intelligence-assisted approaches may support selected steps of the SLR process, provided that methodological rigor and appropriate human oversight are maintained."

The TF consensus meeting

The TF consensus meeting, either virtual or in person, is the most important event for EULAR QoC projects. At this 1- or 2-day meeting, the results of the SLR are presented, discussed, and interpreted. Although EULAR credits high-level (randomised controlled trial [RCT]) evidence published in high-impact medical journals, it understands that EBM involves more than RCTs alone, and that many relevant clinical questions cannot be solved by RCTs and may rely on observational studies. EBM values the best available research evidence, clinical expertise, and patients' values and preferences similarly and so does EULAR. This means that every deliverable of an EULAR TF should reflect a carefully weighted balance of these 3 pillars of EBM (evidence,

expertise, and preference). It should, in other words, rise above a summary of the published literature alone.

The key deliverable of an EULAR TF is a list of usually 10 to 15 statements of recommendations (ie, 'individual recommendations'). Some statements, which are not necessarily based on the results of the literature search, are also presented. These may be considered 'overarching principles' that set the stage of the project, 'delineate the arena', state facts of highly obvious clinical reality, and provide some context for the recommendations (Table 1).

Recommendations should be unequivocal, concise (1 or 2 sentences), actionable in their formulation, and free from explanatory text (the explanation of the 'why' belongs to the manuscript-text, not to the individual recommendations). Projects should aim at conceiving a shortlist of clinical recommendations that can be understood and followed by everyone (and may eventually form a template for nationwide QIs or reimbursement policies).

The distinction between recommendations and PtCs is not sharp, mainly driven by the amount and strength of the underlying evidence, and the final choice rests with the EULAR Council. In general, recommendations reflect a higher level of scientific evidence and maturity and refer more directly to patient care in clinical practice than PtCs. In addition, the term 'recommendations' is reserved for the most prevalent and impactful diseases in the sphere of rheumatology (such as the pharmacological treatment of people living with rheumatoid arthritis).

Of note, when updating existing recommendations, the majority agreement that has been obtained in the past should be respected. An existing recommendation cannot be deleted right away. Changing existing recommendations (text and content) requires careful consideration. However, evolving evidence (as justified in the accompanying SLR) and changing opinions may justify a reconsideration of an existing recommendation or a change of content. Similarly, new recommendations can be added, or existing recommendations can be merged.

Consensus building and voting

TFs decide on the number and content of their individual recommendations by building broad consensus among TF members (Table 1). Deliberately, the process to build consensus is not highly structured or prescribed. TFs, under the leadership of the convenor and the methodologist, can relatively autonomously decide how they will best achieve consensus about the choice and final wording of their recommendations, as long as the final voting result for each recommendation is transparently reported (by providing aggregated levels of agreement) and reflects the best obtainable majority consensus among all voting members. For that reason, EULAR has provided detailed voting instructions and procedures, described in detail in the online 'living' document [4].

Levels of evidence

The level of evidence reflects the extent to which an individual recommendation is supported by the evidence presented in the SLR. EULAR TFs should use the 2011 Oxford Centre for EBM Levels of Evidence for grading evidence [13], and to report these levels in the main manuscript. The grading is performed by the SLR team (usually the fellows with support of the convenor and methodologist), based on all available evidence. In instances where different components within a single recommendation are informed by distinct levels of evidence, these differences must be justified and clearly explained in the manuscript.

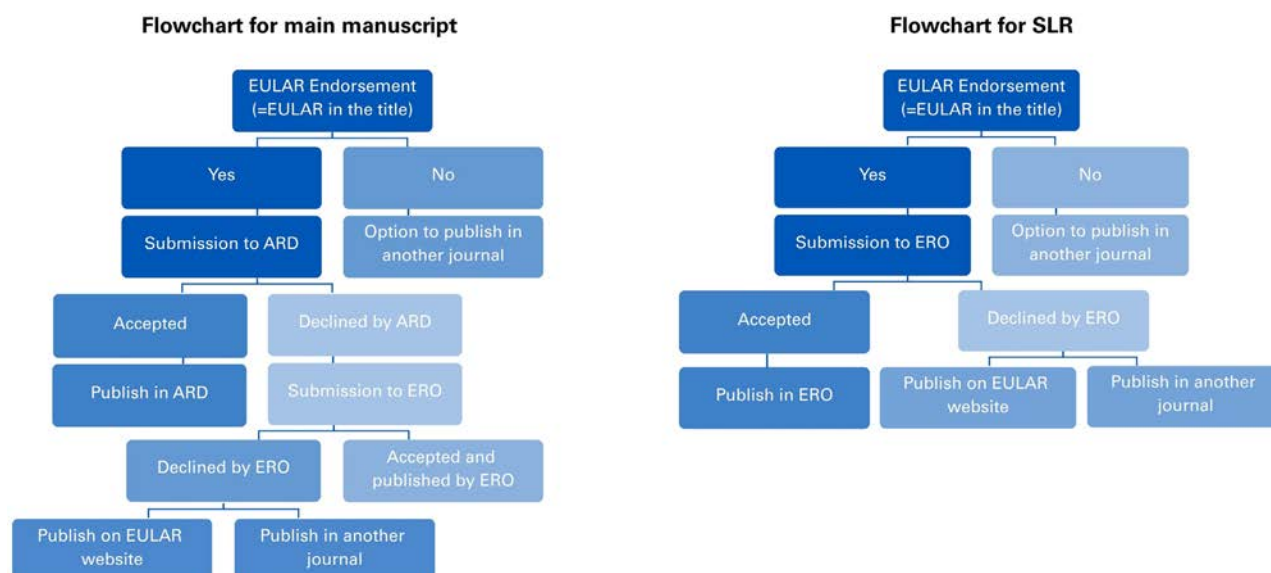


Figure 2. Flowchart of the main manuscript and systematic literature review (SLR) submission process. ARD, Annals of the Rheumatic Diseases; ERO, EULAR Rheumatology Open, EULAR, European Alliance of Associations for Rheumatology.

Levels of agreement

For each recommendation, including overarching principles, the level of agreement (on a 0–10 scale) among TF members is obtained after the final TF meeting. In the main manuscript, levels of agreement are presented for each recommendation as the mean (SD) and the proportion of TF members assigning a score of at least 8 of 10. These values illustrate the degree of heterogeneity in expert opinion (and, indirectly, the intensity of prior discussions) and the extent to which each recommendation is supported by the TF.

Quality Indicators

Alongside levels of evidence and agreement, TFs are asked to formulate QIs for 2 to 3 of their recommendations. Recommendations with greatest feasibility for implementation or with the highest potential impact on health care should be selected. These recommendations will be the templates for the formulation of the respective indicators. QIs are measurable constructs that are used to quantify health care gaps and can be used to assess change in health care quality over time. The operationalisation of QIs may vary across countries and health care settings, depending on available resources, infrastructure, and data collection systems. Therefore, measurement of the QIs may be adapted to ensure feasibility and relevance in different countries.

The manuscript

The main manuscript describes the rationale for the project, the compilation of the TF, the target audience, the methods used for obtaining consensus, the recommendations plus levels of agreement, and a concise discussion in the context of work by other organisations worldwide. A research agenda that poses questions for future updates is also part of the manuscript. Apart from the bullet list, TFs are invited to provide a brief explanation per bullet-point about their motivations and the course of the discussion that has taken place within the TF (Table 1).

Separate from the main manuscript, the SLR team is asked to compile 1 (or more) SLR report(s). The SLR report is a systematic report, preferably according to guidelines spelled out in the Cochrane Handbook for Systematic Reviews of Interventions

[10] or equivalent documents [14,15]. The SLR report is considered an important deliverable but does not necessarily have to be published in peer-reviewed literature. If not, the SLR report(s) will be made available on the EULAR website or provided as Supplementary material to the main manuscript. The manuscript, SLR, and a slide deck summarising the main results (which is also a prerequisite) will be made available on the EULAR website.

Obtaining EULAR endorsement of the final recommendations/PtCs

TF products are made public under the responsibility of EULAR, and the EULAR Council is the decisive institutional body for endorsement (Fig 1). This is why all manuscripts are reviewed by all EULAR Council members, discussed internally, and sent back to the authors for any revisions. Afterwards, the QoC Chair decides whether the work is approved. In case of discrepancies the EULAR Board is asked to give advice. EULAR-endorsed manuscripts are submitted to one of the EULAR journals (or, alternatively, to the EULAR website; see a relevant flowchart in the ‘living document’) (Fig 2). Thereafter, each manuscript undergoes standard editorial procedures, including peer review.

DISCUSSION

EULAR has more than 20 years of experience with QoC projects, executed by expert TFs, culminating in concise lists of recommendations or PtCs. Over the years, almost all RMDs have been subjected to thorough SLR and discussion within TFs and have often been repeated for updates. Procedures and methodologies have been gradually professionalised over the years, but the fundamental idea underlying EULAR QoC projects has remained intact: providing solid yet practical guidance for practitioners who diagnose and treat people living with RMDs. EULAR recommendations are internationally recognised for being practical, clear, and reasonable, and are often used to develop national guideline documents, which testifies to their broad applicability. An additional advantage of EULAR’s iterative process of updating existing recommendations is the

gradually increasing level of maturity: many of the EULAR recommendations have now been updated several times, and increasing maturity is reflected by fewer changes in consecutive versions [16–24].

Recent innovations, among which the incorporation of qualitative research into SLRs, attention to successful implementation of recommendations, and development of QIs, to mention a few, have seen the light and will be further worked on in the future. Implementation is a key aspect of the EULAR recommendations. Implementation plans should be included in the initial proposal for development of EULAR recommendations and QIs are also based in the feasibility for implementation among others. EULAR, recognising the importance of implementation, has recently introduced a new route of funding for projects (EULAR post-TF implementation projects) that aim to estimate and facilitate the implementation of the produced recommendations [25]. Furthermore, the potential role of artificial intelligence in SLRs is increasingly recognised and is currently being explored, with the aim of informing future methodological developments.

EULAR recommendations have also faced criticism. One of the most frequently heard complaints is that the process by which recommendations are developed is not structured and embedded in a robust and comprehensive framework, such as the GRADE framework. Another frequently expressed concern is that, within relatively small TFs, key opinion leaders, who often have declared conflicts of interest, may represent a substantial proportion of the group. EULAR's response to these critiques has always been that producing practical and clinical recognisable recommendations should prevail over methodological comprehensiveness (without detracting from scientific rigour). Although EULAR acknowledges the value of the GRADE framework as a rigorous and transparent approach to guideline development, its highly structured nature may not always be practical, given the broad scope of many EULAR TF projects and the need for feasibility and clinical applicability. Besides, post hoc methodological quality audits of published recommendations could be considered in future developments of the EULAR SOP framework.

In addition, obtaining completely unbiased experts is difficult to achieve in practice, since it is inherent to the role of 'field experts' in TFs that they maintain knowledge about relevant pharmacological developments, by (among others) taking part in trials and therefore having potential conflicts of interest.

Another type of critique is that 'the individual recommendations' usually involve actionable items that urge action: 'to start something' rather than 'to not act', to wait a while, or to forgo a previously initiated action (eg, stopping a drug). It may also be valuable to explore 'stopping' recommendations, 'do-not-act' recommendations, or 'postpone-to-act' recommendations, especially since, in many European countries, there is now increasing attention to overtreatment and 'less-is-more' initiatives.

In summary, more than 20 years after the conception of the first set of EULAR recommendations, EULAR's QoC projects have proven successful overtime. EULAR has kept up with the times and has professionalised its procedures and methods over the years, without compromising on practicability and clinical recognisability. This development has now been laid down in a 'living document' on EULAR's website, with more changes expected over time.

Competing interests

All authors currently work for, or have previously worked for the European Alliance of Associations for Rheumatology (EULAR) on a voluntary basis as members of the EULAR Council.

CRedit authorship contribution statement

George E. Fragoulis: Writing – original draft. **Robert B. M. Landewé:** Writing – review & editing. Writing – original draft. **Daniel Aletaha:** Writing – original draft. **Jose M. Álvaro-García:** Writing – original draft. **Xenofon Baraliakos:** Writing – original draft. **Christopher J. Edwards:** Writing – original draft. **Laure Gossec:** Writing – original draft. **Carina Haupt:** Writing – original draft. **Simona Lupatin:** Writing – original draft. **Souzi Makri:** Writing – original draft. **Rikke Helene Moe:** Writing – original draft. **Uta Kiltz:** Writing – review & editing, Writing – original draft.

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Ethics approval

Not applicable.

Provenance and peer review

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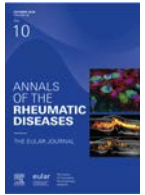
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Rheumatoid arthritis

Effects of air pollution on disease activity in patients with rheumatoid arthritis

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ABSTRACT

Objectives: This study aimed to evaluate the associations of air pollution with disease activity and flare rate in patients with rheumatoid arthritis (RA).

Methods: This prospective cohort study included patients with RA who were treated at a tertiary medical centre in South Korea between January 2021 and December 2024. Air pollution exposure was estimated using monthly mean concentrations of 6 air pollutants (sulfur dioxide, nitrogen dioxide, ozone, carbon monoxide, particulate matter [PM]₁₀, and PM_{2.5}). Disease activity and flares were recorded longitudinally at each outpatient visit. Associations between air pollution and RA disease activity or flare were analysed using linear and logistic generalised estimating equations, adjusting for demographic characteristics, serologic status, medication use, socioeconomic factors, and meteorological variables. As a sensitivity analysis, a case-crossover design using daily air pollutant concentrations preceding each visit was applied, with conditional logistic regression to assess within the same patient.

Results: A total of 12,583 outpatient visits from 1070 patients were analysed. Among 6 pollutants, PM_{2.5} was significantly associated with an increased risk of flare (adjusted odds ratio [OR]: 1.113 [95% CI: 1.017–1.218]) and with higher Disease Activity Score based on 28 joints (DAS28) with C-reactive protein (CRP), Clinical Disease Activity Index, 28-tender joint count, and 28-swollen joint count. In addition, the association between PM_{2.5} and DAS28-CRP was more pronounced among women and nonsmokers. In the case-crossover analysis, prolonged cumulative exposure to PM_{2.5} over >2 weeks was associated with an increased risk of RA flare.

Conclusions: Exposure to air pollutants, particularly PM_{2.5}, was associated with increased RA disease activity and flare risk. Further studies are warranted to determine whether improving air quality can reduce disease activity in patients with RA.

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WHAT IS ALREADY KNOWN ON THIS TOPIC

- Previous epidemiologic studies have shown an association between air pollution and an increased risk of developing rheumatoid arthritis (RA). However, the effects of air pollution on disease activity and flare occurrence in patients with established RA remain unclear, and the underlying mechanisms have not been fully elucidated.

WHAT THIS STUDY ADDS

- In this prospective cohort study, we demonstrated that higher particulate matter (PM)_{2.5} levels in the air were associated with an increased risk of RA flare and higher disease activity. Prolonged exposure to elevated PM_{2.5} concentrations over >2 weeks was associated with a significantly increased risk of RA flare.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- This study provides real-world evidence that air pollution, especially PM_{2.5}, may be associated with worsening RA disease activity. These findings underscore the importance of health-care providers and policymakers to recognise its impact on the management of RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by symmetric, polyarticular joint inflammation and various extra-articular manifestations. Many factors, including genetics, mucosal inflammation, protein modification, auto-antibody production, and environmental exposures, contribute to RA pathogenesis [1,2]. Among these, environmental factors, many of which are modifiable, play a critical role in both the onset and exacerbation of RA. Smoking is a well-established risk factor [3], whereas climatic factors, such as air temperature and humidity, have also been implicated in RA pathogenesis [4].

Air pollution consists of a mixture of gaseous pollutants and particulate matter (PM), which are generated from natural sources and human activities [5]. Major gaseous pollutants include sulfur dioxide (SO₂), nitrogen dioxide (NO₂), ozone (O₃), and carbon monoxide (CO). PM is classified based on aerodynamic diameter, with PM₁₀ referring to particles 10 µm or smaller, and PM_{2.5} referring to particles 2.5 µm or smaller. These fine and coarse particles can enter the lower respiratory tract, inducing oxidative stress and promoting systemic inflammatory responses [6,7].

Consistent with these mechanisms, several epidemiologic studies have reported associations between air pollution exposure and an increased risk of developing RA [8–10]. However, the impact of air pollution on disease activity in patients with established RA remains insufficiently investigated.

In this study, we investigate the longitudinal relationship between ambient air pollutants and RA disease activity, aiming to clarify whether fluctuations in environmental exposure contribute to disease aggravation over time.

METHODS

Patients

This prospective cohort included patients diagnosed with RA who were treated at a tertiary medical centre in South Korea

between January 2021 and December 2024. All patients met the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for RA at diagnosis [11]. Among 1118 patients with RA enrolled, those who (i) were followed up for <6 months or (ii) had <3 outpatient visits were excluded (Supplementary Fig S1). For each patient, follow-up began at the first outpatient visit during the study period (defined as the index date) and continued until the earliest of the following events: last outpatient visit, death, or December 31, 2024.

This study was approved by the Institutional Review Board of the Seoul National University Hospital (IRB no. H-2105-085-1219) and conducted in accordance with the principles of the Declaration of Helsinki [12]. All patients in the prospective cohort signed written informed consent before any data collection.

Clinical data collection

Baseline demographic characteristics, clinical characteristics, and socioeconomic factors were recorded at the index date. Smoking status, serologic status (seropositive vs seronegative RA), disease duration, and concomitant medications were collected at baseline. In addition, socioeconomic factors, including health insurance type and area deprivation index (ADI), were assessed.

South Korea has a well-established National Health Insurance (NHI) system that provides universal healthcare coverage. Although most individuals are covered by the NHI system, low-income individuals are supported through the Medical Aid (MA) program, which covers approximately 3% of the total population [13]. Health insurance type was categorised as NHI or MA. The ADI at the provincial and municipal levels was reported by the Busan Public Health Policy Institute every 5 years [14,15]. The ADI is a composite score based on 10 socioeconomic indicators designed to quantify relative regional socioeconomic deprivation in South Korea, with higher ADI values indicating greater socioeconomic deprivation. The ADI was constructed using the proportions of the following: (i) households with low socioeconomic status, (ii) households owing a house, (iii) households with poor housing conditions, (iv) individuals with less than a high school education, (v) households without a car, (vi) divorced or widowed individuals, (vii) single-person households, (viii) female-headed households, (ix) older adults (≥65 years), and (x) apartment households. We used the ADI derived from the 2020 Population and Housing Census data.

Clinical data, including patient and physician global assessment, 28-tender joint counts (TJC28) and swollen joint counts (SJC28), and C-reactive protein (CRP) levels, glucocorticoid dose, and use of disease-modifying antirheumatic drugs (DMARDs), were obtained at each follow-up visit. DMARD use was categorised as none, conventional synthetic DMARDs (csDMARDs), or biologic DMARDs (bDMARDs)/targeted synthetic DMARDs (tsDMARDs).

Air pollution data collection

Air pollution data were obtained from the Korea National Institute of Environmental Research. For the main analysis, monthly mean concentrations were collected for the following pollutants: SO₂ (ppb), NO₂ (ppb), O₃ (ppm), CO (ppm), PM₁₀ (µg/m³), and PM_{2.5} (µg/m³). Pollutant data from 17

administrative regions across South Korea were matched to each patient's residential area (Supplementary Fig S2). Although the original Air Quality Index (AQI), in which higher values indicate poorer air quality, is calculated based on 24-hour average values or the maximum 1-hour or 8-hour average values within a 24-hour period, we constructed a modified AQI using monthly mean pollutant concentrations as previously described [16]. Climate variables, including monthly mean air temperature and humidity, were obtained from the Korea Meteorological Administration for the same regions and time periods. To facilitate comparison of the effects of different air pollutants on RA disease activity, all pollutant concentrations were standardised to have a mean of 0 and an SD of 1.

For sensitivity analysis, daily mean air pollution data and climate variables were collected from the same sources.

Outcomes

The main outcome of the study was the occurrence of flare, defined as an increase in Disease Activity Score based on 28 joints with CRP (DAS28-CRP) >1.2 from the previous visit, or >0.6 if the current DAS28-CRP $\geq$ 3.2 [17]. Secondary outcomes included TJC28, SJC28, Clinical Disease Activity Index (CDAI), and DAS28-CRP [18].

Patient and public involvement

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

Statistical analysis

Baseline characteristics were summarised as mean $\pm$ SD, median (IQR), or counts (%). As most patients returned to the clinic at relatively regular intervals of approximately 3 to 4 months, flare incidence was expressed both as rates per 100 person-years with 95% CIs and as visit-based incidence per 100 outpatient visits. Clinical data from each visit were linked to the corresponding monthly mean air pollution concentrations, based on each patient's residential area and visit month. To assess the association between air pollution and RA disease activity while accounting for within-patient clustering, we used generalised estimating equations (GEEs) with either linear or logistic link functions, as appropriate. Repeated measurements were accounted for by clustering models at the patient level with an autoregressive correlation structure of order 1 [19].

Logistic GEE was used to estimate odds ratios (ORs) with 95% CIs for flare occurrences. For RA disease activity parameters, the β coefficient with 95% CIs was estimated using a linear GEE model. All models were adjusted for prespecified covariates, including age, sex, smoking status, serologic status, time-varying glucocorticoid dose, and DMARD use (none, csDMARDs, or bDMARDs/tsDMARDs), defined according to prescriptions preceding each visit, as well as socioeconomic factors (insurance type and ADI) and meteorological variables (temperature and humidity).

In addition, the association between air pollution and RA disease activity was evaluated across prespecified clinical subgroups defined by clinically relevant factors for RA, such as age, sex, smoking status, comorbidities, baseline concomitant medication for RA, and baseline TJC28 and SJC28 [20–22].

For sensitivity analysis, to better control for time-invariant confounding and to avoid potential reverse causation inherent in the monthly analysis, we conducted a case-crossover study

using daily exposure data to further evaluate the association between RA flare and air pollution. In a case-crossover study, exposure levels during a hazard period associated with the outcome are compared with those during a control period within the same individual [23]. Among 1070 patients with RA included in the main analysis, those who experienced at least 1 RA flare during the study period were eligible for the sensitivity analysis (Supplementary Fig S1). For each patient, outpatient visits during the study period were classified as case visits if the visit met the RA flare, whereas all other nonflare outpatient visits within the same individual were defined as control visits. In this visit-based case-crossover design, exposure windows were defined relative to each visit date. The hazard period was defined using variable-width cumulative exposure windows ranging from 1 to 28 days preceding each case visit (eg, lag 1, lag 1-2, lag 1-3, ..., lag 1-28). Similarly, the control period was defined using the same cumulative exposure windows preceding each control visit. Daily mean air pollution data and meteorological variables, including daily mean temperature and humidity, were assigned to each individual based on their residential area. Conditional logistic regression models were used to estimate the association between air pollution exposure and RA flare within individuals. To account for potential unmeasured time-varying confounding, we adjusted for calendar time using a smooth function, as well as prespecified covariates, including meteorological variables (temperature and humidity) during each exposure window, and time-varying concomitant medications, such as glucocorticoid dose and DMARD use (none, csDMARDs, or bDMARDs/tsDMARDs).

To examine potential nonlinear dose–response relationships between PM_{2.5} concentrations and RA flare, multivariable conditional logistic regression models incorporating restricted cubic splines with 3 degrees of freedom were used.

All statistical analyses were performed using R software (R package: geepack, survival, version 4.4.3), and a *P* value of <.05 was considered statistically significant. In addition, to evaluate the potential impact of unmeasured confounding, the *E*-value was calculated to estimate the minimum strength of unmeasured confounding needed to explain an association [24].

RESULTS

Baseline characteristics

A total of 12,583 outpatient visits from 1070 patients were analysed in this study. Baseline demographic and clinical characteristics are summarised in Table 1. Briefly, 86.2% of patients were women, the mean $\pm$ SD age was 61.3 $\pm$ 12.6 years, and RA duration was 9.1 $\pm$ 7.9 years. Baseline median (IQR) DAS28-CRP was 2.4 (1.7–3.3). Patients had a median of 11.0 (9.0–14.0) outpatient clinic visits during 2021 to 2024. Approximately 50% patients used glucocorticoids, with a daily dose of 4.3 $\pm$ 2.3 mg of prednisolone equivalent among users. csDMARDs were used in 892 patients (83.4%), and 118 patients (11.0%) received bDMARDs/tsDMARDs, with or without concomitant csDMARDs.

Regional and temporal air pollution trends during the observation period

During the observation period from 2021 to 2024, air pollution levels were generally higher in winter and spring, showing an inverse trend compared with temperature (Fig 1). Regional differences in air pollution were also observed: during the

Table 1
Baseline characteristics of the study population

Characteristics	RA (N = 1070)
Age, y, mean ± SD	61.3 ± 12.6
Sex, female, n (%)	922 (86.2)
Smoker, n (%)	70 (6.5)
Seropositive RA, n (%)	865 (80.8)
Hypertension, n (%)	349 (32.6)
Diabetes mellitus, n (%)	129 (12.1)
Chronic kidney disease, n (%)	35 (3.3)
Interstitial lung disease, n (%)	40 (3.7)
Medical aid, n (%)	26 (2.4%)
Area deprivation index, mean ± SD	−2.6 ± 5.0
RA duration, years, mean ± SD	9.1 ± 7.9
Outpatient clinic visits per patient, median (IQR)	11.0 (9.0–14.0)
TJC28 at index date, median (IQR)	1.0 (0.0–3.0)
SJC28 at index date, median (IQR)	0.0 (0.0–2.0)
DAS28-CRP ^a at index date, median (IQR)	2.4 (1.7–3.3)
Concomitant RA treatment	
Glucocorticoids, n (%)	543 (50.7)
Prednisolone-equivalent, ^b mg/d, mean ± SD	4.3 ± 2.3
csDMARD, n (%)	892 (83.4)
bDMARD/tsDMARD, n (%)	118 (11.0)

bDMARD, biologic disease-modifying antirheumatic drug; csDMARD, conventional synthetic disease-modifying antirheumatic drug; DAS28-CRP, Disease Activity Score based on 28 joints with C-reactive protein; RA, rheumatoid arthritis; SJC28, 28-swollen joint count; TJC28, 28-tender joint count; tsDMARD, targeted synthetic disease-modifying antirheumatic drug.

^a DAS28-CRP at the index date was calculated for 979 patients who had CRP levels available on the index date.

^b Prednisolone-equivalent dose was presented as mean ± SD among glucocorticoid users only.

spring, Incheon showed the highest levels of SO₂ and CO; Gyeonggi-do and Chungcheongnam-do had the highest levels of PM₁₀ and PM_{2.5}; and Seoul exhibited the highest concentration of NO₂ (Supplementary Table S1).

The mean ± SD concentrations of air pollutants matched to patient visits during the study period were as followed: SO₂ (ppb), 2.72 ± 0.45; NO₂ (ppb), 17.94 ± 6.87; O₃ (ppm), 0.03 ± 0.01; CO (ppm), 0.41 ± 0.09; PM₁₀ (µg/m³), 34.86 ± 13.23; and PM_{2.5} (µg/m³), 18.49 ± 6.00. In addition, the modified AQI

values matched to patient visits during the study period averaged 68.35 ± 11.51, with the highest in January (83.70 ± 5.12) and lowest in September (51.25 ± 5.91).

Air pollution and RA flare

During the 3235.43 person-years of follow-up, 1278 flare events occurred among 604 patients with RA, corresponding to an incidence rate of 39.5 per 100 person-years (95% CIs: 37.36–41.73) and 10.16 per 100 outpatient visits. Among 12,583 outpatient visits, the monthly ratio of flare events to outpatient visits was highest in January (0.124; 84 flares out of 679 outpatient visits), and lowest in April (0.086; 90 flares out of 1050 outpatient visits) (Supplementary Fig S3).

The risk of RA flare was increased with higher modified AQI (adjusted OR: 1.115 [1.020–1.219]) (Supplementary Table S2). Among the 6 air pollutants examined, PM_{2.5} was significantly associated with an increased risk of flare in the multivariable GEE model (adjusted OR: 1.113 [1.017–1.218]). The point estimate of the E-value for PM_{2.5} was 1.47. PM₁₀ and O₃ also showed positive, but nonsignificant associations with flare risk (adjusted ORs: 1.060 [0.980–1.148] and 1.023 [0.934–1.121], respectively) (Fig 2, Supplementary Table S3).

Air pollution and other RA disease activity measures

Higher modified AQI was associated with increased overall disease activity in patients with RA (Supplementary Table S2). In univariable analyses, high PM_{2.5} concentrations were significantly associated with higher TJC28 and SJC28. These associations remained consistent after adjustment for covariates (adjusted β: 0.064 [0.010–0.119] and 0.046 [0.005–0.088], respectively). In addition, higher PM_{2.5} levels were significantly associated with increased DAS28-CRP and CDAI (adjusted β: 0.031 [0.009–0.053] and 0.171 [0.039–0.302], respectively) (Table 2, Supplementary Table S4).

DAS28-CRP increased with high PM₁₀ concentrations (adjusted β: 0.020 [0.001–0.039]), and SJC28 increased with high O₃ levels (adjusted β: 0.045 [0.003–0.088]). Among the 6

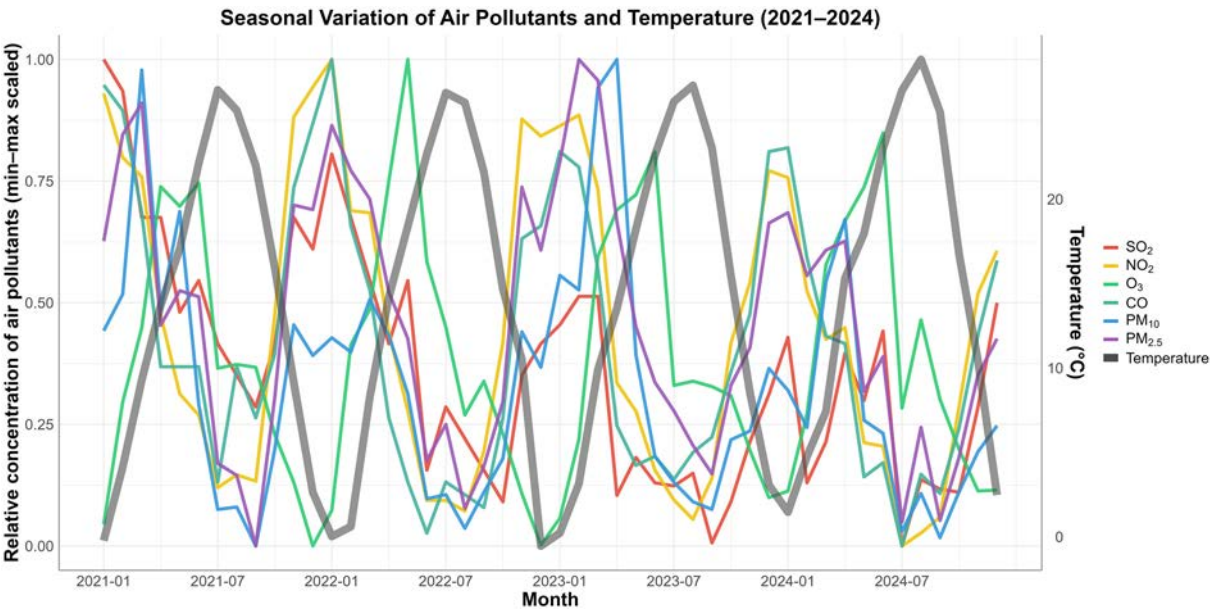


Figure 1. Monthly trends in air pollutants during 2021 to 2024. CO, carbon monoxide; NO₂, nitrogen dioxide; O₃, ozone; PM, particulate matter; SO₂, sulfur dioxide.

Association of Air Pollutants with RA Flare

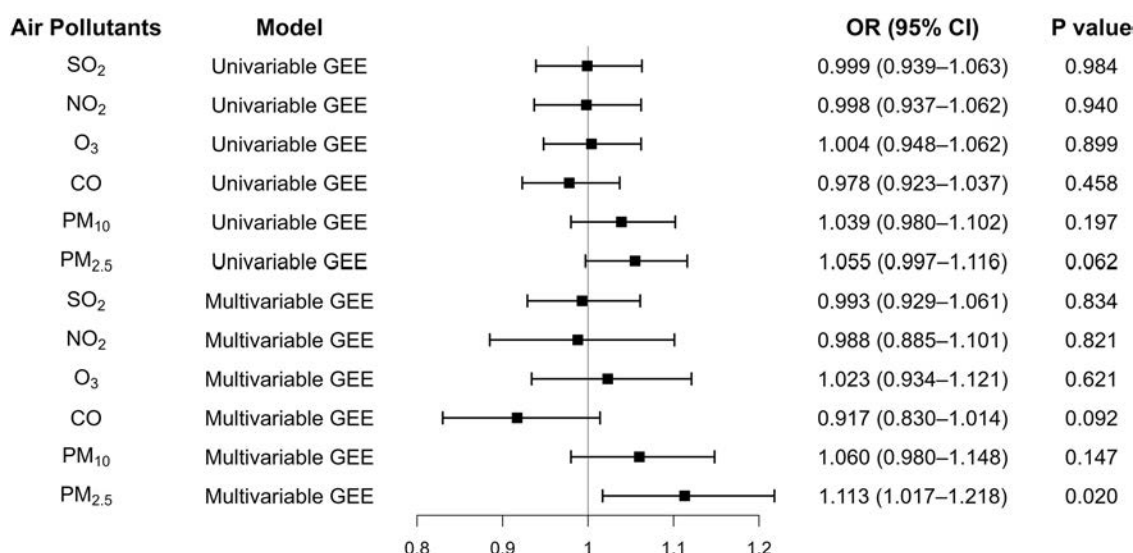


Figure 2. Odds ratios (ORs) for RA flare associated with air pollutants. Results from generalised estimating equation (GEE) models were presented as univariable and multivariable analyses. Multivariable models were adjusted for age, sex, smoking status, serologic status, time-varying concomitant medications (glucocorticoid dose and DMARD use), socioeconomic factors (insurance type and area deprivation index), and meteorological variables (temperature and humidity). Concentrations of air pollutants were standardised to have a mean of 0 and an SD of 1; therefore, effect estimates represent the association per 1-SD increase in monthly mean air pollutant concentrations. CO, carbon monoxide; DMARD, disease-modifying antirheumatic drug; NO₂, nitrogen dioxide; O₃, ozone; PM, particulate matter; RA, rheumatoid arthritis; SO₂, sulfur dioxide.

air pollutants, PM_{2.5} showed the largest effect size and was the only pollutant significantly associated with all disease activity parameters (DAS28-CRP, CDAI, TJC28, and SJC28).

Supplementary Figure S4 presents the predicted disease activity (DAS28-CRP and CDAI) across PM_{2.5} levels, using multivariable GEE with spline smoothing. After adjustment for covariates, both DAS28-CRP and CDAI showed an increasing trend with rising PM_{2.5} levels.

Interaction between PM_{2.5} and clinical factors on RA disease activity

The association between PM_{2.5} and DAS28-CRP was more prominent in female patients (*P* for interaction .026) and non-smoking patients (*P* for interaction .012). In addition, patients with a higher baseline TJC28 (TJC28 ≥ 2) showed a relatively greater increase in DAS28-CRP with higher PM_{2.5} (Fig 3). In

Table 2
Association of air pollutants with RA disease activity

	DAS28-CRP		CDAI	
	Univariable analysis Crude β (95% CI)	Multivariable analysis ^a Adjusted β (95% CI)	Univariable analysis Crude β (95% CI)	Multivariable analysis ^a Adjusted β (95% CI)
Gas				
SO ₂	0.001 (−0.017 to 0.019)	−0.001 (−0.023 to 0.021)	0.016 (−0.088 to 0.121)	0.009 (−0.120 to 0.138)
NO ₂	0.004 (−0.010 to 0.017)	0.022 (−0.018 to 0.062)	0.002 (−0.073 to 0.076)	0.109 (−0.139 to 0.358)
O ₃	0.003 (−0.008 to 0.015)	0.016 (−0.007 to 0.039)	0.050 (−0.014 to 0.113)	0.129 (−0.003 to 0.261)
CO	−0.003 (−0.015 to 0.009)	−0.015 (−0.044 to 0.013)	−0.018 (−0.086 to 0.050)	−0.017 (−0.183 to 0.148)
Particle				
PM ₁₀	0.008 (−0.003 to 0.020)	0.020 (0.001–0.039)	0.032 (−0.035 to 0.099)	0.069 (−0.045 to 0.183)
PM _{2.5}	0.012 (−0.000 to 0.024)	0.031 (0.009–0.053)	0.060 (−0.008 to 0.128)	0.171 (0.039–0.302)
	TJC28		SJC28	
	Univariable analysis Crude β (95% CI)	Multivariable analysis ^a Adjusted β (95% CI)	Univariable analysis Crude β (95% CI)	Multivariable analysis ^a Adjusted β (95% CI)
Gas				
SO ₂	0.021 (−0.023 to 0.065)	−0.002 (−0.056 to 0.052)	0.035 (0.002–0.068)	0.034 (−0.006 to 0.075)
NO ₂	0.022 (−0.009 to 0.053)	0.030 (−0.075 to 0.136)	0.005 (−0.017 to 0.027)	0.018 (−0.060 to 0.095)
O ₃	0.005 (−0.021 to 0.032)	0.031 (−0.023 to 0.084)	0.019 (−0.002 to 0.039)	0.045 (0.003–0.088)
CO	0.008 (−0.021 to 0.037)	−0.023 (−0.091 to 0.046)	0.000 (−0.020 to 0.021)	0.000 (−0.053 to 0.053)
Particle				
PM ₁₀	0.037 (0.009–0.065)	0.042 (−0.007 to 0.091)	0.020 (−0.000 to 0.040)	0.023 (−0.013 to 0.058)
PM _{2.5}	0.041 (0.013–0.070)	0.064 (0.010–0.119)	0.023 (0.003–0.044)	0.046 (0.005–0.088)

CDAI, Clinical Disease Activity Index; CO, carbon monoxide; DAS28-CRP, Disease Activity Score based on 28 joints with C-reactive protein; DMARD, disease-modifying antirheumatic drug; NO₂, nitrogen dioxide; O₃, ozone; PM, particulate matter; RA, rheumatoid arthritis; SJC28, 28-swollen joint count; SO₂, sulfur dioxide; TJC28, 28-tender joint count.

Concentrations of air pollutants were standardised to have a mean of 0 and a SD of 1; therefore, effect estimates represent the association per 1-SD increase in monthly mean air pollutant concentrations.

^a Adjusted for age, sex, smoking status, serologic status, time-varying concomitant medications (glucocorticoid dose and DMARDs use), socioeconomic factors (insurance type and area deprivation index), and meteorological variables (temperature and humidity).

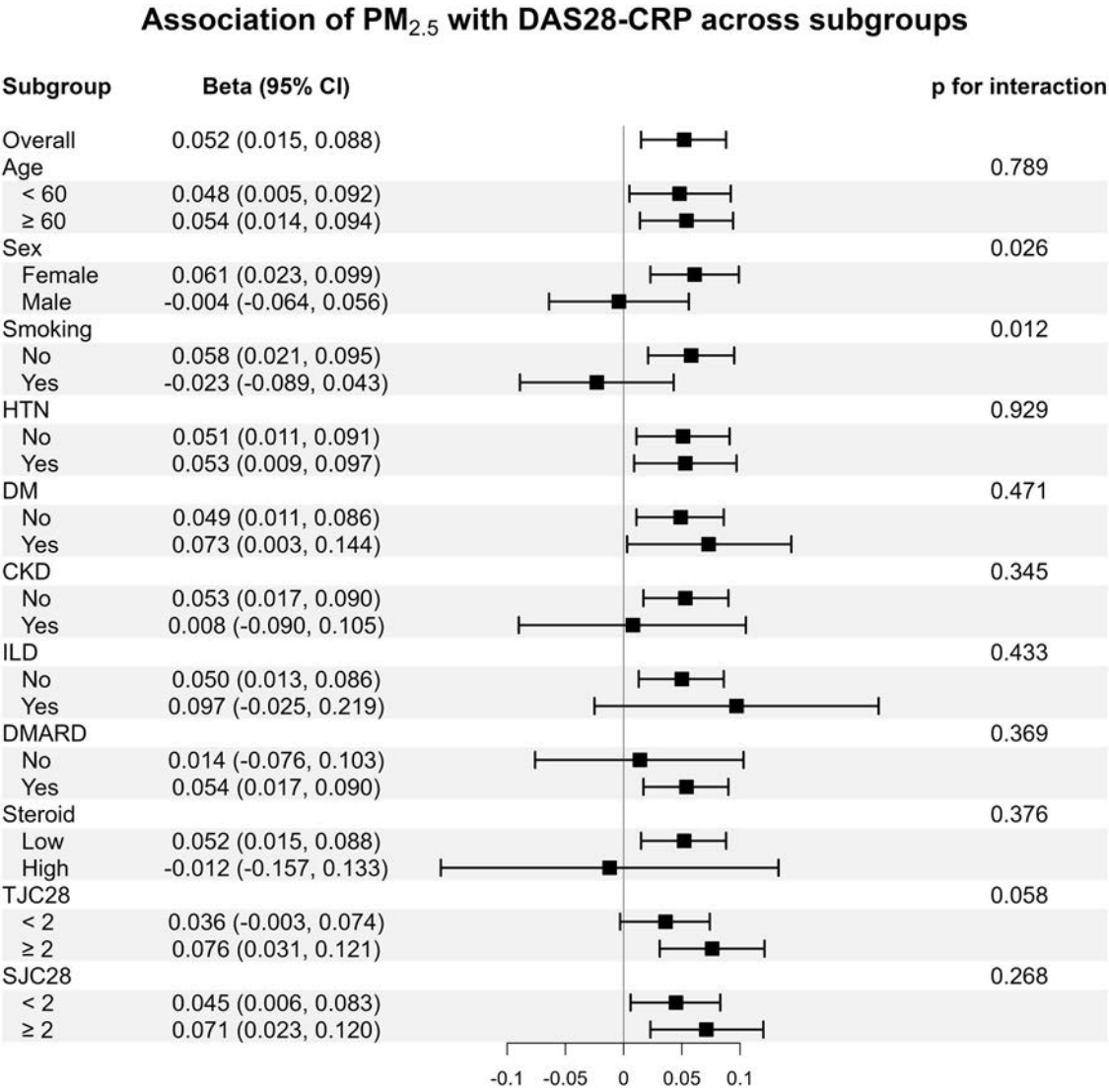


Figure 3. Association of PM_{2.5} with DAS28-CRP across clinical subgroups. High dose of steroid was defined as a daily dose of ≥ 20 mg of prednisolone. Effect estimates represent the association per 10 $\mu\text{g}/\text{m}^3$ increase in monthly mean PM_{2.5} concentration. Adjusted for age, sex, smoking status, serologic status, time-varying concomitant medications (glucocorticoid dose and DMARD use), socioeconomic factors (insurance type and area deprivation index), and climate variables (temperature and humidity). CKD, chronic kidney disease; DAS28-CRP, Disease Activity Score based on 28 joints with C-reactive protein; DM, diabetes mellitus; DMARD, disease-modifying antirheumatic drug; HTN, hypertension; ILD, interstitial lung disease; PM, particulate matter; SJC28, 28-swollen joint count; TJC28, 28-tender joint count;

contrast, the association of PM_{2.5} with RA flare was not significantly modified by any clinical factors (Supplementary Fig S5).

Sensitivity analysis using case-crossover design

Among 603 patients with RA who experienced at least 1 RA flare, a total of 8024 outpatient visits were included, comprising 1278 case visits and 6746 control visits (Supplementary Fig S1). Overall, the findings were highly consistent with the main analysis, supporting the robustness of the association between PM_{2.5} exposure and RA flare. Specifically, higher PM_{2.5} exposure was associated with an increased risk of RA flare across most lag periods, except for lag 1 and lag 1 to 7 period (Supplementary Table S5). Notably, longer cumulative lag periods of daily mean PM_{2.5} exposure preceding each visit were associated with a higher likelihood of RA flare, with more pronounced associations observed when the cumulative lag period extended beyond approximately 14 days (Fig 4A). Exposure–response curves

further demonstrated positive dose-dependent associations between PM_{2.5} levels and the risk of RA flare (Fig 4B).

DISCUSSION

In this prospective study, we observed a significant association between ambient air pollution and RA disease activity over 4 years of longitudinal follow-up in patients with RA. Among the evaluated pollutants, PM_{2.5} showed the strongest and most consistent association with RA flare. Previous studies have focused primarily on the contribution of air pollution to the development of RA rather than to the exacerbation of RA [25]. Although recent research has suggested that air pollution may aggravate RA disease activity [26], findings across studies have been inconsistent. Some reported that exposure to SO₂ and NO₂ was associated with increased DAS28, whereas PM₁₀, O₃, and CO were not [27]. In contrast, another study implicated that O₃ increased DAS28, whereas PM₁₀ and NO₂ showed negative associations [28]. Other investigations have shown that CO, NO₂, and O₃ could significantly

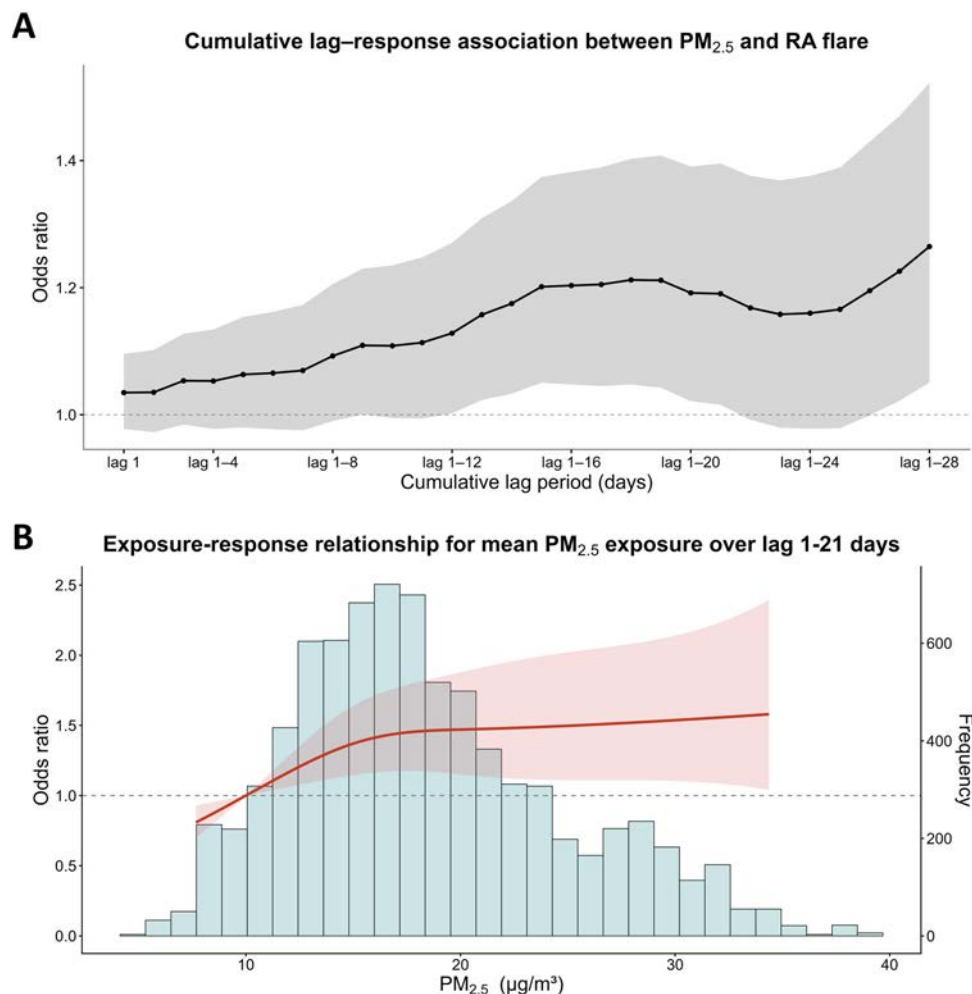


Figure 4. Cumulative PM_{2.5} exposure and the risk of RA flare in the case-crossover cohort. (A), Cumulative lag-response associations across variable-width cumulative lag periods. PM_{2.5} exposure was defined as the mean concentration over each cumulative lag window, with progressively extended lag windows from lag 1 to lag 1-28 days (i.e., lag 1, lag 1-2, lag 1-3, ..., lag 1-28). (B) Exposure-response relationship for cumulative PM_{2.5} exposure (lag 1-21 days) estimated using restricted cubic spline models with multivariable conditional logistic regression. Models were adjusted for daily mean temperature and humidity over each corresponding lag period, time-varying concomitant medications (glucocorticoid dose and DMARD use) and calendar time using a smooth function. In panel (A), effect estimates represent the association per 10 µg/m³ increase in daily mean PM_{2.5} concentration. In panel (B), histograms represent the distribution of PM_{2.5} concentrations. Solid lines indicate adjusted ORs, and shaded areas represent 95% CI. The reference value was set at 10 µg/m³ for the cumulative lag period. Flare was defined as an increase in DAS28-CRP >1.2, or >0.6 if the DAS28-CRP ≥3.2. DAS28-CRP, Disease Activity Score based on 28 joints with C-reactive protein; DMARD, disease-modifying antirheumatic drug; OR, odds ratio; PM, particulate matter; RA, rheumatoid arthritis.

increase DAS28 [29,30], whereas others found no significant associations [31]. These heterogeneous results may reflect variations in study design, pollutant composition, regional meteorological factors, and patient populations.

Air pollution may influence disease activity through the generation of reactive oxygen species (ROS). ROS can activate nuclear factor kappa B and stimulate the production of tumour necrosis factor- α and interleukin-1, promoting synovial inflammation. Air pollution has been implicated in lung inflammation and the formation of inducible bronchus-associated lymphoid tissue, which may promote the generation of anticitrullinated peptide antibodies. Furthermore, pollutants may directly contribute to joint inflammation and erosion [32–35].

In this study, we identified PM_{2.5} as a key contributor to increased RA disease activity. PM_{2.5} particles are smaller than red blood cells and can penetrate deeply into the respiratory tract, accumulating in alveolar ducts and capillaries, and subsequently disseminate to distant organs through the circulation [36]. These particles can induce ROS production through several pathways: the direct metal-cell interaction [37], increased oxidase activity, and calcium channel activation in the cytoplasmic membrane or

endoplasmic reticulum [38], and disruption of mitochondrial homeostasis, leading to mitochondrial DNA damage [39]. Excess ROS can result in DNA damage, oxidative stress, and inflammatory responses in multiple organs [40], which may aggravate synovial and systemic inflammation in RA.

Our subgroup analysis further revealed that sex, smoking status, and baseline TJC28 influenced the association between air pollution and RA disease activity. Specifically, women, non-smokers, and patients with higher baseline TJC28 showed a stronger association between PM_{2.5} and DAS28-CRP. Similar interactions were observed in chronic obstructive pulmonary disease (COPD), where PM_{2.5} induced airway neutrophilic inflammation predominantly in nonsmokers, suggesting that chronic smoking-related airway inflammation may mask the acute effects of PM_{2.5} [41]. Lungs of nonsmokers, being less chronically inflamed and not repeatedly exposed to irritants, may be more vulnerable to the oxidative and inflammatory effects of ambient PM_{2.5}. Because cigarettes contain a much higher concentration of PM_{2.5} than ambient air, smokers may already have maximally activated airway inflammatory pathways. As a result, further exposure to environmental PM_{2.5} may

have a diminished or “saturated” effect on RA disease activity. In contrast, patients with high TJC28 in baseline may represent a more inflammatory state, making them more responsive to additional inflammatory stimuli such as air pollution. This may reflect biological pathways through which PM_{2.5}, after translocating from the alveoli into systemic circulation, can amplify pre-existing synovial inflammation.

In the main analysis, we evaluated the association between air pollution and RA disease activity using monthly averaged air pollutant concentrations. Although it can show the overall trends between air pollution and disease activity, it may obscure short-term fluctuations in air pollution that occur between clinical assessments. To address this, we additionally conducted a case-crossover analysis to evaluate temporal associations at the individual level. Notably, short-term increases in PM_{2.5} concentrations were not associated with an immediate increase in RA flare risk. Rather, prolonged exposure to higher PM_{2.5} levels was associated with a significantly increased risk of RA flare, suggesting that cumulative exposure over time may be more relevant than transient peaks in influencing RA disease activity.

South Korea has experienced relatively high levels of ambient air pollution compared with many other Organization for Economic Co-operation and Development (OECD) countries, particularly with respect to PM_{2.5} concentrations. According to OECD reports, South Korea had the highest mean population exposure to PM_{2.5} among OECD countries in 2013 [42]. In addition, the 2024 World Air Quality Report ranked South Korea 55th among 138 countries in annual average PM_{2.5} concentration worldwide [43]. Therefore, given the widespread and persistent exposure to ambient air pollution in South Korea, increases in RA disease activity at the individual level may still have meaningful public health implications.

This study has some limitations. First, actual individual exposure to air pollution may differ from assigned exposure levels. For instance, outdoor activity time and personal behaviours such as mask use and the use of indoor air purification systems vary among individuals, introducing potential unmeasured confounding. Second, we did not have information on relevant lung diseases, such as pneumonia, asthma, or COPD, which might mediate pulmonary inflammatory response to air pollution. Third, the exposure assignment was based on residential area, and discrepancies between residence and workplace locations, as well as relocations during follow-up, may have led to exposure misclassification. Fourth, the estimated E-value suggested that an unmeasured confounder associated with both PM_{2.5} and RA flare >1.47 could potentially explain away the observed association. In addition, as our study included only Korean patients, the generalizability of our findings to other regions with different environmental, geographic, or ethnic backgrounds might be limited. Last but not least, although the observed associations were statistically significant, the effect sizes (ORs and β coefficients) were relatively modest. Given that most patients in our cohort had well-controlled RA, further studies are needed to determine whether the impact of air pollution on disease activity is more pronounced in patients with higher baseline disease activity. In addition, considering the pulmonary and systemic inflammatory effects of air pollution, future studies investigating the association between air pollution exposure and RA-associated interstitial lung disease flare may provide clinically relevant insights.

CONCLUSION

This study provides real-world evidence that higher levels of air pollution, particularly PM_{2.5}, are related to worsened RA

disease activity and a higher risk of flare. Although further external validation is needed, these findings have important clinical and public health implications. Physicians should encourage patients with RA to minimise exposure to poor air quality, and policymakers should prioritise air quality improvement to reduce the burden of chronic illnesses, including RA flares and elevated disease activity.

Competing interests

EBL received a speaker fee from Boehringer Ingelheim and a consultant fee from Priovant Therapeutics. All other authors declare no competing interests.

CRediT authorship contribution statement

Sung Cheol Jung: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Jeffrey R Curtis:** Writing – review & editing, Formal analysis, Conceptualization. **Seojin Yang:** Writing – review & editing, Investigation, Data curation. **Yunhee Choi:** Writing – review & editing, Methodology, Formal analysis. **Min Hyuk Lim:** Writing – review & editing, Data curation. **Saram Lee:** Writing – review & editing, Data curation. **Sung Ik Cho:** Writing – review & editing, Investigation. **Jin Kyun Park:** Writing – review & editing, Investigation. **Jun Won Park:** Writing – review & editing, Methodology, Investigation. **Eun Bong Lee:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization.

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Patient consent for publication

All patients gave their written informed consent before their participation.

Ethics approval

This study was approved by the institutional review board at Seoul National University Hospital (IRB No. H-2105-085-1219).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Not applicable.

Supplementary materials

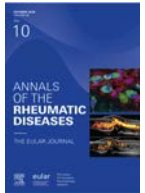
Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2026.06.018.

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Rheumatoid arthritis

Reduced versus standard dosing of Janus kinase inhibitors in rheumatoid arthritis: a propensity score –matched real-world study accounting for poor prognostic factors

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ABSTRACT

Objectives: This study aimed to compare 24-month drug retention between standard-dose and reduced-dose Janus kinase inhibitors (JAKi) in rheumatoid arthritis (RA) and to examine whether poor prognostic factor (PPF) burden and renal function modify treatment persistence, with primary inference restricted to patients with preserved renal function, in whom reduced dosing reflects discretionary clinical decision-making.

Methods: We conducted a multicentre observational study using a Japanese real-world registry of patients with RA who initiated JAKi between 2013 and 2025. Patients were classified according to their initial JAKi dosing strategy. Primary analyses were performed in patients with preserved renal function. Propensity score matching was used to adjust for confounding by indication. Drug retention over 24 months was assessed using Kaplan-Meier analyses and Cox proportional hazards models, with prespecified stratification by PPF burden (0-2 vs 3-4). Supportive analyses were conducted in patients with reduced renal function.

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Results: Among 1135 patients, 24-month drug retention was comparable between standard-dose and reduced-dose JAKi in the matched cohort with preserved renal function. Disease activity improved similarly in both groups, and glucocorticoid use remained low without compensatory escalation. Reduced dosing performed comparably in patients with low PPF burden, whereas standard-dose initiation showed more favourable persistence in patients with multiple PPFs. In patients with reduced renal function, standard dosing was associated with higher drug retention.

Conclusions: In patients with preserved renal function, reduced-dose JAKi initiation was associated with 24-month treatment persistence comparable with standard dosing. Findings in PPF-stratified analyses and in patients with reduced renal function should be interpreted cautiously and warrant further investigation.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Janus kinase inhibitors (JAKi) are effective treatments for rheumatoid arthritis, but dose reduction is often selected in routine clinical practice because of safety concerns and patient-related risk factors.
- In patients with reduced renal function, initial JAKi dosing may reflect label-mandated dose adjustment, complicating interpretation of real-world comparisons between standard and reduced dosing.

WHAT THIS STUDY ADDS

- In patients with preserved renal function, in whom reduced-dose initiation most plausibly reflects discretionary clinical decision-making rather than label-mandated adjustment, reduced initial dosing was associated with 24-month drug retention comparable with standard dosing.
- By contrast, standard-dose initiation showed a signal of more favourable treatment persistence in patients with multiple poor prognostic factors and in those with reduced renal function, although these subgroup findings require cautious interpretation.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- These findings suggest that interpretation of real-world JAKi dosing strategies should distinguish discretionary dose reduction from label-mandated renal dose adjustment.
- Reduced-dose initiation may be a reasonable option in selected patients with preserved renal function, whereas adequate initial drug exposure may warrant consideration in patients with higher prognostic risk or reduced renal function.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease that, if inadequately controlled, leads to progressive joint damage, functional disability, systemic complications, and impaired quality of life [1,2]. Over the past decade, treat-to-target strategies and the expanded use of biologic and targeted synthetic disease-modifying antirheumatic drugs (b/tsDMARDs) have substantially improved disease outcomes [1–3]. Among these agents, Janus kinase inhibitors (JAKi) represent an effective oral treatment option for patients with an inadequate response to conventional synthetic DMARDs (csDMARDs) and/or bDMARDs, with rapid onset of action and broad efficacy across disease activity states [3–5].

Despite their established efficacy, the benefit-risk balance of JAKi has been subject to increasing scrutiny, particularly in older patients and those with cardiovascular risk factors, an increased risk of venous thromboembolism, or a history of malignancy [6]. Regulatory warnings and postmarketing safety

data have heightened awareness of potential adverse outcomes, leading clinicians to adopt more cautious and individualised treatment strategies. In routine clinical practice, initial JAKi dosing is therefore frequently tailored according to patient characteristics such as age, comorbidities, renal function, concomitant medications, and overall risk profile. Consequently, reduced initial dosing is often selected for patients perceived to be at higher risk, although pivotal clinical trials and regulatory evidence are largely based on fixed, label-recommended dosing regimens in selected populations [3,6,7].

Drug retention is a pragmatic composite outcome reflecting treatment effectiveness, safety, tolerability, and patient preference in routine care, and has been widely used to evaluate the real-world performance of b/tsDMARDs [8]. However, the impact of reduced initial JAKi dosing on long-term treatment persistence remains unclear. Interpretation of observational data is challenging because reduced dosing in routine practice may reflect both disease context and broader treatment-selection considerations, resulting in substantial confounding by indication. Poor prognostic factors (PPFs)—including seropositivity for rheumatoid factor (RF) or anticitrullinated protein antibodies (ACPAs), high disease activity, elevated inflammatory markers, and functional impairment—are well-established predictors of aggressive disease and unfavourable long-term outcomes in RA [9]. Current treatment recommendations emphasise early therapeutic escalation in patients who fail to achieve meaningful improvement within 3 months, particularly in the presence of multiple PPFs [1]. Although PPFs themselves are not safety-related factors, reduced initial JAKi dosing in routine practice should not be interpreted as a direct consequence of disease activity or prognostic burden alone. Rather, patients with a high disease burden may also have other clinical characteristics or treatment-related considerations that lead clinicians to choose a more cautious initial JAKi dosing strategy, including concern for extra-articular manifestations, organ function, concomitant glucocorticoid (GC) exposure, and overall safety risk. This clinical paradox complicates the evaluation of dosing strategies and underscores the need for robust adjustment methods in observational studies.

In this multicentre real-world cohort of Japanese patients with RA initiating JAKi therapy, our primary objective was to compare 24-month treatment persistence between standard-dose and reduced-dose initiation among patients with preserved renal function, with primary inference focused on this subgroup, in whom reduced dosing reflects discretionary clinical decision-making rather than label-mandated adjustment. We used propensity score methods to mitigate confounding by indication and prespecified stratification by PPF burden to support interpretation. Analyses in patients with reduced renal function and PPF-stratified comparisons were prespecified as exploratory and are presented to generate hypotheses for future studies.

METHODS

Study design and setting

This multicentre observational cohort study included patients with RA who initiated JAKi during routine clinical care at participating rheumatology centres in Japan. Data were retrospectively collected from March 2013, following the approval of tofacitinib in Japan, through October 2025. The study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines [10].

Patients

Patients were identified from the Kansai Consortium for the Well-being of Rheumatic Disease Patients (ANSWER) cohort, a multicentre registry comprising university-affiliated hospitals in Japan. RA was defined according to the 1987 American College of Rheumatology (ACR) or the 2010 ACR/European Alliance of Associations for Rheumatology (EULAR) classification criteria [11,12]. Eligible patients were those who initiated any JAKi for RA during the study period. JAKi agents included tofacitinib, baricitinib, peficitinib, upadacitinib, and filgotinib. Patients were included regardless of prior exposure to b/tsDMARDs. Exclusion criteria were as follows: (1) absence of follow-up data after JAKi initiation, (2) JAKi prescription for indications other than RA, or (3) missing key baseline variables required for propensity score estimation. The date of JAKi initiation was defined as baseline (month 0). Patients were followed until JAKi discontinuation, death, loss to follow-up, or 24 months after baseline, whichever occurred first. Remission was defined using the ACR/EULAR index-based Clinical Disease Activity Index (CDAI) remission definition.

Data collection

Baseline variables included age, sex, disease duration, RF and ACPA status and titres, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), 28-joint tender and swollen joint counts, patient and physician global assessments (0–100 mm visual analogue scale), CDAI, estimated glomerular filtration rate (eGFR), and routine haematologic parameters. Radiographic and functional status were assessed using the Steinbrocker stage, the Steinbrocker functional class, and the Health Assessment Questionnaire Disability Index (HAQ-DI), rather than the van der Heijde-modified total Sharp score. Concomitant medications at baseline included csDMARDs (methotrexate [MTX], sulfasalazine, iguratimod, bucillamine, and tacrolimus), oral GCs (prednisolone-equivalent dose, mg/d), and the index JAKi agent. During follow-up, JAKi continuation or discontinuation (including date and primary reason) and oral GC dose were recorded at baseline and at months 1, 3, 6, 9, 12, 15, 18, 21, and 24.

Definition of renal function status

Baseline renal function was assessed using eGFR at JAKi initiation (month 0). Reduced renal function was defined as an eGFR <60 mL/min/1.73 m² documented at 2 consecutive time points (month 0 and month 3), whereas preserved renal function was defined as an eGFR ≥60 mL/min/1.73 m² at baseline. This approach was adopted to minimise misclassification due to transient or acute changes in renal function. For descriptive purposes, renal function was further categorised according to

Kidney Disease: Improving Global Outcomes G stages (G1–G5) based on baseline eGFR [13]. Because renal function was assessed over a limited time frame, this subgroup is referred to as having reduced renal function rather than confirmed chronic kidney disease.

Definition of JAKi dose groups

At baseline, patients were categorised according to the prescribed daily dose of JAKi. In patients with preserved renal function, initial dosing was classified as either standard dose or reduced dose. The standard dose was defined as the label-recommended initial dose for RA in Japan, whereas the reduced dose was defined as any lower starting dose selected at the discretion of the treating physician. Standard-dose regimens included tofacitinib 5 mg twice daily, baricitinib 4 mg once daily, peficitinib 150 mg once daily, upadacitinib 15 mg once daily, and filgotinib 200 mg once daily. Reduced-dose regimens included tofacitinib 5 mg once daily, baricitinib 2 mg once daily, peficitinib 100 mg once daily, upadacitinib 7.5 mg once daily, and filgotinib 100 mg once daily. In patients with reduced renal function, agents without renal function-based dose restrictions (peficitinib and upadacitinib) were classified as standard dose or reduced dose using the same definitions as in patients with preserved renal function. For agents with label-recommended renal dose adjustment (tofacitinib, baricitinib, and filgotinib), the label-recommended dose based on renal function was classified as the renal-adjusted (label-recommended) dose. Although the absolute dose of renal-adjusted regimens overlapped numerically with reduced-dose regimens, they were analysed separately because the prescribing rationale differed, reflecting mandatory label-based adjustment rather than discretionary dose reduction. Analyses in patients with reduced renal function were prespecified as supportive.

Definition of poor-prognosis factors

PPFs were predefined based on current recommendations and prior literature [1,9], and included seropositivity for RF or ACPA, high disease activity (CDAI >22), elevated CRP (>1.0 mg/dL), and functional impairment (HAQ-DI ≥1.0). Each factor present at baseline was counted (range, 0–4), and patients were stratified into a low PPF burden group (0–2 PPFs) and a high PPF burden group (3–4 PPFs).

Outcomes

The primary endpoint was 24-month all-cause drug retention, defined as the time from JAKi initiation to permanent discontinuation for any reason. Patients who remained on therapy at 24 months or were lost to follow-up were censored at the last available visit.

Secondary endpoints included drug retention stratified by PPF burden, longitudinal changes in CDAI, rates of clinical remission and low disease activity according to standard CDAI cut-offs, and longitudinal oral GC dose in patients with preserved renal function. Exploratory supportive analyses were performed in patients with reduced renal function, in whom drug retention and GC dose were evaluated descriptively.

Propensity score matching

The initial dosing strategy was not randomised and may have reflected clinical caution and treatment context. Therefore, we

used propensity score matching to mitigate measured confounding by indication. We emphasise that propensity score methods cannot eliminate residual confounding due to unmeasured factors; rather, they are intended to improve comparability on measured covariates. We also acknowledge that matching may reduce sample size and cohort variability in exchange for improved balance.

To address confounding by indication, propensity scores were estimated using logistic regression with treatment group (standard dose vs reduced dose) as the dependent variable. Covariates included age, sex, disease duration, RF/ACPA positivity, baseline CDAI, prior exposure to targeted therapy, and concomitant MTX and GC use. One-to-one nearest-neighbour matching without replacement was performed using a calliper width of 0.2 of the SD of the logit of the propensity score. Covariate balance was assessed using standardised mean differences (SMDs), with values <0.1 indicating acceptable balance. [Tables 1 and 2](#) present baseline characteristics before and after matching together with SMDs to show the degree of balance achieved in observed covariates; however, these measures do not exclude residual confounding from unmeasured factors. Propensity score matching was conducted in the overall cohort and repeated within PPF strata when feasible. To assess the representativeness of the propensity score–matched cohort, baseline characteristics were also compared between matched and unmatched patients. Before matching, the unmatched cohort showed a lower prevalence of ACPA positivity and a lower PPF burden, whereas age, sex, baseline disease activity, renal function, and concomitant MTX and GC use were broadly comparable between groups ([Supplementary Table S1](#)). In patients with reduced renal function, propensity score matching was not performed because of limited sample size and event counts, which could result in unstable models and poor overlap; analyses in this subgroup were therefore interpreted as supportive ([Supplementary Table S2](#)). Because the proportion of missing data for baseline covariates was low, analyses were conducted using a complete-case approach, and patients with missing values required for propensity score estimation were excluded from the matched analyses.

Statistical analysis

Continuous variables are presented as mean $\pm$ SD or median (IQR), and categorical variables as number (percentage). Between-group comparisons were performed using the Mann-Whitney U test, χ^2 test, or Fisher's exact test, as appropriate. Drug retention was evaluated using Kaplan-Meier curves and compared using the log-rank test. Hazard ratios (HRs) with 95% CIs were estimated using Cox proportional hazards models. In matched cohorts, Cox models were fitted with robust (sandwich) SEs clustered by matched pair. The proportional hazards assumption was assessed using log-minus-log plots and time-dependent covariates. Prespecified subgroup analyses were considered exploratory. A 2-sided *P* value of <.05 was considered statistically significant. All analyses were performed using JMP Pro version 18 (SAS Institute) and GraphPad Prism version 10 (GraphPad Software).

Ethics approval

The representative facility for this registry was Kindai University School of Medicine (approval no. 2023-053), Kyoto University (granted on 2016-03-24/approval No. R053), Osaka University (granted on 2015-11-04/approval no. 15300), Osaka Metropolitan University (granted on 2021-6-09/approval no.

2021-074), Osaka Medical and Pharmaceutical University (granted on 2014-07-14/approval no. 1529), Kansai Medical University (granted on 2017-11-21/approval no. 2014625), Kobe University (granted on 2015-03-20/approval no. 1738), Nara Medical University (granted on 2018-01-23/approval no. 1692), and Osaka Red Cross Hospital (granted on 2015-09-1401/approval no. 644). This observational study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committees of all participating institutions. The ethics committee at Kindai University School of Medicine waived the requirement for written informed consent, with study information disclosed via an opt-out procedure on the institutional website.

Patient and public involvement

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

RESULTS

Baseline characteristics before and after propensity score matching

Before propensity score matching, some clinically relevant differences were observed between patients initiating standard-dose and reduced-dose JAKi ([Table 1](#)). In the overall cohort, patients receiving reduced-dose therapy were older and had longer disease duration, higher inflammatory markers, worse patient-reported outcomes, and greater functional impairment than those receiving standard-dose therapy, suggesting potential confounding by indication. These differences were particularly evident among patients with a high PPF burden (PPF 3–4), in whom reduced-dose initiation was associated with higher CRP and ESR levels, higher CDAI, worse HAQ-DI, and more frequent concomitant GC use.

After 1:1 propensity score matching in patients with preserved renal function, baseline characteristics were generally comparable between the standard-dose and reduced-dose groups in the overall cohort and within each PPF stratum ([Table 2](#)). SMDs for most demographic, clinical, laboratory, and treatment-related variables were reduced to <0.1, indicating acceptable covariate balance. Disease activity measures (CDAI, joint counts, CRP, and ESR), functional status (HAQ-DI), renal function, and concomitant therapies were closely aligned after matching. Balance improved for key clinical covariates included in the propensity model; however, some residual imbalances remained, including the distribution of the index JAKi. Overall, the balance achieved supported the validity of subsequent comparative analyses of drug retention and clinical outcomes between dosing strategies. Additional descriptive subgroup analyses according to baseline concomitant MTX and GC use are presented in [Supplementary Tables S3 and S4](#). Compared with patients not receiving MTX, those receiving MTX at baseline were younger, had higher body mass index (BMI) and eGFR, and were less likely to receive concomitant GC. Conversely, compared with patients not receiving GC, those receiving GC at baseline had lower BMI and eGFR, lower MTX use, and a lower proportion of standard-dose initiation. These findings support the presence of clinically distinct treatment-initiation profiles according to concomitant MTX and GC use in routine practice and suggest that baseline concomitant therapy may also have contributed to differences in the selection of standard-dose vs reduced-dose initiation.

Table 1

Baseline characteristics of patients with rheumatoid arthritis initiating JAKi, stratified by PPF burden and initial dose (standard vs reduced), before propensity score matching

JAKi Number (N)	All patients			PPF 0-2			PPF 3-4		
	Standard dose N = 891	Reduced dose N = 244	SMD	Standard dose N = 719	Reduced dose N = 190	SMD	Standard dose N = 172	Reduced dose N = 54	SMD
Age (y)	63.8 ± 14.1	65.6 ± 14.5	0.1	63.1 ± 14.3	64.4 ± 14.5	0.1	66.9 ± 13.1	70.8 ± 13.6	0.3
Female (%)	83.6	85.3	0.1	83.0	86.8	0.0	86.1	78.7	0.2
Body mass index (kg/m ²)	23.7 ± 4.2	22.6 ± 3.5	0.3	23.9 ± 4.6	23.1 ± 4.8	0.2	23.5 ± 4.3	22.9 ± 3.9	0.1
Disease duration (mo)	146.1 (51.3-275.1)	171.8 (66.4-353.2)	0.2	164.1 (57.3-282.1)	178.8 (76.4-343.2)	0.2	156.3 (55.2-311.2)	159.8 (61.2-313.4)	0.0
RF positive (%)	77.8	78.1	0.0	71.9	72.7	0.0	94.4	93.0	0.0
RF titres (IU/mL)	73.0 (17.7-236.5)	61.0 (17.7-219.7)	0.1	52.8 (12.3-190.0)	39.0 (13.2-162.5)	0.1	129.7 (48.0-335.7)	191.5 (65.6-373.0)	0.2
ACPA positive (%)	67.9	76.9	0.2	60.8	68.8	0.2	85.0	90.0	0.1
ACPA titres (U/mL)	28.6 (0.7-228.5)	80.1 (10.4-196.0)	0.1	21.4 (0.5-171.5)	76.7 (2.3-197.0)	0.1	59.0 (11.4-301.4)	96.6 (34.7-335.2)	0.2
CRP (mg/dL)	0.3 (0.1-1.3)	0.5 (0.1-2.2)	0.3	0.2 (0.1-0.8)	0.3 (0.1-1.3)	0.3	1.5 (0.7-2.9)	2.7 (1.1-5.4)	0.7
ESR (mm/h)	26.0 (12.0-49.0)	31.0 (14.0-58.3)	0.2	21.0 (9.9-41.0)	25.0 (11.0-48.0)	0.2	53.0 (32.0-86.0)	64.0 (39.1-85.0)	0.2
Tender joints, range 0-28	2.0 (1.0-7.0)	3.0 (1.0-6.0)	0.0	2.0 (0.0-5.0)	2.0 (1.0-4.0)	0.0	3.0 (1.0-6.0)	2.0 (1.0-6.0)	0.1
Swollen joints, range 0-28	2.0 (0.0-5.0)	2.0 (0.0-5.0)	0.0	2.0 (0.0-4.0)	1.5 (0.0-4.0)	0.0	5.0 (2.0-9.0)	4.0 (2.0-8.0)	0.1
Patient VAS, 0-100 mm	30.0 (16.0-53.0)	36.0 (18.0-57.0)	0.1	28.0 (14.0-49.0)	32.0 (15.0-49.0)	0.1	49.0 (28.0-70.0)	57.0 (35.0-80.0)	0.3
Physician VAS, 0-100 mm	51.0 (26.0-72.0)	51.0 (29.0-72.0)	0.0	46.0 (21.0-67.8)	49.5 (23.3-67.0)	0.0	66.0 (50.0-84.0)	68.0 (50.0-83.0)	0.0
CDAI	14.7 (9.1-22.7)	15.1 (9.0-23.1)	0.0	12.2 (7.7-18.1)	12.7 (8.0-18.4)	0.0	25.0 (16.6-32.7)	23.7 (15.3-32.7)	0.1
eGFR (mL/min/1.73 m ²)	81.6 ± 19.3	81.1 ± 16.7	0.0	81.1 ± 19.5	80.9 ± 16.3	0.0	83.9 ± 18.6	81.7 ± 18.4	0.1
WBC, /μL	7.4 (5.3-10.9)	7.8 (5.7-11.6)	0.1	6.9 (5.0-10.1)	7.4 (5.5-11.0)	0.1	9.2 (7.1-18.8)	9.6 (6.6-12.3)	0.4
Lymphocyte (%)	22.0 (12.2-30.0)	18.2 (8.0-28.0)	0.2	22.8 (12.0-30.5)	19.5 (8.0-28.6)	0.0	21.0 (13.6-26.7)	13.1 (7.1-22.0)	0.6
Hb (g/dL)	12.4 ± 1.6	12.0 ± 1.5	0.3	12.6 ± 1.6	12.2 ± 1.4	0.2	11.9 ± 1.5	11.4 ± 1.4	0.3
Plt (× 10 ⁴ /μL)	30.4 (17.1-38.5)	31.0 (16.0-51.0)	0.2	28.7 (20.9-44.5)	34.5 (25.6-47.0)	0.3	39.5 (28.5-55.3)	45.1 (29.0-59.0)	0.1
Naive/2nd/3rd/others (%)	20.8/24.2/19.8/35.2	30.2/27.3/15.3/27.3	0.3	19.0/24.9/21.0/35.0	28.7/25.6/16.9/28.7	0.3	27.9/20.9/15.1/36.0	36.2/34.0/8.5/21.3	0.4
JAKi: TOF/BAR/PEF/UPA/FIL (%)	16.6/43.5/1.6/24.7/13.6	20.5/24.2/34.4/10.2/10.7	0.9	15.9/44.9/1.8/25.5/12.0	20.3/24.4/35.5/11.2/8.6	0.9	19.8/37.8/0.6/21.5/20.3	21.2/23.4/29.8/6.4/19.2	0.9
MTX use (%) and dose (mg/wk)	59.3, 6.0 (0.0-8.0)	54.9, 4.0 (0.0-8.0)	0.1/0.3	59.1, 0.0 (0.0-3.0)	55.8, 0.0 (0.0-5.0)	0.3	59.9, 6.0 (0.0-10.0)	51.2, 4.0 (0.0-10.0)	0.2/0.1
SASP/IGU/BUC/TAC (%)	22.7/16.8/4.6/10.2	18.0/24.2/7.4/13.9	0.2	23.0/16.8/4.3/9.3	16.2/22.8/7.6/14.7	0.3	21.5/16.9/5.8/7.0	25.5/29.8/6.4/10.6	0.3
GC use (%) and dose (mg/d)	35.3, 0.0 (0.0-3.0)	40.0, 0.0 (0.0-5.0)	0.1/0.0	35.3, 0.0 (0.0-4.0)	37.8, 0.0 (0.0-6.0)	0.4	35.1, 0.0 (0.0-4.0)	48.9, 0.0 (0.0-6.0)	0.3/0.2
Steinbrocker stage I/II/III/IV (%)	29.7/22.6/19.8/28.0	37.5/21.2/14.7/26.6	0.2	31.1/23.9/20.4/24.6	39.7/19.9/16.4/24.0	0.3	25.3/18.7/18.0/38.0	29.0/26.3/7.9/36.8	0.3
Steinbrocker class 1/2/3/4 (%)	28.8/49.7/17.9/3.6	29.7/42.2/22.7/5.4	0.2	32.5/46.6/17.2/3.7	32.2/42.0/19.5/5.4	0.1	17.5/59.1/20.1/3.4	19.4/38.9/36.1/5.6	0.4
HAQ-DI, range 0-3	0.9 (0.4-1.5)	0.8 (0.3-1.3)	0.2	0.6 (0.1-1.1)	0.8 (0.4-1.1)	0.3	1.3 (0.9-2.0)	1.6 (0.9-2.3)	0.2

ACPA, anticitrullinated protein antibody (anti-CCP antibody); ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAR, baricitinib; BUC, bucillamine; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; FIL, filgotinib; GC, glucocorticoid; Hb, haemoglobin; HAQ-DI, Health Assessment Questionnaire Disability Index; IGU, iguratimod; JAKi, Janus kinase inhibitor; MTX, methotrexate; PEF, peficitinib; Plt, platelet; PPF, poor prognostic factor; RF, rheumatoid factor; SASP, salazosulfapyridine; TAC, tacrolimus; TOF, tofacitinib; UPA, upadacitinib; VAS, visual analogue scale; WBC, white blood cell. Values are presented as median (25th-75th percentiles) or mean (SD), unless otherwise indicated. Standardised mean differences (SMDs) <0.1 are provided to describe between-group differences. SMDs for non-normally distributed variables were calculated using available summary statistics and should be interpreted as approximate measures of balance.

Table 2

Baseline characteristics after propensity score matching, stratified by PPF burden and initial JAKi dose (standard vs reduced)

JAKi Number (N)	All patients			PP = 0-2			PPF 3-4		
	Standard dose N = 178	Reduced dose N = 178	SMD	Standard dose N = 124	Reduced dose N = 124	SMD	Standard dose N = 54	Reduced dose N = 54	SMD
Age (y)	65.1 ± 14.6	65.1 ± 15.4	0.0	63.1 ± 14.9	63.7 ± 15.5	0.0	70.3 ± 11.4	69.5 ± 13.1	0.1
Female (%)	86.2	84.5	0.0	88.1	85.1	0.0	84.1	84.2	0.0
Body mass index (kg/m ²)	23.5 ± 4.1	22.4 ± 3.7	0.2	23.6 ± 3.9	23.2 ± 4.2	0.1	23.1 ± 4.2	22.6 ± 4.7	0.1
Disease duration (mo)	166.1 (81.4-315.3)	191.8 (86.4-391.2)	0.1	164.1 (57.3-282.1)	178.8 (76.4-343.2)	0.1	166.3 (58.2-303.2)	189.8 (62.1-321.4)	0.1
RF positive (%)	79.0	78.4	0.1	70.9	70.0	0.1	83.0	81.7	0.1
RF titres (IU/mL)	58.4 (14.5-263.3)	62.5 (17.4-234.0)	0.0	54.7 (11.8-196.5)	59.0 (13.0-212.8)	0.2	151.0 (50.1-607.5)	191.5 (63.8-397.0)	0.1
ACPA positive (%)	75.0	81.0	0.1	70.0	72.7	0.2	85.7	90.0	0.1
ACPA titres (U/mL)	82.6 (3.0-409.5)	82.0 (18.1-201.0)	0.0	82.6 (5.9-199.5)	82.0 (3.0-211.0)	0.2	59.0 (11.4-301.4)	96.6 (34.7-335.2)	0.2
CRP (mg/dL)	0.4 (0.1-1.6)	0.5 (0.1-2.2)	0.0	0.2 (0.1-0.7)	0.5 (0.1-0.8)	0.1	2.0 (0.9-3.7)	2.6 (1.1-5.3)	0.2
ESR (mm/h)	28.0 (12.0-50.8)	29.5 (13.0-57.3)	0.0	20.0 (7.8-40.3)	22.0 (9.8-45.0)	0.0	47.0 (33.8-72.8)	59.6 (37.5-83.8)	0.2
Tender joints, range 0-28	2.0 (1.0-8.0)	3.0 (1.0-6.0)	0.0	2.0 (1.0-5.0)	2.0 (1.0-4.0)	0.0	7.0 (4.0-16.0)	6.0 (3.0-11.8)	0.1
Swollen joints, range 0-28	2.0 (0.5-5.0)	2.0 (0.0-4.5)	0.0	2.0 (0.0-4.0)	1.5 (0.0-4.0)	0.2	5.0 (2.3-10.0)	4.0 (2.0-8.0)	0.2
Patient VAS, 0-100 mm	50.0 (29.0-72.0)	51.0 (29.0-72.0)	0.0	28.0 (14.0-49.0)	32.0 (15.0-49.0)	0.0	76.5 (57.3-85.0)	70.0 (51.3-86.0)	0.3
Physician VAS, 0-100 mm	30.0 (18.0-50.0)	36.0 (17.0-57.0)	0.2	28.5 (15.0-47.3)	32.0 (14.8-48.3)	0.2	53.0 (27.0-77.3)	57.0 (35.0-80.8)	0.1
CDAI	14.5 (9.7-23.3)	15.0 (8.9-22.9)	0.0	12.3 (8.8-18.3)	12.3 (7.9-18.0)	0.0	23.8 (16.7-33.3)	27.5 (21.1-34.0)	0.3
eGFR (mL/min/1.73 m ²)	81.5 ± 21.7	80.5 ± 16.7	0.1	79.0 ± 15.3	79.6 ± 15.7	0.1	85.4 ± 21.3	81.8 ± 18.9	0.2
WBC, /μL	7.6 (5.9-11.6)	7.8 (5.8-18.0)	0.0	6.6 (5.0-9.3)	7.5 (5.7-8.3)	0.0	9.2 (7.6-12.5)	9.6 (6.6-12.5)	0.0
Lymphocyte (%)	24.9 (15.2-31.3)	19.9 (11.1-29.4)	0.4	25.1 (16.4-33.2)	22.2 (15.1-29.9)	0.2	19.9 (11.7-27.6)	18.1 (10.9-22.2)	0.2
Hb (g/dL)	12.5 ± 1.5	12.1 ± 1.5	0.3	12.5 ± 1.5	12.3 ± 1.4	0.1	12.2 ± 1.4	11.4 ± 1.4	0.2
Plt (× 10 ⁴ /μL)	30.3 (17.8-38.5)	39.2 (23.5-48.5)	0.5	30.6 (18.3-39.5)	36.5 (22.6-47.3)	0.4	36.4 (23.6-48.8)	45.4 (29.2-62.5)	0.4
Naive/2nd/3rd/others (%)	30.9/29.8/17.7/21.5	32.6/29.8/16.0/21.5	0.1	29.1/27.6/21.6/21.6	31.3/28.4/18.7/21.6	0.1	36.4/15.9/18.2/29.5	34.1/36.4/6.8/27.7	0.2
JAKi: TOF/BAR/PEF/UPA/FIL (%)	13.8/49.2/1.7/22.1/13.3	20.4/20.4/37.6/10.5/11.1	1.0	14.9/44.8/2.2/26.9/11.2	20.0/18.7/39.6/12.7/8.2	1.0	29.6/38.6/0.0/15.9/15.9	22.7/20.5/31.8/6.8/20.5	0.9
MTX use (%) and dose (mg/wk)	56.9, 4.0 (0.0-10.0)	56.4, 6.0 (0.0-8.0)	0.0/0.1	59.7, 4.0 (0.0-10.0)	58.9, 6.0 (0.0-8.0)	0.0/0.1	54.6, 5.0 (0.0-9.5)	54.6, 5.0 (0.0-10.0)	0.0/0.1
SASP/IGU/BUC/TAC (%)	24.9/21.6/7.4/7.2	18.2/26.5/15.7/13.8	0.4	21.6/18.7/4.5/6.0	16.4/26.1/9.0/14.2	0.4	18.2/13.6/9.1/9.1	25.0/25.0/6.8/11.4	0.3
GC use (%) and dose (mg/d)	26.8, 0.0 (0.0-2.0)	39.9, 0.0 (0.0-5.0)	0.3/0.3	32.6, 0.0 (0.0-2.5)	37.1, 0.0 (0.0-4.0)	0.3/0.3	31.8, 0.0 (0.0-4.0)	45.5, 0.0 (0.0-5.0)	0.3/0.2
Steinbrocker stage I/II/III/IV (%)	29.7/24.2/21.1/25.0	39.2/21.0/14.0/25.9	0.2	33.7/24.9/27.0/14.6	43.4/18.9/16.0/21.7	0.2	20.0/25.0/17.5/37.5	28.6/22.9/8.6/40.0	0.3
Steinbrocker class 1/2/3/4 (%)	32.6/47.3/17.8/2.3	31.0/45.5/20.7/2.8	0.1	34.8/48.9/14.1/2.2	33.9/47.7/16.5/1.8	0.1	18.4/57.9/21.1/2.6	21.2/42.4/30.3/6.1	0.3
HAQ-DI, range 0-3	0.8 (0.4-1.6)	0.8 (0.3-1.3)	0.0	0.5 (0.2-1.4)	0.6 (0.1-1.0)	0.0	1.6 (1.0-2.1)	1.6 (0.9-2.4)	0.0

ACPA, anticitrullinated protein antibody (anti-CCP antibody); BAR, baricitinib; b/tsDMARD, biologic/targeted synthetic disease-modifying antirheumatic drug; BUC, bucillamine; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; FIL, filgotinib; GC, glucocorticoid; HAQ-DI, Health Assessment Questionnaire Disability Index; Hb, haemoglobin; IGU, iguratimod; JAKi, Janus kinase inhibitor; MTX, methotrexate; PEF, peficitinib; Plt, platelet; PPF, poor prognostic factor; RF, rheumatoid factor; SASP, salazosulfapyridine; SMD, standardised mean difference; TAC, tacrolimus; TOF, tofacitinib; UPA, upadacitinib; VAS, visual analogue scale; WBC, white blood cell.

Values are presented as mean ± SD, median (IQR), or percentage, as appropriate. MTX use was defined at baseline at JAK inhibitor initiation.

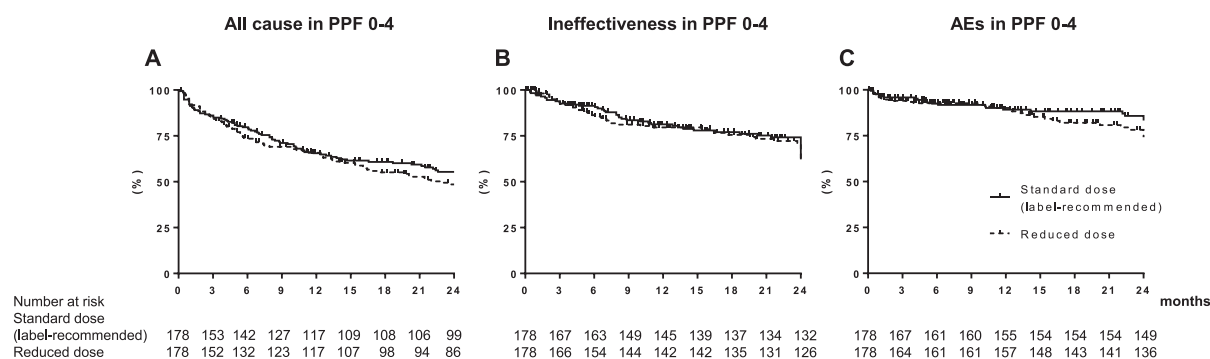


Figure 1. All-cause drug retention by initial Janus kinase inhibitor (JAKi) dose in patients with preserved renal function. Kaplan-Meier curves for 24-month all-cause drug retention comparing standard dose (label-recommended) vs reduced-dose JAKi. Drug retention was defined as the time from JAKi initiation to permanent discontinuation for any reason. Patients remaining on treatment at 24 months were censored at 24 months, and patients lost to follow-up were censored at the last available visit. *P* values were calculated using the log-rank test. HRs with 95% CIs were estimated using Cox proportional hazards models. AE, adverse event; PPF, poor prognostic factor.

Drug retention (primary endpoint)

In the propensity score–matched cohort of patients with preserved renal function, Kaplan-Meier curves for 24-month all-cause drug retention were similar between standard-dose and reduced-dose JAKi (Fig 1A). In Cox proportional hazards models, no significant difference in all-cause discontinuation was observed between dosing strategies. Cause-specific analyses similarly showed no significant differences in discontinuation due to ineffectiveness (Fig 1B) or adverse events (Fig 1C). Discontinuation reasons were classified according to the primary reason recorded by the treating physician and treated as mutually exclusive categories; other reasons (eg, relocation, remission, or patient preference) were included only in the overall all-cause retention analysis. To facilitate interpretation of the role of matching, we additionally compared retention outcomes between the unmatched (crude) cohort and the propensity score–matched cohort in patients with preserved renal function (Supplementary Table S5). Both analyses yielded the same qualitative conclusion, with no statistically

significant difference between reduced-dose and standard-dose initiation. Visual inspection of log-minus-log plots suggested no substantial violation of the proportional hazard's assumption for the primary comparisons.

When analyses were stratified by PPF burden (Fig 2), distinct patterns emerged. Among patients with 0 to 2 PPFs, Kaplan-Meier curves for standard-dose and reduced-dose JAKi were nearly superimposable for all-cause, ineffectiveness-related, and adverse event-related discontinuation (Fig 2A–C), with no statistically significant differences between dosing strategies.

Among patients with 3 to 4 PPFs, overall drug retention remained comparable between standard-dose and reduced-dose regimens (Fig 2D). However, cause-specific analyses demonstrated a lower risk of discontinuation due to ineffectiveness (HR, 0.43; 95% CI, 0.19–0.96; *P* = .03) and adverse events (HR, 0.83; 95% CI, 0.60–0.99; *P* = .04) with standard-dose initiation compared with reduced-dose JAKi (Fig 2E,F). Given the limited number of matched pairs and wide CIs, these findings were interpreted as exploratory.

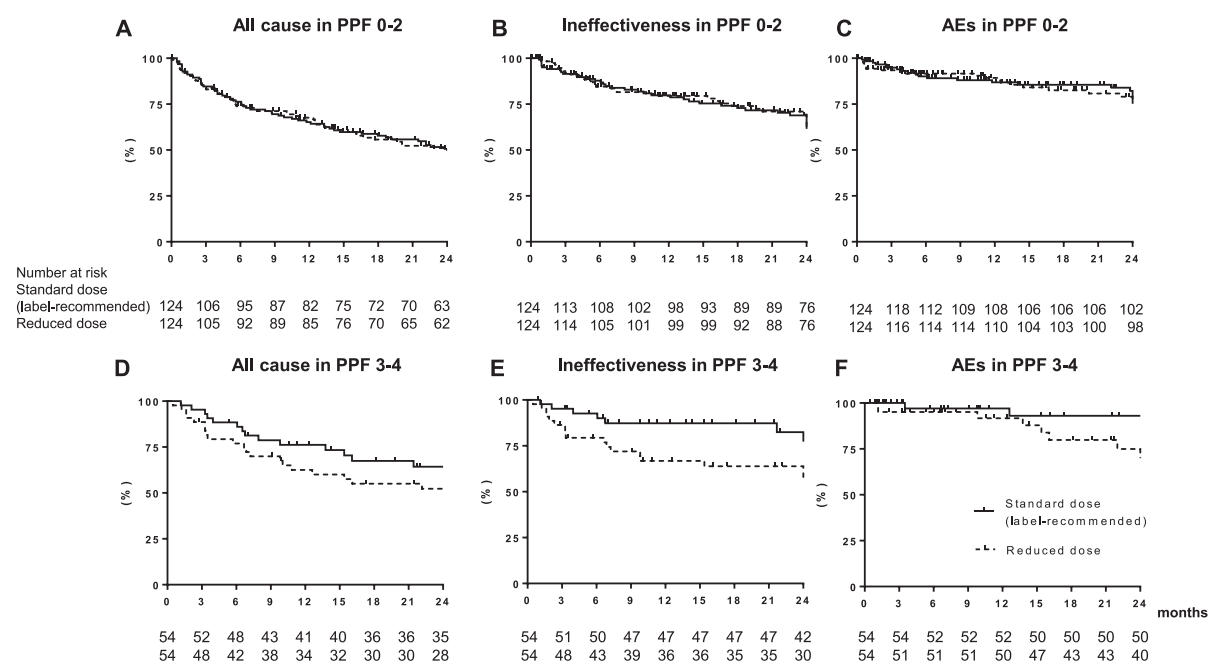


Figure 2. Drug retention by initial Janus kinase inhibitor (JAKi) dose stratified by poor prognostic factor (PPF) burden. Kaplan-Meier curves for 24-month (A,D) all-cause, (B,E) ineffectiveness-free, and (C,F) adverse event (AE)-free drug retention comparing standard dose (label-recommended) vs reduced-dose JAKi in patients with preserved renal function. Panels show patients with 0 to 2 PPFs (A–C) and 3 to 4 PPFs (D–F). Censoring, statistical tests, and HR estimation were performed as described in Figure 1. HR, hazard ratio; PPF, poor prognostic factor.

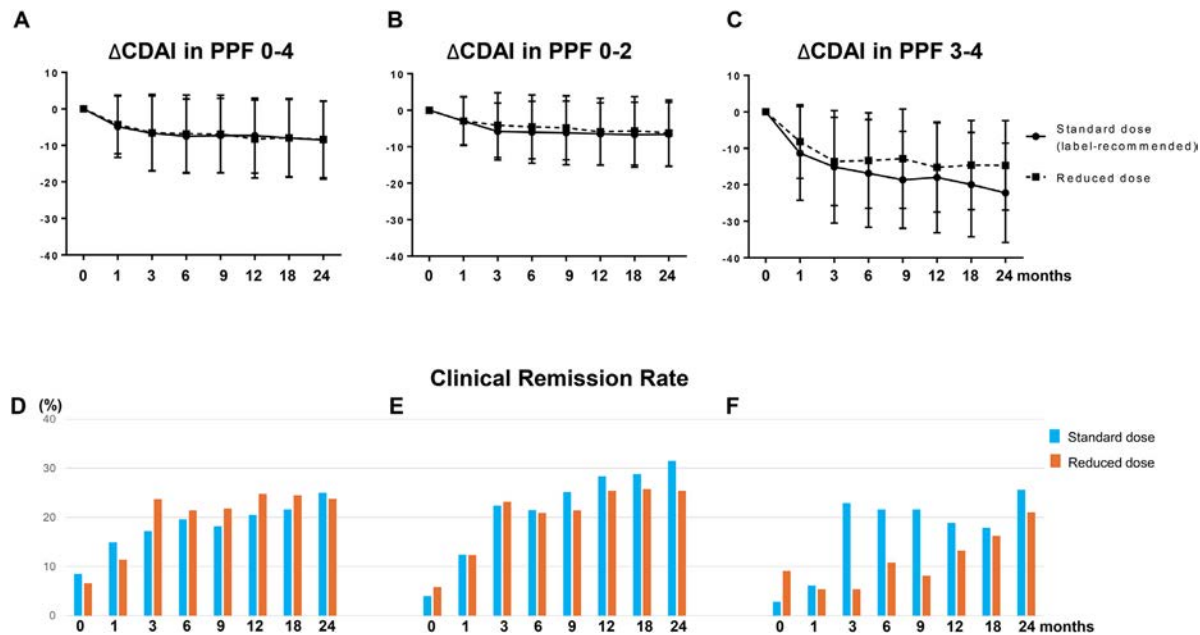


Figure 3. Longitudinal Clinical Disease Activity Index (CDAI) and remission rates by initial Janus kinase inhibitor (JAKi) dose. Mean CDAI over time (baseline and months 1, 3, 6, 9, 12, 15, 18, 21, and 24) in (A) all patients, (B) patients with 0 to 2 PPFs, and (C) patients with 3 to 4 PPFs, comparing standard dose (label-recommended) vs reduced-dose JAKi in patients with preserved renal function. Clinical remission rates at the same time points are shown in (D) all patients, (E) 0 to 2 PPFs, and (F) 3 to 4 PPFs. Remission was defined according to the CDAI remission cut-off. PPF, poor prognostic factor.

Disease activity

In the matched cohort with preserved renal function, both dosing strategies were associated with rapid improvement in CDAI after JAKi initiation, followed by sustained disease control over 24 months (Fig 3A). Overall, CDAI trajectories were similar between standard-dose and reduced-dose JAKi. In patients with 0 to 2 PPFs, CDAI decreased promptly and remained low throughout follow-up in both dosing groups, with comparable remission rates at all time points (Fig 3B,E). In contrast, patients with 3 to 4 PPFs showed more heterogeneous responses. Although CDAI trajectories largely overlapped, standard-dose JAKi showed a tendency toward more sustained disease control and numerically higher remission rates during the early to intermediate treatment phase (3-12 months) (Fig 3C,F).

GC use

Longitudinal oral GC exposure remained low and stable over 24 months in the matched cohort with preserved renal function (Table 3). No evidence of compensatory GC escalation was observed in the reduced-dose group. Similar patterns were

observed across PPF strata. Although baseline GC doses were slightly higher in patients with 3 to 4 PPFs, standard-dose JAKi tended to be associated with numerically lower GC exposure throughout follow-up.

Supportive analyses in patients with reduced renal function

In patients with reduced renal function, 24-month drug retention differed according to the initial JAKi dosing strategy (Supplementary Figs S1 and S2). Overall, standard-dose JAKi was associated with higher all-cause and ineffectiveness-free drug retention compared with reduced-dose and renal-adjusted (label-recommended) dosing, whereas adverse event-related discontinuation did not differ substantially across strategies.

In the overall reduced renal function cohort (PPF 0-4), estimated 24-month all-cause retention was highest with standard dosing and significantly lower with both reduced and renal-adjusted dosing. Relative to standard dosing, Cox models demonstrated a higher risk of discontinuation with reduced and renal-adjusted dosing, while no significant difference was observed between the 2 nonstandard strategies. Ineffectiveness-free retention similarly favoured standard dosing, with reduced

Table 3
Longitudinal glucocorticoid dose after propensity score matching, stratified by PPF burden and initial Janus kinase inhibitor dose (standard vs reduced)

GC dose (mg/d)		Baseline	1 mo	3 mo	6 mo	9 mo	12 mo	18 mo	24 mo
All patients	Standard dose	1.4 ± 2.9	1.4 ± 2.9	1.4 ± 2.6	1.4 ± 2.7	1.3 ± 2.5	1.3 ± 2.7	1.3 ± 2.5	1.3 ± 2.4
	Reduced dose	2.7 ± 4.4	2.5 ± 3.8	2.2 ± 3.4	2.0 ± 3.2	2.0 ± 3.1	2.0 ± 4.2	2.0 ± 3.7	2.0 ± 3.1
PPF 0-2	Standard dose	1.7 ± 3.7	1.7 ± 3.8	2.0 ± 7.0	1.5 ± 3.5	1.7 ± 4.1	1.5 ± 3.8	1.7 ± 3.9	1.6 ± 3.8
	Reduced dose	2.4 ± 4.4	2.3 ± 3.8	2.0 ± 3.0	1.9 ± 2.9	1.7 ± 2.7	1.7 ± 2.8	1.8 ± 3.0	1.9 ± 2.9
PPF 3-4	Standard dose	2.0 ± 3.4	1.6 ± 2.7	1.8 ± 3.0	1.7 ± 2.9	1.6 ± 2.7	1.6 ± 2.7	1.6 ± 2.7	1.6 ± 3.0
	Reduced dose	2.9 ± 4.1	2.8 ± 4.0	2.9 ± 4.5	2.9 ± 4.5	2.7 ± 4.0	2.7 ± 4.2	2.4 ± 3.7	2.5 ± 3.5

GC, glucocorticoid; PPF, poor prognostic factor.
Values are mean (SD), unless otherwise indicated. Baseline denotes month 0 (JAKi initiation). Values are in the propensity score-matched cohort (N = 178 pairs overall, 124 pairs for PPF 0-2 and 54 pairs for PPF 3-4). GC dose is expressed as prednisolone-equivalent mg/d.

dosing showing a significantly higher risk of discontinuation due to ineffectiveness, whereas renal-adjusted dosing did not.

Stratified analyses by PPF burden showed consistent patterns. Among patients with 0 to 2 PPFs, both reduced and renal-adjusted dosing were associated with a higher risk of all-cause discontinuation compared with standard dosing. Among patients with 3 to 4 PPFs, differences were more pronounced, with markedly lower drug retention in both nonstandard dosing groups, largely driven by ineffectiveness-related discontinuation, whereas adverse event-related discontinuation remained comparable across strategies.

Longitudinal oral GC exposure remained low and stable over 24 months regardless of dosing strategy, with no evidence of compensatory GC escalation in the reduced-dose or renal-adjusted groups ([Supplementary Table S6](#)). GC doses tended to be consistently lower in the standard-dose group, particularly among patients with a high PPF burden.

Because propensity score matching was not performed in patients with reduced renal function due to limited sample size and event counts, these findings should be interpreted as supportive and hypothesis generating rather than confirmatory.

DISCUSSION

In this multicentre real-world cohort of patients with RA initiating JAKi, we evaluated the association between reduced vs standard initial dosing and 24-month treatment persistence, with particular attention to PPF burden, renal function, and longitudinal GC use. Several clinically relevant insights emerge.

First, in patients with preserved renal function—where reduced dosing reflects discretionary real-world dose selection rather than label-mandated adjustment—overall drug retention over 24 months was comparable between reduced-dose and standard-dose initiation after propensity score matching. In parallel, disease activity improved similarly over time in both dosing strategies, and GC exposure remained low and stable during follow-up, with no clear evidence of compensatory escalation in the reduced-dose group. Because drug retention reflects a pragmatic composite of effectiveness, safety, and tolerability rather than efficacy alone, it provides a clinically meaningful endpoint for real-world evaluation of JAKi dosing strategies [14,15].

Second, PPF burden appeared to modify clinical patterns. In patients with preserved renal function and a low PPF burden (0–2 PPFs), reduced-dose and standard-dose initiation showed comparable treatment persistence and disease control, suggesting that discretionary initial dose reduction may be a reasonable strategy in this lower-risk subgroup. In contrast, among patients with a high PPF burden (3–4 PPFs), we observed a signal favouring standard-dose initiation for selected endpoints, although estimates were imprecise and should be interpreted cautiously as exploratory. These observations are consistent with the concept that patients with multiple PPFs may require sufficient initial drug exposure to achieve durable disease control and long-term treatment continuation [16,17], and they align with prior real-world findings from the same registry showing that greater cumulative PPF burden is associated with lower JAKi retention and earlier discontinuation [18].

Third, the higher inflammatory markers, worse HAQ-DI, and more frequent GC use observed in the reduced-dose group should not be interpreted as evidence that disease activity itself directly led to dose reduction. Rather, this pattern likely reflects confounding by indication, whereby patients with a higher inflammatory burden also had other characteristics associated with more cautious prescribing. Initial dosing decisions in

routine practice may be influenced by factors such as age, comorbidity burden, renal function concerns, frailty, concomitant medications (including GC), and clinician risk assessment. Because ORAL Surveillance-related safety concerns were followed by heightened regulatory and clinical risk awareness, initial dosing decisions in the post-ORAL Surveillance era may not have been fully discretionary in at-risk patients. Consistent with this possibility, descriptive pre/post analyses showed era-related differences in baseline profiles and prescribing patterns, including shifts in the distribution of the index JAKi and concomitant medication use ([Supplementary Table S7](#)). Taken together, these findings suggest that the interpretation of initial JAKi dosing strategies in routine clinical practice should consider both disease-related prognostic factors and patient-specific characteristics, including renal function. Importantly, in routine practice, the decision to initiate a JAKi at a reduced or standard dose is typically based on pretreatment safety considerations and clinical caution rather than on expectations of differential efficacy. Accordingly, the present findings should be interpreted as descriptive evidence on treatment persistence under real-world dosing decisions, not as prescriptive guidance for dose selection.

Renal function emerged as an additional modifier of treatment persistence. Although dosing strategies were broadly comparable in patients with preserved renal function, patients with reduced renal function showed distinct patterns, including differences between standard-dose, reduced-dose, and renal-adjusted (label-recommended) dosing. These observations are biologically plausible given that renal impairment can affect the clearance and systemic exposure of several JAKi [19,20]. In patients with reduced renal function, particularly those with a high PPF burden, descriptive analyses suggested heterogeneity in treatment persistence across dosing categories, including standard-dose, reduced-dose, and renal-adjusted regimens. Because these analyses were not propensity matched and may have been strongly influenced by residual confounding and label-based dose selection, they should be interpreted cautiously and viewed as hypothesis generating only. In addition, GC exposure was consistently higher in the reduced-dose group than in the standard-dose group among patients with reduced renal function ([Supplementary Table S6](#)). This pattern may help explain the apparently higher risk of adverse event-related discontinuation in the reduced-dose group and is more likely to reflect greater baseline vulnerability and treatment burden than dose-related toxicity alone.

The strengths of this study include the use of a large multicentre real-world registry, inclusion of patients with heterogeneous disease severity and comorbidity profiles, and a prespecified analytical framework incorporating PPF stratification and renal function. We also evaluated longitudinal GC exposure to provide additional context beyond retention alone.

Several limitations merit consideration. Propensity score matching was used to improve comparability between standard-dose and reduced-dose initiators in this observational setting; however, it can reduce confounding only for measured variables and cannot account for unmeasured determinants of dose selection. Accordingly, the matched analyses should be interpreted as supportive evidence to improve comparability rather than as definitive causal inference. Residual confounding may therefore remain, including factors such as frailty, comorbidity burden, infection history, clinician and patient risk preferences, or detailed cardiovascular and malignancy risk profiles. In particular, important clinical factors such as cardiovascular risk profile, malignancy risk, prior major adverse cardiovascular events, thromboembolic history, and prior malignancy were not systematically collected in the ANSWER registry and therefore could

not be incorporated into the present analysis. These factors may have influenced not only the decision to initiate JAKi at a reduced dose but also whether to initiate or discontinue JAKi therapy at all, particularly in more recent years. Analyses were based on the initial dosing strategy, and subsequent dose modifications were not modelled as time-varying exposures. Drug retention is a composite endpoint and cannot fully disentangle inefficacy from toxicity, and cause-specific discontinuation analyses may be subject to competing risks. In addition, this study was conducted exclusively in Japan, and the findings may not be fully generalisable to other populations. The Japanese population is relatively homogeneous, and the average BMI in this cohort was generally lower than that reported in many Western populations; however, BMI was comparable between the standard-dose and reduced-dose groups within the present study. Japan-specific concomitant csDMARD use (eg, iguratimod, bucillamine, and tacrolimus) may further limit generalisability to non-Japanese settings. Moreover, prescribing behaviour may have evolved after the ORAL Surveillance study and subsequent regulatory communications; therefore, reduced-dose initiation in the post-ORAL Surveillance era may not always reflect fully discretionary dose selection in higher-risk patients. Although we added descriptive pre/post analyses and concomitant MTX/GC stratifications to contextualise this issue, these analyses are non-causal and cannot disentangle regulatory-era effects from other secular changes in case-mix and clinical practice. Finally, because reduced dosing is agent- and renal function-dependent, dosing in renal impairment may reflect label-mandated adjustment rather than discretionary underdosing.

Conclusion

In patients with RA and preserved renal function, reduced-dose JAKi initiation was associated with 24-month treatment persistence comparable with standard dosing. Because reduced-dose initiation in this subgroup most plausibly reflects discretionary clinical decision-making rather than label-mandated renal dose adjustment, this subgroup was the primary inferential focus of the present study. Findings from PPF-stratified analyses and from patients with reduced renal function should be interpreted cautiously as exploratory and hypothesis generating. These findings require further validation and do not provide a basis for prescriptive dosing recommendations.

Competing interests

YN has received speaker fees from Astellas Pharma, Asahi Kasei Pharma, Eisai, Eli Lilly, AstraZeneca, and Tanabe Pharma. RW has received a research grant from AbbVie and speaker fees from AbbVie, Asahi Kasei Pharma, AstraZeneca, Chugai Pharmaceutical, Eli Lilly, and Taisho Pharmaceutical. MH has received research grants and/or speaker fees from Asahi Kasei Pharma, Astellas Pharma, AstraZeneca, Ayumi Pharmaceutical, Bristol Myers Squibb, Chugai Pharmaceutical, Eisai, Eli Lilly, Gilead Sciences Japan, Janssen Pharmaceuticals, Ono Pharmaceutical, Otsuka Pharmaceutical, Taisho Pharmaceutical, Mitsubishi Tanabe Pharma, and UCB Japan. KE has received research grants from Asahi Kasei Pharma and Teijin Pharma, and speaker fees from AbbVie, Amgen, Asahi Kasei Pharma, Astellas Pharma, Chugai Pharmaceutical, Daiichi Sankyo, Eisai, Eli Lilly, Gilead Sciences, Ono Pharmaceutical, Pfizer, Taisho Pharmaceutical, Mitsubishi Tanabe Pharma, and Teijin Pharma. YE and KE are affiliated with the Department of Sports Medical Biomechanics, Osaka University Graduate School of Medicine, which is supported by Asahi Kasei Pharma. TTA has received research grants from Boehringer Ingelheim and Chugai

Pharmaceutical, and speaker fees from Astellas Pharma, Chugai Pharmaceutical, Eisai, Eli Lilly, MSD, Otsuka Pharmaceutical, and Taisho Pharmaceutical. KM is affiliated with a department financially supported by Asahi Kasei Pharma Corp and local governments (Nagahama City and Toyooka City), and has received speaking and/or consulting fees from Asahi Kasei Pharma Corp, Chugai Pharmaceutical Co, Ltd, UCB Japan Co, Ltd, Eli Lilly, AbbVie, and Daiichi Sankyo Co, Ltd. HYM has received payments for lectures from AbbVie, Asahi Kasei, Astellas, Bristol Myers Squibb, Chugai, Eisai, Eli Lilly, Gilead Sciences, Ono, Otsuka, Pfizer, Taiho, and Taisho. All authors declare they have no competing interests.

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Contributors

YN conceptualised and designed the study, analysed the data, and drafted the manuscript. TS, JR, TI, DT, HYa, YM, RF, TTs, SM, HA, KM, AO, KE, IS, HYm, MH, RW, YW, TTA, AH, AT, MK, YE, WY, KK, and SR contributed to converting electronic medical records into datasets for analysis. YN is the guarantor and is responsible for the study. All of the authors actively participated in the data acquisition and read and approved the final manuscript.

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Patient consent for publication

Not applicable.

Ethics approval

The representative facility for this registry was Kindai University School of Medicine (approval no. 2023-053), Kyoto University (granted on 2016-03-24/approval No. R053), Osaka University (granted on 2015-11-04/approval No. 15300), Osaka Metropolitan University (granted on 2021-06-09/approval No. 2021-074), Osaka Medical and Pharmaceutical University (granted on 2014-07-14/approval No. 1529), Kansai Medical University (granted on 2017-11-21/approval No. 2014625), Kobe University (granted on 2015-03-20/approval No. 1738), Nara Medical University (granted on 2018-01-23/approval No. 1692), and Osaka Red Cross Hospital (granted on 2015-09-14/approval No. 644). This observational study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committees of all participating institutions. The

ethics committee at Kindai University School of Medicine waived the requirement for written informed consent, with study information disclosed via an opt-out procedure on the institutional website.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

The datasets used and analysed in this study are available from the corresponding author upon reasonable request.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (OpenAI) to support language editing and improve readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ard.2026.03.029](https://doi.org/10.1016/j.ard.2026.03.029).

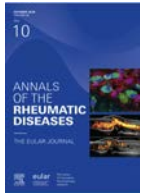
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Rheumatoid arthritis

Predictors of unacceptable pain and the impact of sociodemographic factors and comorbidities—results from a large, nationwide cohort of early rheumatoid arthritis

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ABSTRACT

Objectives: The purpose of this paper is to investigate baseline predictors of unacceptable pain, overall and in patients with low inflammatory activity, as well as of pain over time, in patients with early rheumatoid arthritis (RA).

Methods: We studied patients newly diagnosed with RA in Sweden in 2012–2020 (N = 10297), using data from several national registers. Pain was assessed by a Visual Analogue Scale (VAS; 0–100 mm). Unacceptable pain was defined as VAS pain > 40 mm, and low inflammation as C-reactive protein < 10 mg/L. Baseline predictors of unacceptable pain and unacceptable pain with low inflammation were evaluated using logistic regression analysis. Predictors of pain over time, from baseline to the 2-year follow-up, were investigated using linear mixed-effect models.

Results: Of the 3427 patients with data at 2 years, 1143 (33%) had unacceptable pain, and 808 (26%) had unacceptable pain with low inflammation. Female sex, worse patient-reported outcomes (PROs), low parameters of inflammation, and having many tender compared with swollen joints were baseline predictors of unacceptable pain and unacceptable pain with low inflammation at 1 and 2 years, and of more pain over time. Smoking, non-European origin, and having a psychiatric or pain-related comorbidity were also associated with more pain over time.

Conclusions: Factors beyond inflammation contribute significantly to unacceptable pain at follow-up in RA. Physicians should be aware of the increased risk of unacceptable pain in patients with worse PROs and with discrepancies between the number of tender and swollen joints. Sex and cultural differences also need to be considered in future pain interventions in early RA.

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WHAT IS ALREADY KNOWN ON THIS TOPIC**WHAT IS ALREADY KNOWN ON THIS TOPIC**

- Unresolved pain despite achievement of inflammatory control continues to be a major issue among patients with rheumatoid arthritis (RA).
- Unacceptable pain at long-term follow-up leads to more suffering and limitations in daily life for patients and has been associated with increased morbidity.
- Early detection of patients at risk of pain at follow-up is important for initiation of multimodal therapeutic pain interventions.

WHAT THIS STUDY ADDS

- Unacceptably high pain levels are common in RA, and occur most frequently without inflammation.
- Patient-reported outcomes (PROs) and high tender-swollen joint difference at diagnosis could be useful for predicting unacceptable pain in RA. Female sex, being born abroad, and low level of education, were also factors associated with worse pain.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Screening for patients with discrepancies in the number of tender to swollen joints, high PROs, and low parameters of inflammation at RA diagnosis could help to identify patients at risk of developing long-lasting pain, to support appropriate treatment.

INTRODUCTION

Pain in rheumatoid arthritis (RA) can not only result from active synovitis and structural joint damage but also from changes in central pain regulatory pathways [1–3]. Depression, anxiety, sleep disturbances, and psychological constructs such as pain-catastrophising are other factors that may affect the perception of pain [4–7]. Differentiation between these pain mechanisms is important for appropriate management.

Despite improved treatment options during the last decades with the achievement of inflammatory control in a growing number of patients, unresolved pain continues to be a major problem in RA [8–10]. Pain has been shown to persist also in patients in clinical remission or with (an otherwise) good response to treatment [8,11], strengthening the concept of non-inflammatory mechanisms as contributors to pain in RA. Reduction of pain is considered important by patients, with >70% of patients ranking pain reduction as their top priority [12,13].

Studying predictors of pain in new-onset RA could lead to a better understanding of the pain mechanisms in both early and later stages of the disease, potentially leading to improved and more personalised pain management.

In a previous inception study of patients with early RA from southern Sweden, included 1995–2005, before the implementation of a treat to target strategy, and before more widespread use of treatment with biologic disease modifying antirheumatic drugs (bDMARDs), lower swollen joint count at baseline was associated with unacceptable pain at 5 years, and patients with anticitrullinated peptide antibodies (ACPA) were less likely to experience noninflammatory unacceptable pain at 5 years [14]. In a subsequent study of patients with early RA, with inclusion 2012–2016, female sex and severe self-reported disability and fatigue were the only significant baseline predictors of unacceptable pain at 1 and 2 years [15]. However, in that study, the

groups of patients with unacceptable pain with low inflammation were small, resulting in limited statistical power. Indeed, most previous studies of predictors of pain in RA included smaller single-centre samples, did not adjust for patient background factors, or did not specifically investigate early RA [16–19].

The aims of this study were therefore to further evaluate predictors of unacceptable pain, overall and with a special focus on patients with low inflammatory activity, and to identify predictors of pain over time in patients with early RA.

METHODS*Study population*

Patients with a first diagnosis of RA from January 1, 2012 to March 1, 2020 with available data in the Swedish Rheumatology Quality register (SRQ), and a reported symptom duration <12 months at RA diagnosis were included in this study. The SRQ is a national clinical register of patients with rheumatic diseases based on prospectively registered information on disease activity and antirheumatic therapies entered by the treating physician at visits in standard clinical care. Overall, SRQ has a coverage of around 90% of all patients with RA who are treated actively within rheumatology [20]. The study was approved by the Swedish Ethical Review Authority (reference number 2024-04516-01).

Patient and public involvement

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

Clinical data assessment

Clinical data were retrieved from SRQ at inclusion and at follow-up visits at 3, 6, 12, and 24 months, with time windows defined in accordance with those used in the development of the LUNDEX method [21]. In addition, the time window for baseline measurements was defined as <1 month from inclusion. If there were >1 visit in a time window, the visit closest to the specific follow-up time, among visits with available data on VAS pain, was used. Data retrieved from SRQ included biologic sex, age, patients' assessment of pain, fatigue, and global disease activity (patient global assessment [PGA]) using a visual analogue scale (VAS; 0–100 mm), health-related quality of life using EQ-5D, Health Assessment Questionnaire Disability Index (HAQ-DI, the Swedish validated version [22]), disease activity score in 28 joints calculated with C-reactive protein (DAS28-CRP), swollen and tender joint counts in 28 joints (SJC28 and TJC28), erythrocyte sedimentation rate (ESR), CRP, as well as initiated treatment with conventional synthetic (cs)DMARDs, biologic (b)DMARDs, targeted synthetic (ts)DMARDs, and glucocorticoids. Serologic status (rheumatoid factor [RF] and ACPA (in Sweden, usually from the anti-CCP2 assay)) was based on the registered diagnosis (using International Classification of Diseases, 10th Edition (ICD-10) codes, specific for these serological states) in SRQ. Tender-swollen joint difference (TSJD) was calculated as TJC28 minus SJC28 [23]. 'Fibromyalgic RA' was defined as a TSJD ≥ 7 according to a previously used definition by Pollard et al [24], who found this ratio to be most strongly linked to fibromyalgia in patients with RA.

A substantial improvement/worsening in VAS pain was defined as a change of >11 mm [25].

Demographics and comorbidities

Data on education and country of birth were obtained from registers at Statistics Sweden. Comorbidity data were retrieved from the national Swedish Patient Register, covering inpatient care (since 1987) and specialised outpatient care (since 2001), and were defined based on ICD-codes. In addition, comorbidities were divided into 3 groups as follows: pain-related, psychiatric, and other relevant comorbidities ([Supplementary Table S1](#)), defined as having any of the relevant diagnoses during the 5 years preceding inclusion.

Assessment of unacceptable pain and low inflammation

Unacceptable pain was defined as VAS pain > 40 mm in accordance with the patient acceptable symptom state—a measure captured from patient reports, indicating the cut-off level of acceptable pain [26]. Low inflammation was defined as CRP < 10 mg/L [9,27]. In addition, a stricter definition of low inflammation was used: CRP < 10 mg/L and SJC28 ≤ 1 [9].

Statistical analysis

Normality distribution of data was assessed based on visual inspection of histograms and using the Shapiro–Wilk test. In all models, continuous, normally distributed variables were analysed per standard deviation to facilitate comparisons. Skewed variables were assessed per quartile, with the lowest category as the reference. Due to a suspected nonlinear effect of age, it was assessed per quintile.

Baseline predictors of unacceptable pain and unacceptable pain with low inflammation, as well as unacceptable pain with the strict definition of low inflammation, were evaluated using logistic regression analysis. The results were presented as odds ratios with 95% CI.

Furthermore, baseline predictors of pain, as a continuous measure, over the follow-up time from inclusion to 2 years, were assessed using linear mixed-model analysis, including all VAS pain values at inclusion and at follow-up visits at 3, 6, 12, and 24 months. Models including a random intercept, a random slope, and the combination of both were evaluated. Based on Akaike's information criterion, the models including a random intercept only were considered to fit the data best. Differences in pain at baseline, by baseline characteristics, were estimated as the intercept. Mean differences in pain over the follow-up time and differences in the change of pain per month by baseline characteristics were also estimated.

In the multivariable analyses of predictors of dichotomous pain outcomes and pain over time, age, sex, and baseline pain were included based on a priori decisions. In addition, variables with a *P* value < .10 in an age- and sex adjusted analysis were eligible for multivariable analyses and assessed for bivariate correlations using the Spearman rank correlation test. In case of collinearity, only the covariate with the strongest correlation with the outcome variable was included in the multivariable model. Furthermore, adjustments were made for *background factors* (education level and country of birth), as well as *smoking and comorbidities*. These 2 groups were added to the analysis separately at first, and then together.

All models were complete case analyses, with no imputation. However, patients with a missing baseline visit could still contribute with background data. Furthermore, all patients with ≥ 1 visit contributed to the mixed models. IBM SPSS Statistics version 25 was used for statistical analyses.

RESULTS

Patients

A total of 10,297 patients with early RA (median symptom duration 4 months) were included. The patients had a total of 9506 registered visits at inclusion, and 5200 and 3743 registered visits at the 1- and 2-year follow-up, respectively. A total of 2751 (74%) patients were treated with methotrexate at the 2-year follow-up, and 1124 (30%) were treated with a bDMARD. A somewhat larger fraction of the patients with 2-year follow-up data were ACPA and RF-positive compared with the total sample. Demographics were similar at all follow-up visits ([Table 1](#)). VAS pain scores were missing for 7% to 11% of the patients during the follow-up, whereas EQ-5D was missing for about 50% of the patients ([Supplementary Table S2](#)). Among those with available data at baseline, 5333 of 8540 (62.4%) had unacceptable pain and 2210 of 8180 (27.0%) had unacceptable pain with low inflammation. Out of all included patients, 101 (1.0%) had a registered diagnosis of fibromyalgia before inclusion, which had increased to 191 (1.9%) by 2 years.

Among those with available data, 68.5% had a substantial improvement in pain from inclusion to 3 months. Although average VAS pain scores were thereafter stable, 26.9% had a substantial improvement, and 29.7% worsening, between 3 and 24 months ([Supplementary Table S3](#)).

Predictors of unacceptable pain

Among those with available VAS pain, there were 1478 of 4799 (30.8%) and 1140 of 3427 (33.3%) patients with unacceptable pain at the 1- and 2-year follow-up visits, respectively. Unacceptable pain at baseline and also after 1 and 2 years was reported by 13.7% (260/1891 with available data). Baseline treatment with methotrexate and with bDMARDs were similar in patients with and without unacceptable pain at 1 and 2 years ([Supplementary Table S4](#)).

Univariate

In univariate analysis, significant positive baseline predictors of unacceptable pain at both 1 and 2 years were female sex, higher VAS pain, VAS fatigue, VAS PGA, HAQ-DI, TJC28, DAS28-CRP, and TSJD, as well as TSJD ≥ 7 ([Table 2](#)). The results were similar after adjustment for age and sex ([Supplementary Table S5](#), where details on selection for the multivariable models are given). There was a negative association with unacceptable pain at 1 and 2 years for baseline CRP and EQ-5D in the highest quartile, and age in the fourth quintile (patients aged 67–72 years) ([Table 2](#)). In addition, current smoking, being born outside of Europe, and having a pain-related, psychiatric or other relevant comorbidity were positively associated with unacceptable pain at 1 and 2 years in univariate analysis, whereas formal education of >12 years was a negative predictor of this state ([Table 3](#)).

Multivariable

In multivariable models of predictors of unacceptable pain at 1 and 2 years, including demographics and clinical characteristics, significant associations remained for female sex, VAS pain, TSJD ≥ 7, and low ESR ([Table 4](#)). In addition, age in the second quintile (patients aged 47–58) was associated with unacceptable pain at 1 and 2 years in multivariable analysis ([Table 4](#)). After adjustment for education level, country of birth, smoking status, and comorbidities, there were still significant associations for

Table 1
Patient characteristics at inclusion and at follow-up visits

Characteristic	Inclusion	3 months	6 months	12 months	24 months
N	9506	6438	4358	5200	3743
Female sex, n (%)	6387 (67.2)	4367 (67.8)	2968 (68.1)	3595 (69.1)	2583 (69.0)
Age at diagnosis*	60.0 (15.5)	59.6 (15.4)	58.7 (15.4)	58.8 (15.4)	58.2 (15.5)
RA characteristics					
Symptom duration at diagnosis* (mo)	4.8 (3.1)	4.5 (3.0)	4.7 (3.0)	4.7 (3.0)	4.6 (3.0)
Anti-CCP seropositivity*, n/N (%)	6221/9241 (67.3)	4277/6261 (68.3)	2951/4244 (69.5)	3534/5072 (69.7)	2624/3655 (71.8)
RF seropositivity*, n/N (%)	5683/9266 (61.3)	3901/6274 (62.2)	2696/4256 (63.3)	3193/5086 (62.8)	2381/3662 (65.0)
Methotrexate, n (%)	6804 (71.6)	5513 (85.6)	3565 (81.2)	4074 (78.3)	2751 (73.5)
Non-mtx csDMARD n (%)	691 (7.3)	778 (12.1)	737 (16.9)	946 (18.2)	735 (19.6)
>1 csDMARD, n (%)	167 (1.8)	336 (5.2)	318 (7.3)	469 (9.0)	339 (9.1)
Glucocorticosteroids, n (%)	5208 (54.8)	3704 (57.5)	2326 (53.4)	2306 (44.3)	1308 (34.9)
bDMARD, n (%)	369 (3.9)	709 (11.0)	809 (18.6)	1234 (23.7)	1124 (30.0)
tsDMARD, n (%)	12 (0.1)	16 (0.2)	11 (0.3)	46 (0.8)	68 (1.8)
DAS28-CRP	4.4 (1.4)	2.8 (1.2)	2.8 (1.2)	2.7 (1.2)	2.6 (1.2)
SJC28	6.3 (5.3)	1.9 (3.1)	1.7 (2.9)	1.4 (2.6)	1.3 (2.7)
TJC28	6.6 (5.8)	2.6 (4.1)	2.5 (4.0)	2.2 (3.7)	2.1 (3.8)
EQ-5D, median (IQR)	0.65 (0.26-0.76)	0.76 (0.66-0.80)	0.73 (0.66-0.80)	0.73 (0.66-0.80)	0.73 (0.66-0.80)
HAQ-DI	1.0 (0.6)	0.5 (0.5)	0.5 (0.6)	0.6 (0.6)	0.6 (0.6)
VAS pain	51.2 (26.8)	27.2 (24.9)	29.3 (25.8)	29.2 (27.7)	30.3 (26.6)
VAS PGA	49.2 (26.7)	29.1 (24.8)	30.4 (25.6)	30.0 (25.4)	30.9 (26.5)
Fatigue	47.8 (28.7)	33.5 (27.8)	34.8 (28.7)	34.7 (28.4)	34.9 (29.1)
CRP, median (IQR)	10.0 (4.0-25.0)	4.0 (1.6-8.0)	4.0 (1.3-7.0)	3.2 (1.0-6.6)	3.4 (1.0-6.1)
ESR	31.0 (23.8)	17.8 (15.7)	17.1 (15.6)	16.6 (15.5)	16.6 (15.6)
Tender-swollen joint difference	0.3 (4.8)	0.7 (3.3)	0.8 (3.5)	0.8 (3.2)	0.8 (3.1)
Tender-swollen joint difference ≥ 7 , n (%)	616 (6.7)	296 (4.8)	212 (5.0)	223 (4.5)	160 (4.6)
Smoking status*, n (%)					
Never smoker	3152/8082 (39.0)	2211/5732 (38.6)	1529/3862 (39.6)	1823/4597 (39.7)	1329/3294 (40.3)
Former smoker	3534/8082 (43.7)	2537/5732 (44.3)	1659/3862 (43.0)	1985/4597 (43.2)	1415/3294 (43.0)
Current smoker	1396/8082 (17.3)	984/5732 (17.2)	674/3862 (17.5)	789/4597 (17.2)	550/3294 (16.7)
Education level*, years, n (%)					
≤ 9	2343 (25.4)	1503 (23.9)	1006 (23.6)	1183 (23.4)	841 (23.2)
10-12	4292 (46.6)	2951 (47.0)	1979 (46.5)	2348 (46.5)	1695 (46.7)
>12	2580 (28.0)	1829 (29.1)	1274 (29.9)	1522 (30.1)	1090 (30.1)
Country of birth*, n (%)					
Sweden	8172 (86.0)	5537 (86.0)	3778 (86.7)	4459 (85.8)	3200 (85.5)
Scandinavia, not Sweden	460 (4.8)	332 (5.2)	201 (4.6)	265 (5.1)	206 (5.5)
Europe, not Scandinavia	417 (4.4)	258 (4.0)	171 (3.9)	213 (4.1)	154 (4.1)
Other	455 (4.8)	311 (4.8)	208 (4.8)	263 (5.1)	183 (4.9)
Comorbidities, n (%)					
Pain-related comorbidities*	2063 (21.7)	1416 (22.0)	964 (22.1)	1144 (22.0)	774 (20.7)
Psychiatric comorbidities*	391 (4.1)	254 (3.9)	173 (4.0)	203 (3.9)	152 (4.1)
Other comorbidities*	2398 (25.2)	1588 (24.7)	1057 (24.7)	1261 (24.3)	887 (23.7)

Anti-CCP, anti-cyclic citrullinated peptide; bDMARD, biologic DMARD; csDMARD, conventional synthetic DMARD; tsDMARD, targeted synthetic DMARD; DAS28-CRP, Disease Activity Score in 28 joints calculated with CRP; HAQ-DI, Health Assessment Questionnaire Disability Index; PGA, patient global assessment; RA, rheumatoid arthritis; SJC28, Swollen Joint Count in 28 joints; TJC28, Tender Joint Count in 28 joints; EQ-5D, EuroQol 5 dimensions; ESR, erythrocyte sedimentation rate; VAS, Visual Analogue Scale.

Variables marked with ** are baseline values. Variables are mean (SD) unless otherwise indicated.

female sex, age, VAS pain, and low ESR with unacceptable pain at 1 and 2 years ([Supplementary Tables S6 and S7](#)).

Predictors of unacceptable pain with low inflammation

Among those with available VAS pain and CRP, there were 1051 of 4647 (22.6%) and 808 of 3278 (25.6%) patients with unacceptable pain and low inflammation at 1 and 2 years, respectively. Baseline treatment with methotrexate and with bDMARDs was similar in patients with and without unacceptable pain with low inflammation at 1 and 2 years ([Supplementary Table S8](#)).

Univariate

In univariate analysis, significant associations with unacceptable pain plus low inflammation at 1 and 2 years were seen for female sex, higher VAS pain, VAS fatigue, VAS PGA, HAQ-DI, TJC28, TSJD, and TSJD ≥ 7 , and there were negative

associations for ESR, CRP (quartile 3-4 vs lower), EQ-5D in the fourth quartile, and for age in the fourth quintile ([Table 2](#)). There were also associations for current smoking, birth outside of Europe, and pain-related and psychiatric comorbidities with this pain outcome at 1 and 2 years ([Table 3](#)).

Multivariable

In multivariable analysis, including demographics and clinical characteristics, significant associations with unacceptable pain plus low inflammation at 1 and 2 years remained for female sex, higher VAS pain, TSJD ≥ 7 , and lower ESR. Furthermore, there was an association with age in the second quintile ([Table 4](#)). After further adjustment for education level, country of birth, smoking status, and comorbidities, significant associations remained for female sex, VAS pain, age in the fourth quintile and ESR with this pain state at 1 and 2 years, and for TSJD ≥ 7 at 1 year, with the same tendency seen at 2 years ([Supplementary Tables S9 and S10](#)). In addition, there was a negative

Table 2

Baseline predictors of unacceptable pain and unacceptable pain with low inflammation at 1 and 2 years; demographics and clinical characteristics; univariate analysis

Variable	Unacceptable pain		Unacceptable pain with low inflammation	
	1 y (n = 4799) OR (95% CI)	2 y (n = 3427) OR (95% CI)	1 y (n = 4647) OR (95% CI)	2 y (n = 3278) OR (95% CI)
Age quintile 1 (≤ 46 y)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Age quintile 2 (47–58 y)	1.18 (0.99–1.42)	1.10 (0.89–1.35)	<i>1.20 (0.99–1.46)</i>	1.10 (0.88–1.39)
Age quintile 3 (59–66 y)	0.89 (0.73–1.07)	0.89 (0.71–1.10)	0.75 (0.61–0.93)	<i>0.79 (0.62–1.00)</i>
Age quintile 4 (67–72 y)	0.66 (0.54–0.81)	0.78 (0.62–0.99)	0.58 (0.46–0.73)	0.66 (0.51–0.86)
Age quintile 5 (≥ 73 y)	<i>0.83 (0.68–1.01)</i>	0.95 (0.76–1.19)	0.65 (0.52–0.82)	<i>0.80 (0.62–1.03)</i>
Female sex	1.40 (1.22–1.60)	1.51 (1.28–1.77)	1.56 (1.33–1.83)	1.61 (1.34–1.92)
Symptom duration	1.03 (0.97–1.10)	<i>1.06 (0.99–1.14)</i>	1.06 (0.99–1.13)	1.05 (0.97–1.14)
Anti-CCP	<i>1.12 (0.98–1.29)</i>	0.96 (0.82–1.13)	0.91 (0.78–1.05)	0.88 (0.74–1.05)
RF	<i>1.12 (0.99–1.28)</i>	1.09 (0.93–1.26)	0.96 (0.83–1.11)	1.02 (0.86–1.20)
ESR	0.95 (0.89–1.02)	0.88 (0.81–0.95)	0.81 (0.75–0.88)	0.78 (0.72–0.86)
CRP quartile 1 (≤ 3 mg/L)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
CRP quartile 2 (4–9 mg/L)	0.93 (0.77–1.12)	0.97 (0.78–1.20)	0.75 (0.61–0.91)	<i>0.81 (0.64–1.02)</i>
CRP quartile 3 (10–24 mg/L)	0.77 (0.64–0.93)	<i>0.83 (0.67–1.03)</i>	0.53 (0.43–0.65)	0.57 (0.44–0.72)
CRP quartile 4 (≥ 25 mg/L)	0.83 (0.69–0.99)	0.70 (0.57–0.87)	0.44 (0.35–0.54)	0.47 (0.37–0.60)
SJC28	0.94 (0.88–1.00)	0.94 (0.87–1.01)	<i>0.93 (0.87–1.00)</i>	<i>0.92 (0.84–1.00)</i>
TJC28	1.14 (1.07–1.21)	1.14 (1.06–1.22)	1.19 (1.11–1.27)	1.14 (1.05–1.23)
HAQ-DI	1.34 (1.25–1.44)	1.29 (1.19–1.40)	1.29 (1.19–1.39)	1.22 (1.11–1.33)
VAS pain	1.50 (1.39–1.61)	1.39 (1.28–1.51)	1.44 (1.33–1.56)	1.31 (1.19–1.43)
VAS PGA	1.56 (1.45–1.68)	1.48 (1.35–1.59)	1.48 (1.37–1.60)	1.42 (1.29–1.55)
VAS fatigue	1.61 (1.48–1.74)	1.44 (1.31–1.57)	1.63 (1.49–1.78)	1.42 (1.28–1.58)
DAS28-CRP	1.15 (1.07–1.23)	1.12 (1.03–1.22)	<i>1.08 (1.00–1.17)</i>	1.03 (0.95–1.13)
EQ-5D quartile 1 (≤ 0.25)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
EQ-5D quartile 2 (0.26–0.65)	0.95 (0.72–1.24)	0.89 (0.64–1.24)	1.07 (0.80–1.44)	0.78 (0.54–1.14)
EQ-5D quartile 3 (0.66–0.75)	0.60 (0.46–0.78)	0.67 (0.49–0.92)	0.68 (0.51–0.92)	0.78 (0.54–1.11)
EQ-5D quartile 4 (≥ 0.76)	0.42 (0.32–0.56)	0.56 (0.40–0.77)	0.43 (0.31–0.60)	0.57 (0.40–0.82)
Low disease activity*	0.76 (0.62–0.92)	0.76 (0.61–0.95)	0.85 (0.69–1.06)	0.86 (0.66–1.10)
Tender-swollen joint difference	1.27 (1.19–1.36)	1.26 (1.17–1.36)	1.35 (1.25–1.46)	1.30 (1.19–1.41)
Tender-swollen joint difference >1	1.45 (1.26–1.66)	1.38 (1.17–1.61)	1.70 (1.46–1.98)	1.38 (1.16–1.65)
Tender-swollen joint difference ≥ 7	1.83 (1.45–2.31)	1.76 (1.34–2.32)	2.16 (1.69–2.76)	1.84 (1.37–2.47)

Anti-CCP, anti-cyclic citrullinated peptide; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; DAS28-CRP: disease activity score in 28 joints calculated with CRP; EQ-5D: EuroQol 5 dimensions; HAQ-DI, Health Assessment Questionnaire Disability Index; OR, odds ratio; PGA, patient global assessment; RF, rheumatoid factor; SJC28: swollen joint count in 28 joints; TJC28: tender joint count in 28 joints; VAS: visual analogue scale.

Standard deviations for continuous variables: symptom duration: 3.1, ESR: 23.8, SJC28: 5.3, TJC28: 5.8, HAQ-DI: 0.6, VAS pain: 26.8, VAS PGA: 26.7, VAS fatigue: 28.7, DAS28-CRP: 1.4, tender-swollen joint difference: 4.8.

* Univariate logistic regression analysis. Odds ratios are calculated per standard deviation for continuous variables. Bold text indicates statistical significance with P values $< .05$. Italic text indicates P values $\geq .05$ and $< .10$. Bold plus italic text indicates P values $< .001$. Unacceptable pain: VAS pain ≥ 40 . Low inflammation: CRP < 10 . *DAS28-CRP ≤ 3.2 .

Table 3

Baseline predictors of unacceptable pain and unacceptable pain with low inflammation at 1- and 2-year follow-up visits; patient background factors; univariate analysis

Variable	Unacceptable pain		Unacceptable pain with low inflammation	
	1 y (n = 4799) OR (95% CI)	2 y (n = 3427) OR (95% CI)	1 y (n = 4647) OR (95% CI)	2 y (n = 3278) OR (95% CI)
<i>Smoking status</i>				
Never smoker	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Former smoker	1.28 (1.10–1.48)	1.11 (0.93–1.31)	1.20 (1.02–1.41)	1.07 (0.89–1.30)
Current smoker	1.81 (1.51–2.18)	1.66 (1.34–2.06)	1.41 (1.14–1.73)	1.50 (1.18–1.90)
<i>Education level</i>				
≤ 9 y	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
10–12 y	1.06 (0.90–1.23)	0.98 (0.82–1.18)	1.22 (1.02–1.45)	1.02 (0.84–1.26)
>12 y	0.70 (0.58–0.83)	0.70 (0.57–0.86)	<i>0.83 (0.68–1.02)</i>	0.88 (0.70–1.10)
<i>Country of birth</i>				
Sweden	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Scandinavia, not Sweden	1.22 (0.93–1.60)	1.41 (1.05–1.91)	1.21 (0.89–1.64)	1.28 (0.92–1.79)
Europe, not Scandinavia	1.60 (1.16–2.10)	1.16 (0.81–1.97)	1.75 (1.27–2.41)	1.09 (0.73–1.63)
Other	1.89 (1.44–2.48)	2.45 (1.80–3.42)	1.80 (1.34–2.42)	2.31 (1.65–3.22)
<i>Comorbidities</i>				
Pain-related comorbidities	1.41 (1.22–1.62)	1.45 (1.22–1.72)	1.33 (1.13–1.56)	1.34 (1.10–1.62)
Psychiatric comorbidities	1.78 (1.32–2.39)	1.54 (1.10–2.16)	1.74 (1.27–2.38)	1.74 (1.27–2.38)
Other comorbidities	1.23 (1.06–1.41)	1.33 (1.12–1.56)	1.06 (0.91–1.25)	<i>1.19 (0.99–1.44)</i>

OR, odds ratio; VAS, Visual Analogue Scale; CRP, c-reactive protein.

Univariate logistic regression analysis. Bold text indicates statistical significance with P values $< .05$. Italic text indicates P values $\geq .05$ and $< .10$. Bold plus italic text indicates P values $< .001$. Unacceptable pain: VAS pain ≥ 40 . Low inflammation: CRP < 10 .

Table 4
Baseline predictors of unacceptable pain and unacceptable pain with low inflammation; multivariable analysis

Variable	Unacceptable pain		Unacceptable pain with low inflammation	
	1 y (n = 3780) OR (95% CI)	2 y (n = 2684) OR (95% CI)	1 y (n = 3573) OR (95% CI)	2 y (n = 2510) OR (95% CI)
Female sex	1.37 (1.16-1.61)	1.43 (1.18-1.72)	1.44 (1.19-1.75)	1.56 (1.26-1.94)
Age quintile 1 (≤ 46 y) (ref)	1.00	1.00	1.00	1.00
Age quintile 2 (47-58 y)	1.31 (1.06-1.62)	1.31 (1.03-1.68)	1.31 (1.03-1.65)	1.36 (1.04-1.78)
Age quintile 3 (59-66 y)	1.13 (0.90-1.42)	1.14 (0.88-1.47)	0.98 (0.75-1.26)	1.08 (0.81-1.45)
Age quintile 4 (67-72 y)	<i>0.79 (0.61-1.01)</i>	1.02 (0.77-1.35)	0.67 (0.50-0.89)	0.92 (0.67-1.28)
Age quintile 5 (≥ 73 y)	1.12 (0.89-1.43)	1.23 (0.94-1.62)	0.92 (0.70-1.22)	1.12 (0.82-1.52)
VAS pain	1.57 (1.45-1.70)	1.45 (1.32-1.59)	1.56 (1.42-1.71)	1.42 (1.28-1.57)
Tender-swollen joint difference ≥ 7	1.52 (1.18-1.96)	1.37 (1.01-1.85)	1.75 (1.33-2.30)	1.42 (1.01-1.98)
ESR	0.82 (0.75-0.89)	0.77 (0.70-0.85)	0.72 (0.65-0.79)	0.71 (0.63-0.79)
Anti-CCP seropositivity	NI	NI	0.86 (0.72-1.03)	0.88 (0.71-1.08)

VAS: visual analogue scale; ESR, erythrocyte sedimentation rate; Anti-CCP, anti-cyclic citrullinated peptide; CRP, c-reactive protein; NI, not included.

Models include all variables in each sub-table. Results are presented as odds ratios (95% CI). Odds ratios are calculated per SD for continuous variables. Unacceptable pain: VAS pain ≥ 40 . Low inflammation: CRP < 10 . Bold text indicates statistical significance with P values $< .05$. Italic text indicates P values $\geq .05$ and $< .10$. Bold plus italic text indicates P values $< .001$. SDs for continuous variables: ESR: 23.8, VAS pain: 26.8.

association for ACPA with unacceptable pain plus low inflammation at 1 year in this fully adjusted model (Supplementary Table S9).

Predictors of unacceptable pain with low inflammation, strict definition

At the 1- and 2-year follow-up visits, 597 of 4531 (13.2%) and 473 of 3140 (15.1%) patients, respectively, had unacceptable pain with the strict definition of low inflammation (CRP < 10 mg/L and SJC28 ≤ 1) (Supplementary Table S11).

Univariate

Univariate associations to unacceptable pain with low inflammation (strict) at 1 and 2 years were seen for female sex, lower ESR, higher VAS pain, VAS fatigue, VAS PGA, HAQ-DI, TSJD, and TSJD ≥ 7 , and inverse associations were seen for CRP, SJC28, and EQ-5D in the highest quartile. In addition, we noted positive associations for current smoking and birth outside of Europe (Supplementary Tables S12 and S13). The results were similar after adjustment for age and sex (Supplementary Table S14).

Multivariable

In multivariable analysis of this pain outcome, at 1 and 2 years, significant associations remained for female sex, VAS pain, and low ESR—also after adjustment for level of education, country of birth, smoking status, and comorbidities (Supplementary Tables S15 and S16). TSJD ≥ 7 was a significant baseline predictor of this pain state at 1 year in multivariable analysis, but did not reach statistical significance at 2 years (Supplementary Tables S15 and S16).

Predictors of pain over time

A total of 9880 patients contributed with pain data for this analysis, of which 7895 patients had pain data at ≥ 2 follow-up visits. The distribution of the number of visits with available pain data among the patients is shown in Supplementary Table S17.

Univariate

In univariate mixed-model analysis, higher baseline VAS PGA, VAS fatigue, HAQ-DI, CRP, DAS28-CRP, TJC28, SJC28, TSJD, and female sex and RF seropositivity were associated with higher pain scores over time (expressed as estimated mean differences over time), from inclusion to 2 years, whereas higher EQ-5D and older age were associated with lower pain scores over 2 years (Table 5).

Estimated mean differences in pain scores by patient-reported outcomes (PROs) and disease activity measures were more pronounced at baseline compared with the corresponding differences over time. Patients with worse PROs and disease activity measures at baseline also had greater reductions in pain over time (Table 5). There was a similar pattern for health-related quality of life, with higher EQ-5D at baseline associated with less pain reduction over time (Table 5). Furthermore, current smoking, being born outside of Europe, and pain-related and psychiatric comorbidities were associated with higher pain scores at baseline and over time, but were not associated with a reduction in pain over time (Table 6). Education level > 12 years was a predictor of lower estimated mean pain at baseline and over time (Table 6).

Multivariable

In multivariable mixed-model analysis, significant baseline predictors of higher estimated mean pain over 2 years were female sex, TSJD ≥ 7 , and higher CRP. However, after adjustment for baseline pain, higher CRP at baseline was associated with lower pain over time (Table 7). Furthermore, older age was associated with lower estimated mean pain over time, in particular, patients aged between 67 and 72 years (quintile 4) (Table 7). Significant associations with pain over time remained for female sex, TSJD ≥ 7 , age, and CRP—also after adjustment for education level, country of birth, smoking status and comorbidities (Table 8).

DISCUSSION

We performed a large nationwide cohort study on prospectively collected data on patients with early RA, investigating baseline predictors of unacceptable pain and unacceptable pain with low inflammation, as well as baseline predictors of estimated mean pain over time, during a 2-year follow-up period.

Table 5

Predictors of pain over time, from inclusion to 2 years, in patients with early RA; demographics and clinical characteristics

Variable	Estimated mean difference at baseline (95% CI)	Estimated mean difference over time (95% CI)	Difference in change/month (95% CI)
Female sex	4.81 (3.81, 5.81)	5.28 (4.43, 6.14)	0.07 (-0.01, 0.16)
Age quintile 1 (≤46 y) (ref)	1.00	1.00	1.00
Age quintile 2 (47–58 y)	-0.11 (-1.59, 1.37)	0.48 (-0.77, 1.73)	0.09 (-0.03, 0.20)
Age quintile 3 (59–66 y)	-2.61 (-4.11, -1.11)	-2.43 (-3.70, -1.17)	0.03 (-0.09, 0.14)
Age quintile 4 (67–72 y)	-5.39 (-6.94, -3.48)	-5.46 (-6.78, -4.15)	-0.01 (-0.14, 0.11)
Age quintile 5 (≥73 y)	-4.96 (-6.44, -3.48)	-4.54 (-5.80, -3.29)	0.07 (-0.06, 0.19)
Anti-CCP seropositivity	0.59 (-0.44, 1.62)	0.73 (-0.16, 1.61)	0.02 (-0.06, 0.11)
RF seropositivity	1.19 (0.20, 2.17)	1.22 (0.38, 2.06)	0 (-0.08, 0.09)
ESR	3.68 (3.18, 4.18)	1.78 (1.36, 2.21)	-0.30 (-0.35, -0.26)
CRP quartile 1 (ref) (≤3 mg/L)	1.00	1.00	1.00
CRP quartile 2 (4–9 mg/L)	2.32 (0.92, 3.71)	0.87 (-0.33, 2.08)	-0.22 (-0.33, -0.10)
CRP quartile 3 (10–24 mg/L)	6.19 (4.77, 7.61)	2.63 (1.41, 3.85)	-0.56 (-0.68, -0.45)
CRP quartile 4 (≥25 mg/L)	11.46 (10.05, 12.87)	5.72 (4.51, 6.93)	-0.91 (-1.02, -0.79)
SJC28	3.71 (3.22, 4.20)	1.78 (1.36, 2.20)	-0.31 (-0.35, -0.27)
TJC28	6.74 (6.26, 7.22)	4.86 (4.44, 5.27)	-0.30 (-0.34, -0.26)
HAQ-DI	11.56 (11.09, 12.04)	8.79 (8.39, 9.19)	-0.46 (-0.50, -0.42)
VAS PGA	15.17 (14.74, 15.60)	11.61 (11.24, 11.97)	-0.57 (-0.61, -0.53)
VAS fatigue	12.97 (12.47, 13.46)	10.46 (10.04, 10.88)	-0.41 (-0.46, -0.37)
DAS28-CRP	10.99 (10.51, 11.48)	7.55 (7.13, 7.97)	-0.57 (-0.61, -0.52)
EQ-5D quartile 1 (ref) (≤0.25)	1.00	1.00	1.00
EQ-5D quartile 2 (0.26–0.65)	-13.84 (-15.78, -11.90)	-9.59 (-11.22, -7.97)	0.67 (0.50, 0.84)
EQ-5D quartile 3 (0.66–0.75)	-23.27 (-25.10, -21.45)	-17.31 (-18.84, -15.79)	0.95 (0.79, 1.11)
EQ-5D quartile 4 (≥0.76)	-34.27 (-36.59, -33.03)	-26.13 (-27.63, -24.64)	1.40 (1.24, 1.56)
Tender-swollen joint difference	4.02 (3.54, 4.50)	3.90 (3.49, 4.31)	-0.02 (-0.06, 0.02)
Tender-swollen joint difference ≥7	10.41 (8.48, 12.34)	9.76 (8.11, 11.40)	-0.10 (-0.26, 0.06)

Anti-CCP, anti-cyclic citrullinated peptide; ESR, erythrocyte sedimentation rate; CRP, c-reactive protein; DAS28-CRP, disease activity score in 28 joints calculated with CRP; EQ-5D, EuroQol 5 dimensions; HAQ-DI, Health Assessment Questionnaire Disability Index; PGA, patient global assessment; RA, rheumatoid arthritis; SJC28, swollen joint count in 28 joints; TJC28, tender joint count in 28 joints; VAS, visual analogue scale.

Mixed model analysis using data on VAS pain at inclusion and at 3, 6, 12 and 24-month follow-up visits. Estimates for continuous variables are per standard deviation. Bold text indicates statistical significance with P values < .05. Italic text indicates P values ≥ .05 and < .10. Bold plus italic text indicates P values < .001. Standard deviations for continuous variables: symptom duration: 3.1, ESR: 23.8, SJC28: 5.3, TJC28: 5.8, HAQ-DI: 0.6, VAS pain: 26.8, VAS PGA: 26.7, VAS fatigue: 28.7, DAS28-CRP: 1.4, tender-swollen joint difference: 4.8.

Table 6

Predictors of pain over time, from inclusion to 2 years, in patients with early RA; patient background factors

Variable	Estimated mean difference at baseline (95% CI)	Estimated mean difference over time (95% CI)	Difference in change/month (95% CI)
Smoking status			
Never smoker (ref)	1.00	1.00	1.00
Former smoker	0.66 (-0.45, 1.78)	1.00 (0.06, 1.94)	0.05 (-0.04, 0.14)
Current smoker	5.96 (4.49, 7.42)	6.55 (5.31, 7.79)	0.09 (-0.03, 0.21)
Education level			
≤9 y (ref)	1.00	1.00	1.00
10–12 y	-0.30 (-1.49, 0.88)	-0.05 (-1.07, 0.96)	0.04 (-0.06, 0.14)
>12 y	-4.31 (-5.62, -3.00)	-4.27 (-5.38, -3.16)	0.01 (-0.10, 0.12)
Country of birth			
Sweden (ref)	1.00	1.00	1.00
Scandinavia, not Sweden	2.56 (0.39, 4.74)	2.78 (0.94, 4.62)	0.03 (-0.14, 0.20)
Europe, not Scandinavia	7.93 (5.54, 10.32)	7.07 (5.04, 9.10)	-0.14, (-0.33, 0.06)
Other	10.33 (8.06, 12.60)	11.40 (9.49, 13.31)	0.16 (-0.02, 0.35)
Comorbidities			
Pain-related comorbidities	4.38 (3.23, 5.52)	4.71 (3.74, 5.68)	0.05 (-0.04, 0.15)
Psychiatric comorbidities	7.10 (4.68, 9.51)	7.19 (5.13, 9.25)	0.01 (-0.18, 0.21)
Other comorbidities	0.04 (-1.05, 1.14)	0.76 (-0.17, 1.70)	0.11 (0.02, 0.21)

OR: odds ratio; RA, rheumatoid arthritis; VAS, Visual Analogue Scale.

Mixed model analysis using data on VAS pain at inclusion and at 3, 6, 12, and 24-month follow-up visits. Bold text indicates statistical significance with P values < .05. Italic text indicates P values ≥ .05 and < .10. Bold plus italic text indicates P values < .001.

Table 7
Predictors of pain over time, from inclusion to 2 years, in patients with early RA; multivariable analysis

Variable	Basic model	Adjustment for baseline pain
Female sex	5.02 (4.12, 5.92)	1.96 (1.23, 2.70)
Age quintile 1 (≤46 y) (ref)	1.00	1.00
Age quintile 2 (47–58 y)	0.53 (–0.77, 1.82)	<i>0.98 (–0.08, 2.03)</i>
Age quintile 3 (59–66 y)	–2.29 (–3.61, –0.97)	–0.14 (–1.22, 0.94)
Age quintile 4 (67–72 y)	–5.55 (–6.94, –4.16)	–2.60 (–3.73, –1.46)
Age quintile 5 (≥73 y)	–4.56 (–5.90, –3.23)	–0.81 (–1.91, 0.28)
CRP quartile 1 (ref) (≤3 mg/L)	1.00	1.00
CRP quartile 2 (4–9 mg/L)	1.89 (0.68, 3.08)	–1.21 (–2.20, –0.23)
CRP quartile 3 (10–24 mg/L)	4.20 (2.98, 5.43)	–3.11 (–4.12, –2.09)
CRP quartile 4 (≥25 mg/L)	8.00 (6.77, 9.23)	–3.94 (–4.99, –2.89)
Tender-swollen joint difference ≥7	8.99 (7.36, 10.62)	3.10 (1.77, 4.43)

RA, rheumatoid arthritis; VAS, Visual Analogue Scale; CRP, c-reactive protein. Mixed model analysis using data on VAS pain at inclusion and at 3, 6, 12, and 24-month follow-up visits. Estimated mean differences over time, with 95% CIs. Each model includes all variables in the table. Odds ratios are calculated per standard deviation for continuous variables. Bold text indicates statistical significance with *P* values < .05. Italic text indicates *P* values ≥ .05 and < .10. Bold plus italic text indicates *P* values < .001.

We found that female sex was a significant positive predictor of unacceptable pain and of more pain over time in all models. Worse PROs at baseline, as well as having many tender joints in relation to swollen joints, were associated with unacceptable pain and unacceptable pain with low inflammation at 1 and

2 years, and with higher pain scores over time, whereas there was a negative association for CRP. In addition, patients in their late 60s/early 70s (fourth quintile) had less pain over time compared with younger patients (age ≤46 years). Sociodemographic factors, ie country of birth, education level, and smoking, also had an impact on reported pain.

Chronic widespread pain (CWP) is more prevalent in women compared with men in the general population [28], and has also been shown to be more prevalent in women in an RA population [29]. It has been suggested that pain differences by sex in RA exist only in patients with lower disease activity [30]. In this study, however, we found that female sex was associated with higher pain scores already at baseline, that is, in patients with moderate/high disease activity (mean DAS28-CRP 4.4). Nonetheless, mean estimated differences in pain by sex over time were somewhat more pronounced compared with pain at baseline, which could support that differences in pain perception between men and women are more distinct in patients with lower disease activity. Although women had higher reported pain levels at diagnosis compared with men, pain scores did not improve more over time, which could suggest that the increased pain levels expressed by women compared with men are not related to inflammation, but rather from, eg, CWP or differences in pain perception.

Worse PROs at baseline were associated with unacceptable pain and unacceptable pain with low inflammation at 1 and 2 years, and with more pain over time. Associations between pain and PROs have also been found in previous studies [8,14,19]. Furthermore, we found that patients with higher CRP

Table 8
Predictors of pain over time in patients with early RA; multivariable analysis

Variable	Basic model ^a	Basic model ^a + Background factors	Basic model ^a + Smoking and morbidities	Combined ^a
Female sex	1.96 (1.23, 2.70)	2.24 (1.49, 2.99)	1.91 (1.12, 2.70)	2.17 (1.37, 2.97)
Age quintile 1 (≤46 y) (ref)	1.00	1.00	1.00	1.00
Age quintile 2 (47–58 y)	<i>0.98 (–0.08, 2.03)</i>	0.59 (–0.47, 1.66)	0.42 (–0.71, 1.54)	0.11 (–1.02, 1.25)
Age quintile 3 (59–66 y)	–0.14 (–1.22, 0.94)	–0.48 (–1.58, 0.62)	–1.30 (–2.49, –0.11)	–1.52 (–2.73, –0.31)
Age quintile 4 (67–72 y)	–2.60 (–3.73, –1.46)	–2.98 (–4.15, –1.82)	–3.68 (–4.93, –2.43)	–3.93 (–5.21, –2.65)
Age quintile 5 (≥73 y)	–0.81 (–1.91, 0.28)	–1.39 (–2.53, –0.25)	–2.07 (–3.29, –0.85)	–2.55 (–3.82, –1.29)
CRP quartile 1 (ref) (≤3 mg/L)	1.00	1.00	1.00	1.00
CRP quartile 2 (4–9 mg/L)	–1.21 (–2.20, –0.23)	–1.36 (–2.34, –0.37)	–1.36 (–2.39, –0.31)	–1.51 (–2.56, –0.46)
CRP quartile 3 (10–24 mg/L)	–3.11 (–4.12, –2.09)	–3.21 (–4.23, –2.18)	–3.29 (–4.37, –2.21)	–3.39 (–4.47, –2.31)
CRP quartile 4 (≥25 mg/L)	–3.94 (–4.99, –2.89)	–3.98 (–5.03, –2.93)	–4.10 (–5.21, –2.99)	–4.13 (–5.25, –3.01)
Tender-swollen joint difference ≥7	3.10 (1.77, 4.43)	2.49 (1.14, 3.83)	2.80 (1.38, 4.22)	2.14 (0.70, 3.57)
Education level	NI		NI	
≤9 y (ref)	–	1.00	–	1.00
10–12 y	–	–1.05 (–1.92, –0.18)	–	–0.95 (–1.88, –0.01)
>12 y	–	–3.91 (–4.89, –2.93)	–	–3.43 (–4.49, –2.38)
Country of birth	NI		NI	
Sweden (ref)	–	1.00	–	1.00
Scandinavia, not Sweden	–	–0.46 (–2.04, 1.11)	–	–0.14 (–1.81, 1.52)
Europe, not Scandinavia	–	1.92 (0.12, 3.71)	–	1.81 (–0.10, 3.73)
Other	–	5.26 (3.51, 7.02)	–	5.20 (3.36, 7.04)
Smoking status	NI	NI		
Never smoker (ref)	–	–	1.00	1.00
Former smoker	–	–	0.93 (0.11, 1.74)	0.83 (0.01, 1.66)
Current smoker	–	–	3.79 (2.73, 4.85)	3.14 (2.05, 4.23)
Pain-related comorbidities	NI	NI	4.01 (3.11, 4.91)	4.04 (3.14, 4.94)
Psychiatric comorbidities	NI	NI	2.32 (0.49, 4.15)	2.36 (0.52, 4.20)
Other comorbidities	NI	NI	1.68 (0.81, 2.54)	1.50 (0.62, 2.37)

NI, not included; RA, rheumatoid arthritis; CRP, c-reactive protein. Mixed model analysis. Estimated mean differences over time, with 95% CIs. Each model includes all variables in the table. Odds ratios are calculated per standard deviation for continuous variables. Bold text indicates statistical significance with *P* values < .05. Italic text indicates *P* values ≥ .05 and < .10. Bold plus italic text indicates *P* values < .001.

^a With adjustment for baseline pain.

and ESR at baseline were less likely to experience unacceptable pain at 2 years, and had less pain over time after adjustment for baseline pain. The results went in the same direction for having a higher swollen joint count at baseline, but only reached statistical significance at the 1-year follow-up. This is in line with earlier data [8]. Furthermore, after adjustment for age and sex, there was an association between negative ACPA and unacceptable pain plus low inflammation at 1 and 2 years. Taken together, these results indicate that patients with more objective signs of inflammation, and with a more classic disease presentation, have less risk of high unresolved pain levels up to 2 years follow-up.

The prevalence of fibromyalgia in RA has been estimated to 9% to 30% [31]. In our study, 1.9% of the patients had a diagnosis of fibromyalgia at some time, suggesting that fibromyalgia is under-recognised in clinical practice. Having many tender in relation to swollen joints at diagnosis was associated with higher pain levels over time, as well as with unacceptable pain and unacceptable pain with low inflammation at 1 and 2 years. These associations were independent of sex, age, baseline pain and CRP/ESR levels. Whether the TSJD can capture the presence of fibromyalgia in RA could not be specifically concluded from this study, but the dichotomous TSJD of ≥ 7 , adopted in the present study, has been identified by Pollard et al as the best cut-off for this identification. Notably, the TSJD seems to capture some aspect of pain prediction in our study that is not captured by other variables. Furthermore, a TSJD ≥ 7 was associated with higher pain levels at baseline and over time, but not with a decrease in pain over time, suggesting that the pain represented by this measure has a more noninflammatory character, which would correspond well with the presence of fibromyalgia as part of the disease spectrum of RA. Given these results, the assessment of TSJD in early RA may be useful for identifying patients in need of extended observation and management due to a higher risk for development of long-lasting pain.

Patients aged 47 to 58 years were more likely to experience unacceptable pain compared with patients ≤ 46 years in the multivariable analyses, whereas patients aged 67 to 72 years were less likely to have unacceptable pain. A previous study of the general population has shown an increasing prevalence of chronic musculoskeletal pain up to upper middle age [32], followed by a decrease in prevalence after that. Our results suggest that this pattern could also be relevant to patients with RA. It could be noted that this reduction in pain seems to appear around the age of retirement, which might partly be explained by the relief from work and potential stress related to that, as well as less bodily strain for manual workers.

Being born outside of Europe was associated with both unacceptable pain and higher pain levels over time. Potential causes for such associations include differences in economic background, sickness absence, employment status and physical/emotional trauma, and stress related to these factors. In one study of the general population, the association between chronic pain and ethnicity was markedly attenuated after adjustment for income, employment, and adverse life events [33]. We also found that formal education of >12 years was linked to less pain over time. Education level most likely corresponds to differences in income and in proportions of patients with manual work, which has also been associated with chronic pain [34]. Smoking is also a known risk factor for chronic pain, and was associated with more pain over time in the present study. There is evidence that pain may motivate smoking, and a reciprocal relationship between pain intensity and smoking has been proposed, where pain and smoking may exacerbate each other [35].

Comorbidities may also contribute to both pain and pain perception. Having a diagnosis of depression or anxiety before inclusion was associated with increased pain levels over time in the present study. This is in line with several previous studies [5,6,36], and mental illness should be considered when treating patients with RA. Finally, the effect of clinical parameters, socio-demographic factors, and comorbidities on pain in this study appears to be independent, supporting the importance of taking the biopsychosocial model of pain into account when treating patients with early RA. This may include structured stress assessment in early RA, and when indicated, assistance with stress reduction or referral to specialised clinics for this purpose [37].

Several limitations should be mentioned. This was an observational study with a variation in follow-up rate, with a substantial amount of missing data for the 1- and 2-year follow-up visits. Therefore, bias from factors influencing missingness cannot be excluded. It is possible that there is a selection of patients with more severe disease at the follow-up visits. However, demographics and background factors were similar for those with data at all visits. Furthermore, we have only evaluated VAS pain and therefore cannot draw conclusions on more detailed characterisation or localisation of pain. Predictors of pain intensity may differ from predictors of pain distribution. Data on pain medication was not available, and ‘acceptability’ of pain may vary depending on the use of painkillers. Finally, the classification of antibodies was based on ICD-10 codes and not blood sample test results, and it is possible that some ACPA or RF classifications are incorrect.

Strengths of this study include the use of observational data from a nationwide register, with a large sample size yielding adequate statistical power. Patients have been followed according to usual care, reflecting daily practice. Furthermore, we have investigated pain as a dichotomous outcome, as well as longitudinally, and utilised extensive data on background factors through links to validated Swedish registers.

CONCLUSION

In this study, one-third of patients with RA had unacceptable pain 2 years after diagnosis, in most cases despite low inflammation. Factors not directly related to inflammation contributed significantly to unacceptable pain in this early RA population. These include having many tender compared with swollen joints and high scores for PROs at diagnosis. Physicians should be aware of risk factors for unacceptable pain and take these into account when managing patients with early RA. A closer and tailored follow-up may be needed for these patients. Furthermore, sex and pain perception differences related to origin should also be considered in future pain interventions. Finally, known risk factors for chronic pain in the general population appear to be applicable also to patients with early RA.

Competing interests

CT has received consulting fees from AbbVie and Nordic Drugs, and speaking fees from AbbVie, Nordic Drugs, and Pfizer. JA has had or have research agreements with AbbVie, AstraZeneca, BMS, Eli Lilly, Galapagos, MSD, Pfizer, Roche, Samsung Bioepis, Sanofi, and UCB, mainly in the context of safety monitoring of biologics via AntiRheumatic Therapy in Sweden (ARTIS)/Swedish Biologics Register. TO has performed consulting tasks for MSD and UCB. TM is an employee at UCB Pharma. AE, DG, KW, JL and SB report no conflict of interest.

CRedit authorship contribution statement

Anna Eberhard: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Daniela Di Giuseppe:** Writing – review & editing, Formal analysis, Data curation. **Stefan Bergman:** Writing – review & editing, Methodology. **Jon Lampa:** Writing – review & editing, Methodology. **Thomas Mandl:** Writing – review & editing, Methodology. **Tor Olofsson:** Writing – review & editing, Methodology. **Kristin Waldenlind:** Writing – review & editing, Methodology. **Johan Askling:** Writing – review & editing, Methodology, Conceptualization. **Carl Turesson:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization.

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Patient consent for publication

Not applicable.

Ethics approval

This study was approved by the Swedish Ethical Review Authority (reference number 2024-04516-01).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

The individual patient data comes entirely from national Swedish registers. According to Swedish law, access to national register data is granted on a restrictive basis and may not be shared without additional specific permissions from the Swedish register-holding authorities.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2026.05.028.

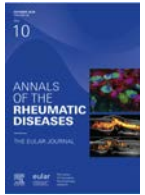
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Rheumatoid arthritis

Enhanced lipolysis in synovial fibroblasts mark early pathogenic changes in RA-risk individuals

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ABSTRACT

Objectives: Rheumatoid arthritis (RA) is a chronic autoimmune disease marked by synovial inflammation and progressive joint destruction, driven in part by the aggressive behaviour of synovial fibroblasts (SFs). Emerging evidence suggests that metabolic dysfunction in SFs contributes to RA pathogenesis. Studying autoantibody-positive at-risk individuals who have not yet developed clinical arthritis (RA-risk) offers a unique opportunity to investigate early disease mechanisms. Our previous work identified a transcriptional downregulation of genes involved in lipid and fatty acid metabolism in synovium samples of RA-risk individuals who later developed RA, even in the absence of overt immune cell infiltration. Here, we hypothesised that these alterations reflect an early metabolic shift in SFs.

Methods: To investigate this, we compared transcriptomic profiles and lipid metabolism in SFs from RA-risk individuals, patients with RA and osteoarthritis (OA), and age-matched noninflammatory orthopaedic injury controls. RA and RA-risk SFs displayed distinct transcriptional signatures compared with controls, including dysregulation of lipid metabolism.

Results: Despite retained adipogenic differentiation potential, RA, RA-risk, and OA SFs showed reduced baseline lipid droplet (LD) content compared with controls. In RA and RA-risk SFs, this reduction was a result of enhanced LD lipolysis, plausibly as a result of decreased G0S2 expression, and could be reversed by adipose triglyceride lipase inhibition. Furthermore, stimulating G0S2 expression coincided with a reduced myeloid-supporting SF phenotype.

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Conclusions: Overall, these findings suggest that early lipid metabolic alterations in SFs represent important shifts in cellular function that precedes clinical disease and highlight the need to further interrogate the role of lipid metabolism in RA progression.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Synovial fibroblasts (SFs) drive joint inflammation and damage in rheumatoid arthritis (RA).
- Metabolic disturbances in SFs are increasingly recognised as contributors to RA pathogenesis.
- Noninflamed synovium from risk of developing RA (RA-risk) individuals who later developed RA shows reduced lipid droplet content and downregulation of lipid metabolism genes.

WHAT THIS STUDY ADDS

- To our knowledge, this is the first study to perform a comprehensive transcriptomic analysis of SFs from RA-risk individuals, providing direct evidence of preclinical cellular alterations.
- It demonstrates that SF dysfunction precedes synovial inflammation and is marked by early dysregulation of lipid metabolism and stress-response pathways.
- It identifies aberrant lipolysis, associated with reduced *G0S2* expression, as a potentially targetable mechanism underlying altered lipid homeostasis in RA-risk and RA SFs.
- It further links *G0S2* regulation to inflammatory features of SFs, showing that induction of *G0S2* is accompanied by reduced expression of key myeloid-supporting mediators.
- Reduced *G0S2* expression in synovial tissue from RA-risk individuals who later developed arthritis suggests that alterations in this pathway may represent an early molecular event associated with disease progression.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- By uncovering the early involvement of SFs, this study invites reconsideration of the synovial stromal niche as an active contributor to early RA pathogenesis rather than merely responding to downstream inflammatory processes.
- This in-depth transcriptomic characterisation of RA-risk SFs provides a reference framework for studying key preclinical RA pathways in laboratories lacking access to these rare samples.
- Identification of *G0S2*-linked lipid metabolic pathways in SFs may inform future studies aimed at targeting stromal cell metabolism in early RA.
- These findings may inform future early detection programmes by incorporating SF-derived cellular markers alongside serological measures to enhance identification of individuals at RA risk.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that primarily affects the synovium of peripheral joints, causing painful swelling that can eventually lead to joint destruction [1,2]. Although current therapies can effectively suppress synovial inflammation and slow disease progression in many patients, continuous treatment is typically necessary to prevent relapse [3–7]. In addition, not all patients respond to treatment or become resistant to their treatment, and early initiation of therapy is crucial to prevent joint destruction [4,8]. Hence, a deeper understanding of the early molecular and cellular events that initiate and perpetuate disease is necessary for identifying new curative strategies that can prevent onset, halt progression, or achieve long-term remission in RA.

RA is considered a multistage disease involving genetic and environmental risk factors, and systemic autoimmunity precedes

clinical onset of disease [1,9–13]. Systemic autoimmunity is reflected by the detection of autoantibodies such as rheumatoid factor (RF) and anticitrullinated protein antibodies (ACPAs) in the blood [1,9,14] even in absence of clinical synovitis [12,15]. These autoantibodies can be present for years before clinical manifestation of disease [1,9,10]. This allows identification of individuals at risk of developing RA (RA-risk individuals) of whom approximately 30% will go on to develop RA within the next 3 to 4 years [1,9,14]. Studying these RA-risk individuals provides novel insights into the earliest phases of RA, even before the development of clinical symptoms, which will aid the discovery of drug targets for preventive therapies and disease remission.

Our recent research using synovial tissue biopsies from RA-risk individuals who developed RA after follow-up (pre-RA), suggested that transcriptional alterations in lipid metabolism are associated with the development of arthritis [16]. Particularly, we observed a downregulation of genes involved in fatty acid and lipid metabolism such as *LPL*, *DGAT2*, *ADIPOQ*, and *PPARG* as well as a lower presence of lipid droplets (LDs) in the noninflamed synovium of pre-RA individuals [16]. Given that synovial inflammation was absent in biopsies from these RA-risk individuals [12,15], the observed alterations are likely driven by intrinsic changes in resident synovial tissue cells rather than secondary effects of inflammation. Synovial fibroblasts (SFs) are 1 of the major resident cell types in the synovium and are the main drivers of pannus formation and joint destruction in RA [17]. A functional genomics atlas of SFs identified SFs as contributing up to 24% of RA heritability, emphasising their crucial role in RA pathogenesis [18]. Typically, cells involved in inflammation face demanding environmental conditions, requiring them to be dynamic and metabolically flexible [19,20]. Interestingly, alterations in the metabolic function of SFs have been associated with RA [21]. A recent study from our group examining the metabolic dynamics of cultured SFs revealed that metabolic flexibility in response to inflammatory triggers was reduced in RA-risk, RA, and osteoarthritis (OA) SFs compared with control SFs, which exhibited the highest adaptability [22]. Notably, control SFs predominantly relied on fatty acid oxidation to maintain basal mitochondrial respiration, whereas RA and RA-risk SFs showed an increased dependence on glucose utilisation and impaired fatty acid β -oxidation. Fatty acid metabolism is closely linked to cellular lipid homeostasis through its involvement in LD dynamics. In addition to serving as energy reserves, LDs function as essential buffers that sequester excess fatty acids, protect against lipotoxicity, and help maintain mitochondrial function during metabolic stress [23]. Furthermore, SFs are multipotent cells with the ability to differentiate into LD-rich adipocytes [24,25]. In healthy individuals, synovium is rich in adipocytes, especially in the sublining regions, where they are dispersed among fibroblasts [26]. However, in RA and OA, these synovial adipocytes progressively disappear and are replaced by inflammatory and fibrotic tissue [27–29]. Of note, a recent study demonstrated that RA SFs exhibit lower lipid content compared with controls [30]. Given our previous observations of altered metabolic flexibility and dependence on fatty acid oxidation in RA-risk and RA SFs [22], we here investigated whether the lipid alterations observed in pre-RA synovial tissue [16] are driven by intrinsic changes in SFs. We hypothesise that

the lower LD content in pre-RA synovium is caused by an altered metabolic state and loss of multipotency of SFs during the at-risk phase of RA. To explore this, we analysed the transcriptome and adipogenic differentiation capacity of SFs obtained during various phases of RA.

METHODS

Synovial tissue collection

Synovial tissues from patients with RA, with a clinical diagnosis and fulfilling the American College of Rheumatology/European Alliance of Associations for Rheumatology (ACR/EULAR) 2010 criteria for RA [31], were obtained during routine joint replacement surgery (n = 4) or standardised knee joint mini-arthroscopy (n = 2) [32]. Furthermore, mini-arthroscopic synovial tissue sampling of the knee was performed in RA-risk individuals. RA-risk was defined as (i) either having arthralgia without current or previously observed clinical arthritis or being an asymptomatic first-degree relative of a patient with RA and (ii) positive for immunoglobulin M–rheumatoid factor and/or ACPAs (anti-cyclic citrullinated peptide), all without an RA diagnosis according to EULAR criteria [31]. RA-risk individuals were identified through RA-specific autoantibody testing of family members of patients with RA, referred from the rheumatology clinic at Reade (Amsterdam, The Netherlands) or recruited from the Amsterdam University Medical Center (UMC) (location Academic Medical Center (AMC)) outpatient clinic. In addition, control synovial samples were obtained from individuals undergoing exploratory orthopaedic surgery due to joint injury, with no history of autoimmune or inflammatory diseases and no prior treatment with disease-modifying antirheumatic drug (DMARDs) or biologics. Furthermore, synovial tissues from patients with OA were obtained during knee, ankle, or hip joint replacement surgery. It includes OA samples served as a disease-

relevant control group, allowing comparison within a similar joint microenvironment and controlling for variables such as joint type and mechanical stress. This enabled assessment of whether observed transcriptional changes were specific to RA or reflected broader responses associated with joint pathology and inflammation. As previously evaluated [33,34], histological assessment was performed to assess tissue architecture and confirm the absence of inflammatory infiltrates in control synovial biopsies. Informed consent was obtained from all participants before their involvement in the study. The research was carried out following the ethical guidelines set by the Declaration of Helsinki [35]. Approval of the study was granted by the Institutional Review Board at the Amsterdam UMC, AMC location, The Netherlands. Patients and the public were not involved in the design, conduct, or reporting of this study. Detailed demographic information of the study participants is outlined in Table 1.

Isolation and in vitro expansion of SFs

Synovial tissue biopsies were collected and enzymatically digested according to established protocols for the isolation and culture of SFs [36]. Following digestion, SFs were cultured in complete cell culture medium composed of low-glucose Dulbecco’s Modified Eagle Medium (Gibco), enriched with 1% penicillin/streptomycin (10,000 U/mL, Gibco), 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (Gibco), and 10% fetal bovine serum (Biowest). Cells were routinely passaged upon reaching > 80% confluency, and all experiments were conducted using cultured SFs between passages 3 and 5, which ensured the absence of nonfibroblast populations. Cells were detached using 0.05% trypsin/5 mM ethylenediaminetetraacetic acid (EDTA) (Thermo Fisher Scientific) and counted with trypan blue (Sigma-Aldrich) in a Bürker-Türk hemocytometer (VWR).

Table 1
Demographics of study subjects

	Controls ^a n = 6	RA-risk individuals ^b n = 6	Patients with RA ^c n = 6	Patients with OA n = 6
Sex (female, n) (%)	1 (16.6)	5 (83.3)	2 (33.3)	6 (100)
Age (y)	54 (27-60)	50 (44-63)	63 (53-66)	64 (54-75)
IgM-RF positive (n) (%)	ND	2 (33.3)	4 (66.6)	ND
ACPA positive (n) (%)	ND	4 (66.6)	5 (83.3)	ND
IgM-RF and ACPA both positive (n) (%)	ND	0 (0)	3 (50)	ND
ESR (mm/h)	ND	8.5 (5.0-11.3)	15.5 (6.7-19.7)	ND
CRP (mg/L)	ND	1.5 (0.8-2.0)	3.45 (2.7-4.9)	ND
DAS28	ND	ND	3.6 (3.2-4.3)	ND
Disease duration (mo)	–	–	137 (83-261)	12 (0-138)
Joint site (n) (%)				
Knee	5 (83.3)	6 (100)	5 (83.3)	3 (50)
Ankle	1 (16.6)	–	1 (16.6)	1 (16.6)
Hip	–	–	–	2 (33.3)
Treatment (n) (%)				
NSAID	2 (33.3)	–	3 (50)	5 (83.3)
Anti-TNF	–	–	3 (50)	–
Corticosteroids	–	–	1 (16.6)	–
Rituximab	–	–	1 (16.6)	–
DMARD	–	–	4 (66.6)	–

ACPA, anticitrullinated protein antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IgM, immunoglobulin M; IgM-RF, IgM rheumatoid factor; ND, not determined; OA, osteoarthritis; RA, rheumatoid arthritis; RA-risk, risk of developing rheumatoid arthritis. DAS28, disease activity score; TNF, tumor necrosis factor; DMARD, disease-modifying antirheumatic drug.
Data are expressed as median (IQR) unless otherwise indicated.
^a Control donors were recruited for noninflammatory orthopaedic indications; therefore, RF, ACPA, ESR, and CRP were not routinely assessed. All synovial tissue biopsies were obtained by joint replacement surgery, except for ^ball RA-risk individuals and ^c2 patients with RA, whose biopsies were obtained by mini-arthroscopies of a knee joint as previously described (32).

RNA sequencing data processing and quality control

Total RNA was extracted from cultured SFs obtained from 6 donors per diagnostic group using the AllPrep DNA/RNA Mini Kit (Qiagen), including on-column DNase digestion, following the manufacturer's guidelines. Bulk RNA sequencing (RNA-seq) was performed at the Genomics Core Facility at Amsterdam UMC. RNA integrity and quantity were determined using the Agilent TapeStation system with RNA ScreenTape, and all RNA samples exhibited an RNA integrity number (RIN) within the range of 5.1 to 8.7, with a median RIN of 6.7. Libraries were prepared using the KAPA mRNA Hyperprep Kit (Roche) and sequenced using the NovaSeqXplus sequencer (Illumina). Sequencing yielded between 33.0 and 60.1 million reads per sample, with a median of 42.15 million. Raw read quality was evaluated using FastQC (v0.12.1) and MultiQC (v1.14) [37]. Quality filtering and adapter trimming were performed using TrimGalore (v0.6.10) in paired-end mode, applying Illumina adapter removal, Phred + 33 encoding, and quality trimming at a Phred score threshold of 30. Filtered reads were aligned to the GRCh38.p14 human reference genome, and gene-level read counts were simultaneously generated using STAR (v2.7.11b) [38]. All raw sequencing reads are available through the European Nucleotide Archive under project accession PRJEB94343.

Differential gene expression analysis

The identification of differentially expressed genes (DEGs) was performed using DESeq2 (v1.45.3) [39] in RStudio (v2024.09.1 + 394). Genes with low counts (< 10 reads in at least 6 samples (smallest group)) were prefiltered before differential expression analysis. Before model fitting, principal component analysis (PCA) of variance-stabilised counts was performed to evaluate potential confounders (age, biological sex, passage number, treatment, and RIN score). As only diagnosis contributed to separation across principal components, the DESeq2 design included diagnosis and sex to account for differences in sex distribution between groups. DESeq2 median ratio count normalisation was used, and log-fold change estimates were shrunk for improved visualisation using the *apeglm* method [40]. Dimensionality reduction and clustering identification were performed using PCA on regularised-logarithm transformed counts. Statistical significance was assessed for each comparison of interest using the Wald test implemented in DESeq2, and *P* values were adjusted for multiple testing using the Benjamini-Hochberg (BH) method to control the false discovery rate. Three differential gene expression analyses were conducted, comparing each diagnostic group (RA, RA-risk, and OA) against the control group. A threshold of $|\log_2 \text{fold change}| \geq 1$, along with an adjusted *P*-value < .05, was applied to select significantly DEGs. A detailed list of the DEGs and associated metrics is available in [Supplementary Tables S1 to S3](#).

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) [41] was performed using the *GSEA* function from the *clusterProfiler* package [42] in R, which implements the *fgsea* method for enhanced computational efficiency [43]. For each comparison, genes were ranked based on $\log_2\text{FC}$ values, organising genes by the direction of their expression changes (up or downregulated). Enrichment analysis was performed using a minimum of 10 and a maximum of 500 genes per gene set, with 1000 permutations and multiple testing correction via the BH method. We conducted GSEA using

2 different established gene sets from the Molecular Signatures Database (v 7.5.1) in RStudio (Posit Software, PBC, Boston, MA, USA) (v2024.09.1 + 394). To obtain a focused and nonredundant overview of major biological processes, we used the Hallmark gene sets (H collection). In parallel, we analysed the data using the canonical pathways gene sets (C2:CP collection), which aggregate pathway annotations from multiple databases including Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, and WikiPathways, providing more granular and comprehensive pathway coverage. This dual analysis strategy was chosen to maximise both interpretability and depth of pathway-level insights. Pathways were considered significantly enriched when the adjusted *P* values were < .05. The full list of enriched pathways and their associated metrics is available in [Supplementary Tables S4 to S9](#). The enriched Reactome pathways identified in each comparison were considered comprehensively and categorised into distinct biological processes, as detailed in [Supplementary Table S10](#).

Adipogenesis differentiation

SFs were seeded in duplicate with 100,000 cells per well in 12-well plates (Corning) for RNA collection, and in triplicate with 12,000 SFs per well in 96-well plates (Corning) for imaging, and were subsequently cultured in the presence of adipogenesis differentiation medium for 14 days at 37°C/5% CO₂ or with the peroxisome proliferator-activated receptor gamma (PPAR γ) agonist rosiglitazone (Sigma-Aldrich) diluted in complete culture medium (10 μM) for 14 days at 37°C/ 5% CO₂. Adipogenesis differentiation medium consisted of complete culture medium as described above, enriched with 5 $\mu\text{g}/\text{mL}$ insulin, 500 μM 3-Isobutyl-1-methylxanthine, 1 μM dexamethasone, and 60 μM indomethacin (all Sigma-Aldrich). Medium was refreshed every 2 to 3 days. On day 14, cells were washed twice with phosphate-buffered saline (PBS) and collected for RNA lysates (12-well plates) or immunohistochemistry staining (96-well plates). Before staining, cells were carefully fixed for 30 min at room temperature (RT) with 10% formalin (Merck KGaA). After incubation, cells were washed twice with distilled water. Next, cells were incubated with 60% isopropanol (Sigma-Aldrich) for 5 min. After removal of the isopropanol, cells were stained with Oil Red O working solution and incubated for 20 min at RT while gently shaking the plate. For the Oil Red O stock solution, 0.25 g of Oil Red O powder (Thermo Fisher Scientific [Alfa Aesar]) was dissolved in 50 mL of isopropanol for 24 h at RT. No more than 2 h before staining, Oil Red O working solution was made by mixing 3 parts Oil Red O stock solution with 2 parts distilled water and filtered through Whatman NO 1 filter paper (Cytiva, Marlborough). After staining, cells were washed with distilled water multiple times until the water ran clear. Finally, nuclei were stained with hematoxylin (Sigma-Aldrich) for 5 min to determine cell numbers. Cells were washed with distilled water and imaged using the Leica DM6 microscope and LAS X software (Leica Microsystems). Images were analysed using ImageJ software (National Institutes of Health (NIH), Bethesda, MD, USA) to quantify Oil Red O-positive cells relative to the total cell count, as determined by haematoxylin staining.

Adipose triglyceride lipase inhibition lipid assay

Six thousand SFs per well were seeded in duplicate into 96-well plates (Corning) to ensure confluency and incubated overnight at 37°C/5% CO₂. The following day, cells were treated for 24 h with either 0.4 mM oleic acid (Sigma-Aldrich) or 0.5 mM

NG-497, an inhibitor of the human adipose triglyceride lipase (ATGL) developed by Grabner et al [44], both diluted in complete cell culture medium. After treatment, cells were washed once with sterile PBS and fixed with 4% paraformaldehyde in PBS (Sigma-Aldrich) for 60 min at RT. Following fixation, cells were rinsed with sterile distilled water and stained with Oil Red O solution and haematoxylin as described for adipogenesis differentiation. Cells were imaged using an EVOS M5000 digital inverted microscope (Thermo Fisher Scientific). Quantification was performed using ImageJ software. Lipid content was quantified in ImageJ by calculating the average mean intensity of Oil Red O staining and determining the ratio of Oil Red O-positive cells to the total number of haematoxylin-stained nuclei.

Quantitative polymerase chain reaction

Total RNA was isolated from cultured SFs obtained from 6 donors per diagnostic group using the RNeasy Micro Kit (Qia-gen), including on-column DNase digestion, following the manufacturer’s guidelines. Complementary DNA synthesis was performed with the RevertAid H Minus First Strand complementary DNA Synthesis Kit (Thermo Fisher Scientific). Quantitative Polymerase Chain Reaction (qPCR) was conducted using either TaqMan Universal PCR master mix combined with Taqman assays or fast SYBR Green PCR master mix (Applied Biosystems, Life Technologies) with custom-designed primers (Thermo Fisher). Primer sequences and Taqman assays are detailed in Table 2. Detection was performed using QuantStudio 3 (Applied Biosystems). The expression levels of each target gene were normalised to the geometric mean of the reference genes *GAPDH* and *POLR2A* for the SYBR Green assays and *GAPDH* and *18S* for the Taqman assays [45]. An arbitrary calibrator sample was used to adjust for interplate variability. The relative quantity was calculated using the standard curve method for SYBR green assays, and the delta-delta cycle threshold (Ct) method was applied to Taqman assays.

Statistical testing

Data are presented as median with IQR. Differences between the study groups were analysed using a Kruskal-Wallis test followed by a post hoc Dunn’s test. GraphPad Prism software (version 10.2.0, La Jolla) was used for statistical analysis. *P* values < .05 were considered statistically significant.

RESULTS

Shared transcriptional signatures distinguish RA and RA-risk SFs from controls

To explore overall gene expression patterns and assess transcriptional differences between RA, RA-risk, and OA SFs, we first examined global transcriptomic variation across samples. A schematic overview of the synovial tissue sampling and experimental workflow is shown in Figure 1A. PCA was performed using the top 500 most variable genes identified from the DESeq2-normalised read counts. Despite all samples being derived from the same cell type (SFs), the PCA plot revealed separation of the samples (Fig 1B). Three distinct clusters were distinguishable from the RA, RA-risk, and control samples, suggesting substantial transcriptional differences among these groups. In contrast, OA samples were more dispersed, indicating greater sample heterogeneity within this group. These transcriptional differences between diagnostic groups were further supported by the total number of significantly DEGs identified in each comparison. RA SFs exhibited the greatest transcriptional divergence from control SFs with 205 DEGs identified, followed by OA SFs with 101 DEGs and RA-risk SFs with 29 DEGs, both relative to control SFs (Fig 1C). To visualise the extent of these transcriptional alterations and explore the magnitude and direction of gene expression changes, volcano plots of each comparison are shown in Figure 1D-F. Notably, 8 DEGs were shared between the ‘RA vs Control’ and ‘RA-risk vs Control’ comparisons, suggesting that transcriptional alterations in SFs may begin at early phases of RA. Overall, these findings point towards shared gene signatures between RA-risk and RA SFs.

Pathway enrichment analyses reveal early metabolic reprogramming and functional dysregulation in SFs

To gain insights into the biological pathways enriched in our gene expression dataset, we conducted 2 separate GSEAs. The first used were the curated Hallmark gene sets, offering a concise, nonredundant view of well-defined biological processes. The second employed a broader collection of canonical pathways, incorporating detailed, pathway-specific gene sets from databases such as KEGG, Reactome, and WikiPathways. Among the 15 significantly enriched Hallmark gene sets in RA SFs compared with controls (Fig 2A), we observed a notable association with invasive and profibrotic features as well as activation of endoplasmic reticulum (ER) stress mechanisms—a condition

Table 2
qPCR primer sequences and Taqman assays

SYBRgreen			
Gene	mRNA transcriptID	Forward sequence	Reverse sequence
<i>GAPDH</i>	ENST00000229239	GCCAGCCGAGCCACATC	TGACCAGGCGCCCAATAC
<i>POLR2A</i>	NM_000937.5	TCCTCGCATGATTGTCACCC	GTTTCATCACTTCACCCGCT
<i>FABP4</i>	NM_001442.2	GGCCAGGAATTGTGACGAAGT	CGCATTCCACCACCACTTTATC
<i>CEBPa</i>	NM_004364.4	AAGCACGATCAGTCCATCCC	GGCACAGAGGCCAGATACAA
<i>GOS2</i>	NM_015714.4	GCCGTGCCACTAAGGTCATT	ATCAGCTCCTGGACCGTTTC
<i>CCL2</i>	NM_002982.4	ATGAAAGTCTCTGCCGCCCT	GGGCATTGATTGCATCTGGCT
<i>GM-CSF</i>	NM_000758.4	GACACTGCTGCTGAGATGAATG	GGCTCTGGAGGTCAAAACAT
TaqMan <i>18S</i>	Hs99999901_s1		
<i>GAPDH</i>	4352934E		
<i>ADIPOQ</i>	Hs00605917_m1		
<i>LPL</i>	Hs00173425_m1		
<i>PNPLA2</i>	Hs00386101_m1		

qPCR, quantitative polymerase chain reaction

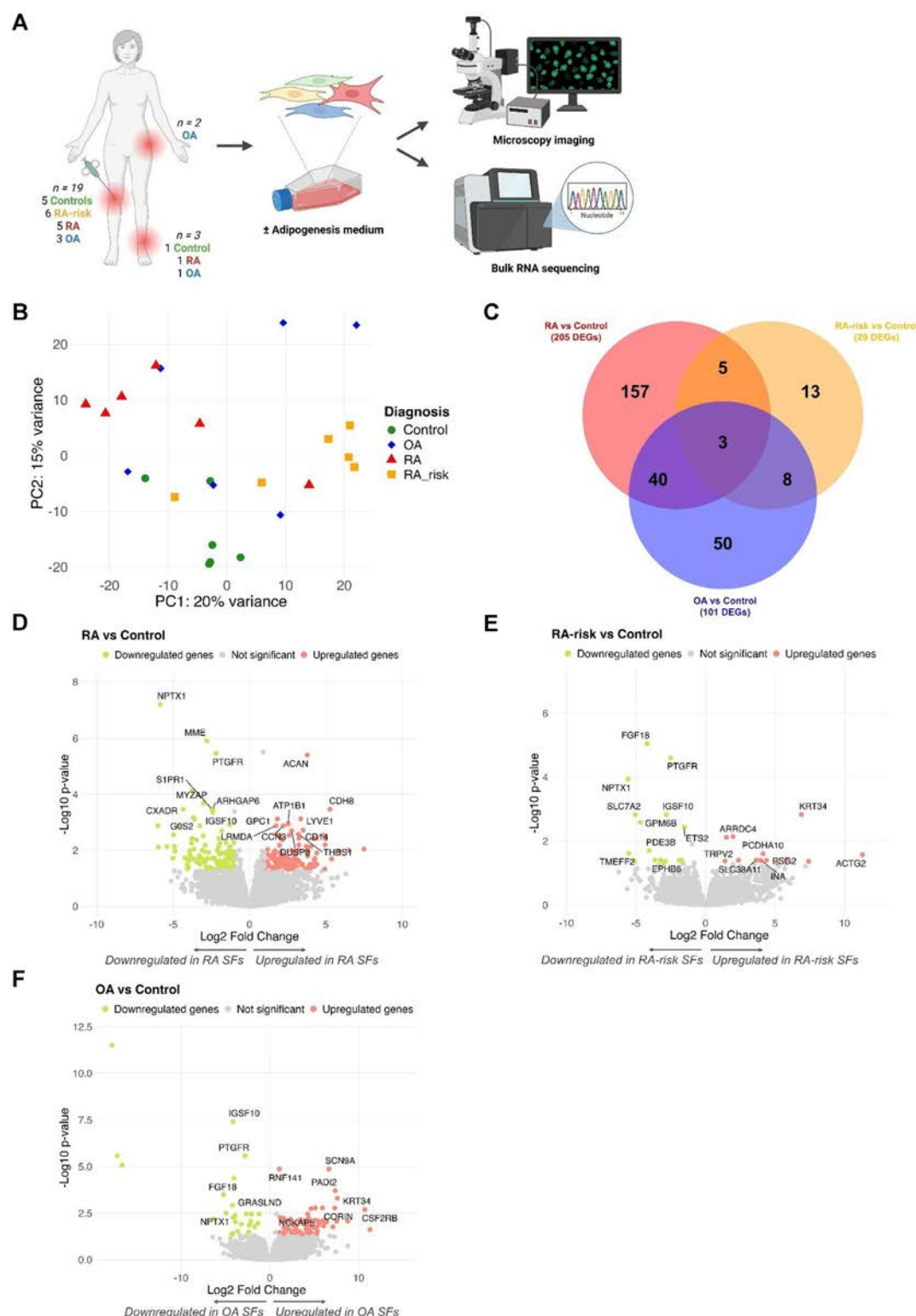


Figure 1. Diagnosis-specific clustering and shared transcriptional signatures differentiate RA and RA-risk SFs from controls. (A) Schematic overview of synovial tissue sampling and experimental workflow. Synovial biopsies were obtained from affected joints (knee, hip, or ankle). Synovial fibroblasts were isolated, expanded, and cultured *in vitro*. Cells were then maintained under basal conditions or stimulated with adipogenic differentiation medium for 14 days, followed by harvesting for gene expression analyses and imaging-based phenotyping. (B) PCA plot of the top 500 most variable genes across all SF samples. Each point represents an individual sample, with diagnostic groups distinguished by both colour and shape: green circles for controls, blue diamonds for OA, red triangles for RA, and orange squares for RA-risk. PC1 (20%) and PC2 (15%) are shown. (C) Venn diagram displaying the overlap of DEGs identified in 3 comparisons: RA vs control (red), RA-risk vs control (orange) and OA vs control SFs (blue). Each circle represents the number of DEGs ($P_{adj} < .05$, $|\log_2 FC| > 1$) found in 1 comparison. Overlapping regions indicate DEGs shared across 2 or more comparisons, whereas unique segments represent DEGs specific to each comparison. (D–F) Volcano plots showing differential gene expression in RA vs control SFs (D), RA-risk vs control (E) and OA vs control SFs (F). Each dot represents a gene. Genes with an absolute $\log_2$ fold change ($|\log_2 FC|$) > 1 and adjusted P -value (P_{adj}) < .05 are considered significantly differentially expressed and are highlighted in red (upregulated) and green (downregulated). Nonsignificant genes are shown in grey. DEG, differentially expressed gene; OA, osteoarthritis; PCA, principal component analysis; RA, rheumatoid arthritis; RA-risk, risk of developing Rheumatoid arthritis; SF, synovial fibroblast.

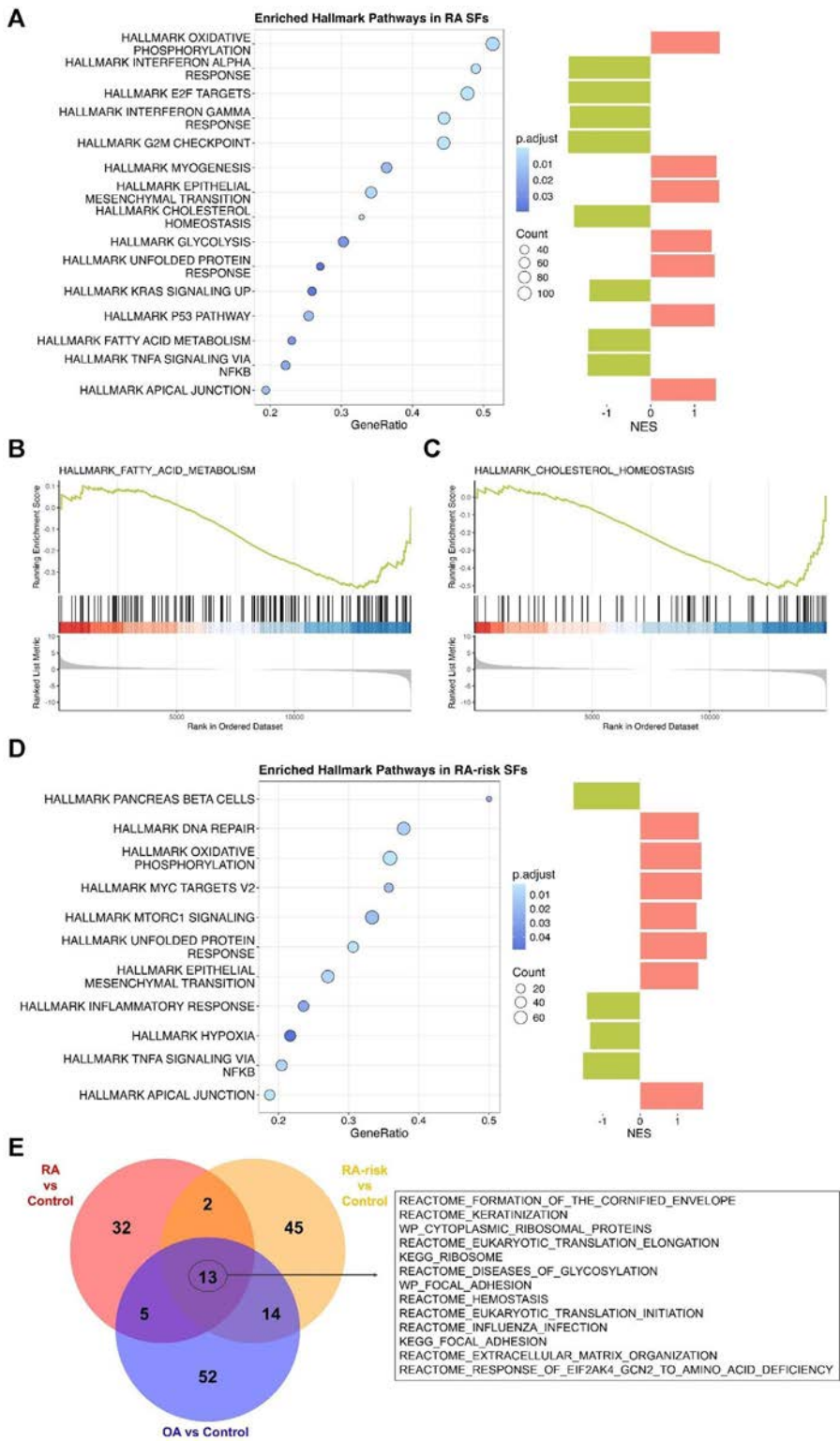


Figure 2. Enriched pathways identified by GSEA are reflective of early metabolic changes in SFs. GSEA was first performed using the Hallmark gene set collection from the Molecular Signatures Database (MSigDB). (A) Significantly enriched Hallmark pathways in RA SFs compared with controls. (B–C) Enrichment score (ES) curves for the HALLMARK_FATTY_ACID_METABOLISM (B) and CHOLESTEROL_HOMEOSTASIS (C) pathways from the RA vs control comparison is shown to illustrate individual gene enrichment patterns within these pathways. (D) Significantly enriched Hallmark pathways in RA-risk SFs compared with controls. Dot plots display enriched pathways ranked by *P*.adj value, with dot size representing gene count. Corresponding bar plots show Normalised Enrichment Scores (NES) for each pathway. (E) Venn diagram showing the overlap of significantly enriched canonical pathways across the 3 comparisons: RA vs control, RA-risk vs control, and OA vs control SFs. Enrichment of canonical pathways was performed using gene sets from WikiPathways, Reactome, and KEGG databases from the MSigDB (Supplementary Fig S1; Supplementary Tables S7–S10). Screening criteria for significant enrichment were set at *P*.adj < .05. GSEA, Gene set enrichment analysis; OA, osteoarthritis; RA, rheumatoid arthritis; RA-risk, risk of developing Rheumatoid arthritis; SF, synovial fibroblast; WP, WikiPathways. E2F, early 2 factor; G2M, gap 2 to mitosis; NFKB, nuclear factor kappa-light-chain-enhancer of activated B cells; MYC, myelocytomatosis; MTORC1, mammalian target of rapamycin complex 1; KEGG, kyoto encyclopedia of genes and genomes.

frequently experienced by SFs within the chronically inflamed RA synovium [46,47]. Moreover, the upregulation of stress- and cell cycle-related pathways along with downregulation of proliferation-associated gene sets (Fig 2A), suggested reduced proliferation capacity and altered stress responses in these cells. In addition, coordinated downregulation of lipid metabolic pathways together with upregulation of central energy-producing pathways underscored a shift in cellular metabolic activity (Fig 2A–C). RA-risk SFs exhibited enrichment in 11 Hallmark gene sets, many of which overlapped with those in RA SFs (Fig 2D). These transcriptional changes are consistent with our previous findings showing impaired mitochondrial function,

increased reliance on glycolysis, and altered fatty acid oxidation in RA and RA-risk SFs [20], supporting the idea that core metabolic dysfunction is already present at the at-risk stage. In addition, OA SFs exhibited enrichment in 6 Hallmark gene sets, including those linked to invasiveness and fibrosis, similarly upregulated in RA SFs (Supplementary Fig S1A). The overlap in enriched pathways between RA-risk and RA SFs supports the notion that core pathogenic processes are already active during the at-risk stage.

To further contextualise these findings, analysis of canonical pathways revealed a set of 13 pathways consistently enriched across all 3 comparisons (Fig 2E; Supplementary

Tables S7–S10). These were broadly linked to invasive and profibrotic features, altered protein synthesis and processing, posttranslational modification, and cellular metabolism (Supplementary Fig S1B–G; Supplementary Table S10). Notably, lipid metabolism alterations were evident across all diagnostic groups: whereas RA and OA SFs exhibited down-regulation of cholesterol metabolism (Supplementary Table S10; Supplementary Fig S1B,C), lipid metabolic changes in RA-risk SFs appeared to be more specifically linked to adipogenesis gene sets (Supplementary Fig S1D). Altogether, these insights revealed that key functional alterations in SFs are not limited to established RA but can be detected in SFs obtained from RA-risk individuals, reflecting early pathogenic mechanisms.

Lower LD content in RA-risk, RA, and OA SFs despite preserved adipogenic differentiation capacity

Our pathway analysis revealed that 1 of the dysregulated processes in RA-risk, RA, and OA SFs is lipid metabolism, which coincides with the previously observed reduced LD content in pre-RA synovium and RA SFs [16,30]. Moreover, G0S2—a physiological inhibitor of LD catabolism—was 1 of the most significantly down-regulated genes in RA SFs compared with controls (Fig 1D, Supplementary Table S1). Therefore, we next sought to experimentally evaluate the capacity of these SFs to undergo adipogenic differentiation and LD formation *in vitro*. SFs were differentiated towards adipocytes, and LD formation was assessed by Oil Red O staining (Fig 3A). LDs were clearly detected in control SFs before

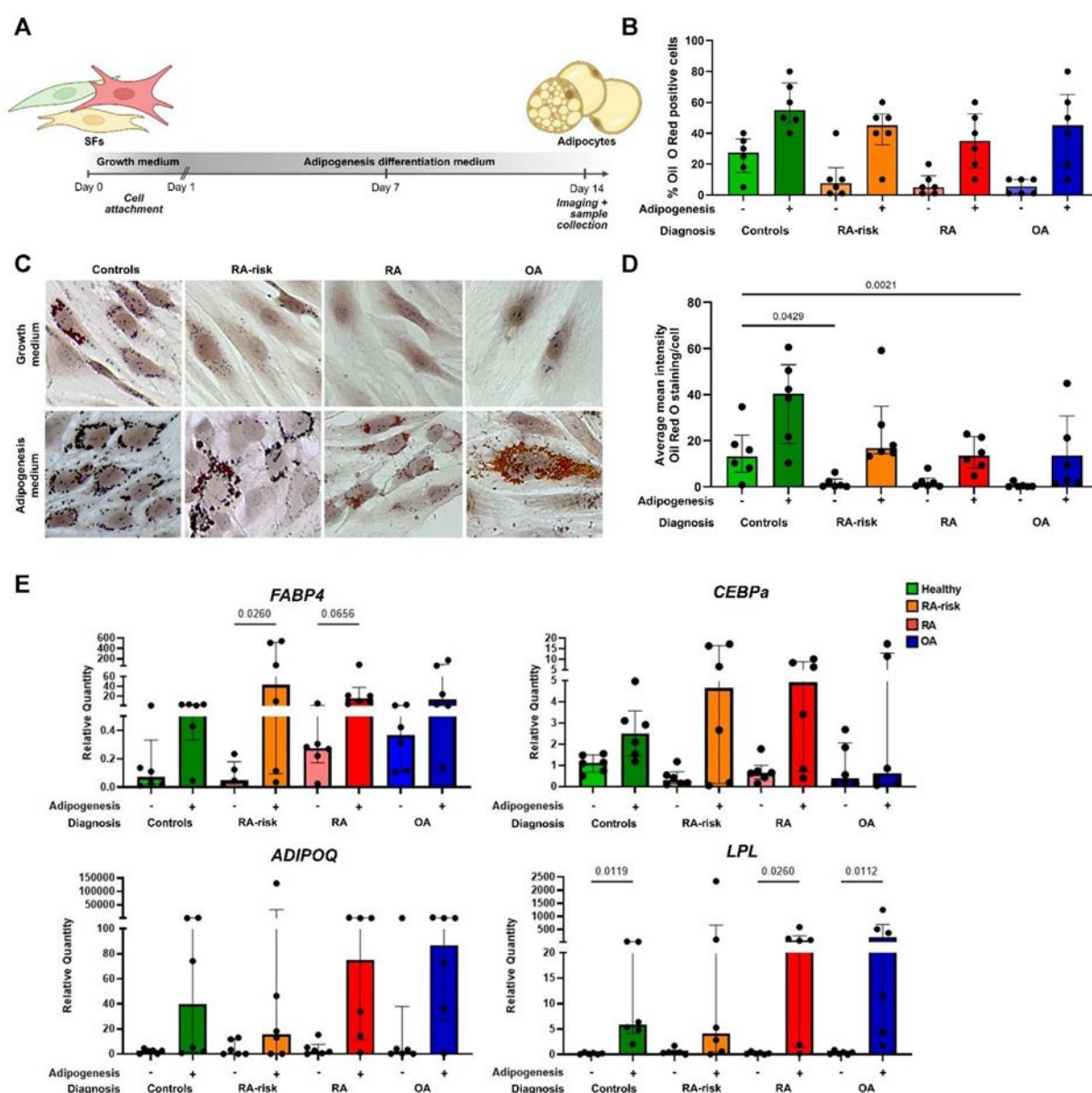


Figure 3. Lower lipid droplet content in RA, RA-risk, and OA SFs at baseline. (A) Graphical overview of adipogenesis differentiation experimental setup. Created in Biorender.com. (B) Representative microscopy images of cultured SFs stained with Oil Red O (dark red) and counterstained with haematoxylin (brown red). (C) Frequency of Oil Red O-positive cells as a percentage of total cells (represented by haematoxylin-stained cells) in SFs treated with or without adipogenesis differentiation medium, analysed using ImageJ. (D) Average mean intensity of Oil Red O signal per cell. A total of 50 cells per condition were analysed using Image J software to determine Oil Red O-positive signal per cell as well as cell quantification. (E) Relative gene expression levels of *FABP4*, *CEBPa*, *ADIPOQ*, and *LPL*. All donors were in passage 4, n = 6 per group. Data are presented as median + IQR. Statistical differences were assessed using a Kruskal–Wallis test with Dunn’s multiple comparisons for unpaired data. Paired comparisons were analysed using either a Wilcoxon matched-pairs signed-rank test (nonnormal distribution) or a paired *t* test (normal distribution) to analyse the effect of adipogenesis stimulation. OA, osteoarthritis; RA, rheumatoid arthritis; RA-risk, risk of developing rheumatoid arthritis; SF, synovial fibroblast.

differentiation, but were largely absent in RA, RA-risk, and OA SFs (Fig 3B-D). However, LDs were detected in all SFs after 14 days of culture in adipogenic differentiation medium (Fig 3B-D). This indicates that SFs from RA-risk individuals, and patients with RA and OA have an intact adipogenic differentiation programme despite having a lower abundance of LDs under homeostatic conditions. To confirm that our adipogenesis differentiation medium induces adipocyte differentiation, and not only LD formation, we analysed the expression of certain key genes known to be upregulated during adipogenesis such as *FABP4*, *CEBP α* , *ADIPOQ*, and *LPL*. All 4 genes were upregulated in SFs upon adipogenic differentiation (Fig 3E). These results indicate that, although RA-risk, RA, and OA SFs maintain their *ex vivo* adipogenic differentiation capacity, an intrinsic lower LD content is present under homeostatic conditions.

RA and RA-risk SFs exhibit enhanced lipolytic activity coupled to *G0S2* downregulation

As adipogenic capacity was unaffected, we turned to investigate the underlying cause of the reduced baseline LD content observed in RA and RA-risk SFs compared with controls. As 1 of the most significantly downregulated genes in RA SFs compared with controls (Fig 1D, Supplementary Table S1), *G0S2* involvement was considered. *G0S2* inhibits the ATGL enzyme, responsible for the initial step of triglyceride hydrolysis in LDs. Control, RA, and RA-risk SFs were incubated with the ATGL inhibitor NG-497 and/or oleic acid for 24 h, and LD formation was assessed using Oil Red O staining. As depicted in Figure 4A,B, both RA and RA-risk SFs were able to form LDs after oleic acid treatment, indicating that lipid storage capacity is intact.

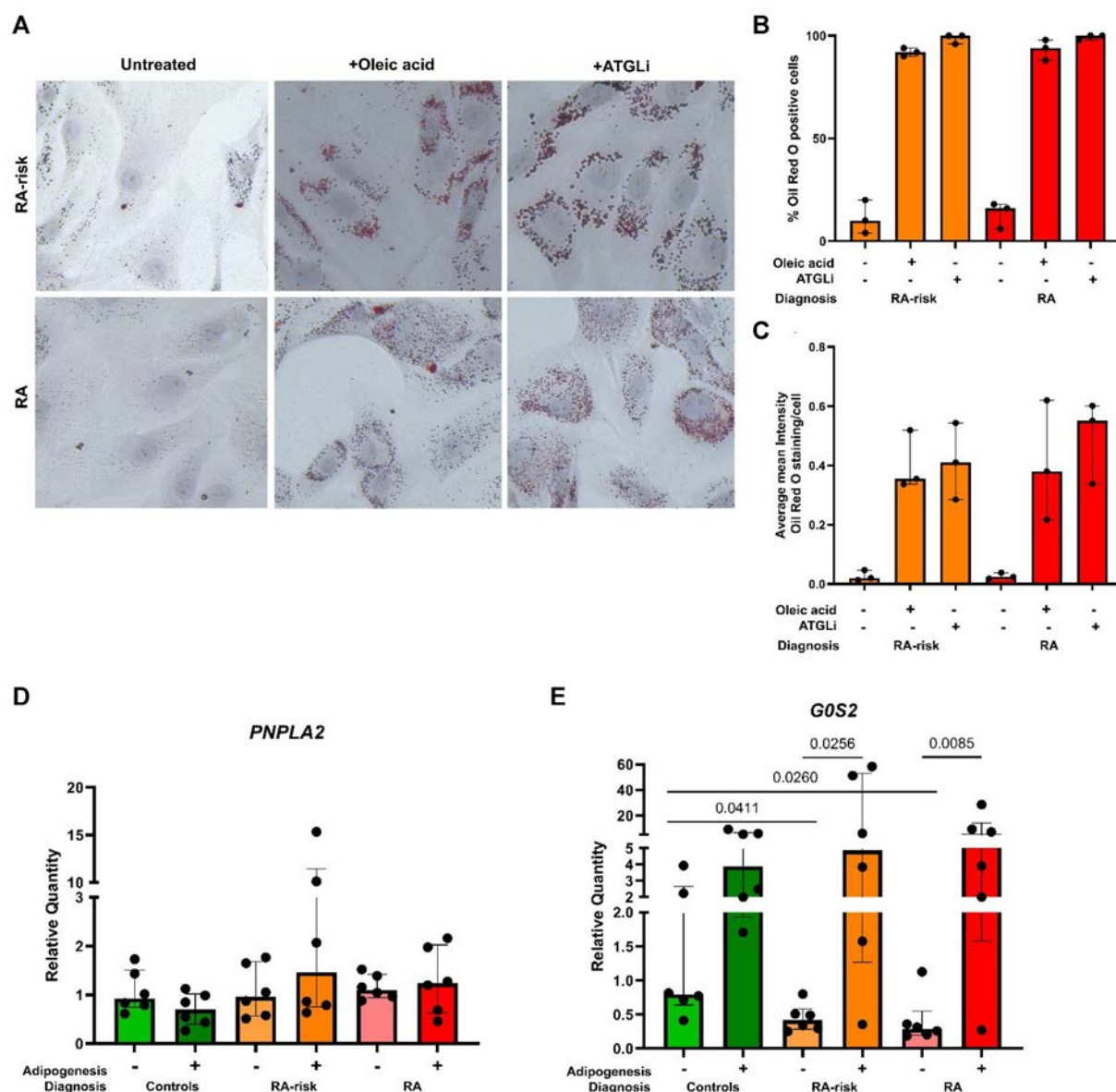


Figure 4. Enhanced lipolytic activity in RA and RA-risk SFs. (A) Representative microscopy images of cultured RA and RA-risk SFs in the presence or absence of oleic acid (0.4 mM) and the ATGL inhibitor (ATGLi) NG-497 (0.5 mM) for 24 h. Cells were first stained with Oil Red O to stain lipid droplets (in red) and counterstained with haematoxylin to stain the nuclei (in blue). (B) Frequency of Oil Red O-positive cells as percentage of total cells (represented by haematoxylin-stained cells). (C) Average mean intensity of Oil Red O signal per cell. A total of 50 cells per condition were analysed using Image J software to determine Oil Red O-positive signal per cell as well as cell quantification. All SFs cells were between passage 4-5, $n = 3$ per group. (D,E) Relative gene expression levels of *PNPLA2* and *G0S2*. All donors were in passage 4, $n = 6$ per group. Data are presented as median + IQR. Statistical differences were assessed using a Kruskal-Wallis test with Dunn's multiple comparisons for unpaired data. Paired comparisons were analysed using either a Wilcoxon matched-pairs signed-rank test (nonnormal distribution) or a paired t test (normal distribution) to analyse the effect of adipogenesis stimulation. ATGL, adipose triglyceride lipase; RA, rheumatoid arthritis; RA-risk, risk of developing rheumatoid arthritis; SF, synovial fibroblast.

Moreover, ATGL inhibition also led to LD accumulation in both RA and RA-risk SFs, showing that the capacity for lipid synthesis is maintained (Fig 4A and C). Control SFs displayed approximately 15% LD content under baseline conditions (Supplementary Fig S2), consistent with our previous observations (Fig 3B–D). ATGL inhibition similarly increased LD accumulation in control cells, further supporting the role of active lipolysis in regulating basal LD levels in SF. To further validate the downregulation of *GOS2* and explore its regulation during adipogenic differentiation in SFs, we analysed the expression of *GOS2* and *PNPLA2* (encoding ATGL) before and after stimulation with adipogenic medium by qPCR. Consistent with our bulk RNA-seq results, *PNPLA2* expression remained stable across diagnostic groups and was not notably affected by adipogenic stimulation (Fig 4D). In contrast, *GOS2* expression was significantly lower in RA and RA-risk SFs compared with controls under basal conditions, but markedly increased following adipogenic stimulation (Fig 4E). Notably, this regulation closely mirrored the LD phenotype described in Figure 3B–D, where RA and RA-risk SFs exhibited reduced LD abundance at baseline that was restored upon adipogenic differentiation. Altogether, these findings suggest that the reduced LD content observed in RA and RA-risk SFs at baseline is driven by downregulation of *GOS2*, which results in enhanced lipolysis.

Reduced *GOS2* levels associate with a myeloid-supporting SF phenotype and arthritis progression

Next, we studied potential upstream mechanisms controlling *GOS2* expression and impact on SF phenotype. Given that PPAR γ , a master regulator of adipogenesis [48], has been reported to directly induce *GOS2* transcription [49] and is required for an efficient lipolytic response [50], we pharmacologically activated PPAR γ using its agonist rosiglitazone in control, RA, and RA-risk SFs. Upon PPAR γ activation, *GOS2* levels were increased (Fig 5A), indicating that *GOS2* is responsive to PPAR γ activation in SFs. We next investigated whether induction of *GOS2* was accompanied by functional changes in the characteristic proinflammatory phenotype of RA SFs, as previous reports have implicated *GOS2* in inflammatory regulation [51]. Induction of *GOS2* by rosiglitazone or adipogenesis treatment coincided with a reduced expression of myeloid cell supporting cytokines *GM-CSF* (only for adipogenesis treatment) and *CCL2* (Fig 5B–E). To further explore the potential relationship between synovial *GOS2* expression and arthritis development, we revisited our previously published microarray dataset derived from *ex vivo* synovial biopsies of RA-risk individuals who later developed RA [16]. As shown in Figure 5F, *GOS2* expression was significantly lower in synovial biopsies of individuals who progressed to arthritis compared with those who did not, indicating an association between reduced levels of *GOS2* and future arthritis development. Taken together, these findings link diminished *GOS2* expression to altered lipid homeostasis and a myeloid-supporting phenotype in SFs, and suggest that reduced *GOS2* levels may represent an early molecular feature associated with arthritis development (graphical abstract).

DISCUSSION

In this study, we provided evidence that SFs from autoantibody-positive individuals at RA-risk exhibit early molecular and metabolic alterations that overlap with those observed in SFs from patients with RA with established disease. This suggests

the existence of a shared early pathogenic programme in this disease. We identified a disruption in lipid metabolism as reflected by reduced LD content in both RA and RA-risk SFs compared with controls. This reduction, which was unrelated to their adipogenic differentiation capacity, was attributed to increased lipolytic activity and enhanced LD catabolism.

Comprehensive transcriptomic profiling of SFs across RA stages revealed significant and substantial differences compared with control SFs. Signatures long observed in RA synovial tissue, such as cell migration, matrix remodelling, and tissue invasiveness [52], were, as expected, prominent in RA SFs. Strikingly, elements of these same pathways were also activated in RA-risk SFs, suggesting early activation of pathogenic fibroblast programmes. Positive enrichment of DNA repair pathways in RA-risk SFs may reflect underlying DNA damage triggering repair processes, consistent with our recent study, where RA and RA-risk synovial tissue present higher DNA damage compared with controls, with lower DNA damage repair capacities of RA and RA-risk SFs [53]. In inflamed RA synovium, SFs face ER stress from protein synthesis exceeding the ER's folding capacity [54]. As cells age, their ability to manage protein folding and degradation diminishes, leading to the accumulation of damaged proteins, which impairs cellular function [55]. Consistent with this, protein synthesis pathways were upregulated in RA-risk and RA SFs, pointing to early changes in protein homeostasis potentially linked to cellular ageing. Furthermore, the mechanistic target of rapamycin complex 1 signalling pathway was enriched in RA-risk SFs, supporting the findings of Barker et al [56] of enhanced expression of mechanistic target of rapamycin complex 1 activators in RA SFs. Of interest, targeting the mTOR pathway has shown promise in reducing synovial inflammation in patients with RA [57]. Altogether, these findings suggest that stress-response and repair mechanisms are engaged early in RA-risk SFs, potentially marking a shift towards a pathogenic phenotype. An important next step will be to determine whether these early molecular changes influence fibroblast behaviour in a way that promotes inflammation or joint tissue remodelling associated with RA onset.

A key insight from this study is the recurrent enrichment of metabolic gene programmes in RA and RA-risk SFs, suggesting that metabolic rewiring constitutes a fundamental and potential early feature of SFs' pathobiology. Metabolic profiling of cultured SFs performed earlier by our group showed that control SFs had the highest metabolic flexibility under inflammatory conditions, relying primarily on fatty acid metabolism for basal respiration. In contrast, RA and RA-risk SFs shifted towards glucose dependency [22]. Supporting this, noninflamed synovial tissue from pre-RA individuals displayed reduced expression of lipid metabolism genes and lower LD content [16]. In the present study, lipid metabolism appeared to be disrupted at both molecular and functional levels in RA and RA-risk SFs. Supporting these observations, recent data from the Accelerating Medicines Partnership Rheumatoid Arthritis and Systemic Lupus Erythematosus (AMP RA/SLE) network reported decreased lipid content in RA SFs relative to controls, suggesting that RA SFs exist in a relatively lipid-depleted state [30]. Our findings reveal a reduced lipid content also in RA-risk SFs, thus preceding the development of inflammatory disease. Together with our previous work on synovial tissue from RA-risk individuals who later developed RA [16], this supports the notion that alterations in lipid metabolism in resident cells such as SFs are present before clinical disease onset and may contribute to RA pathogenesis.

LDs play a crucial role in cellular lipid homeostasis, acting as key organelles involved in the storage and regulation of lipids

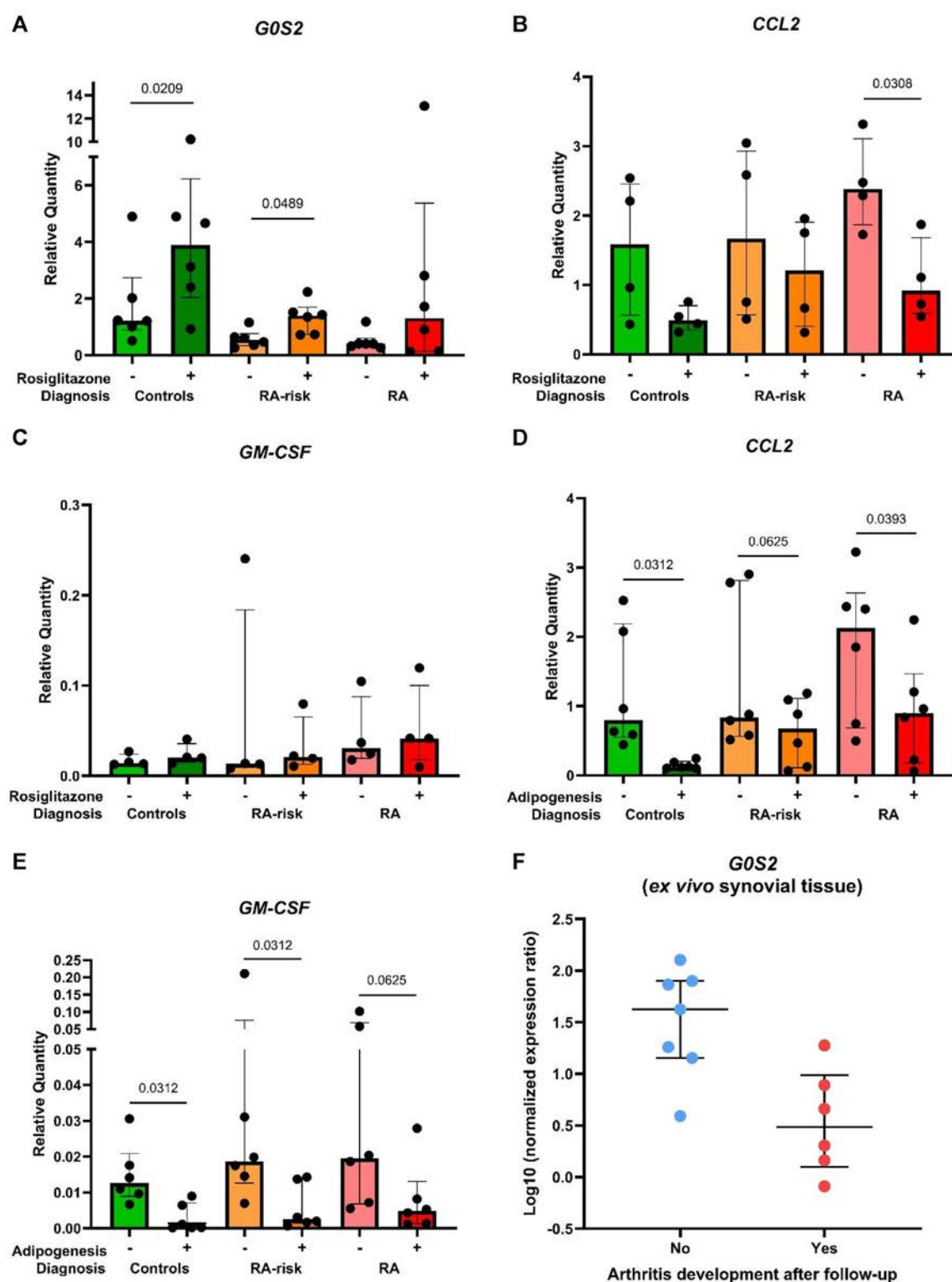


Figure 5. *G0S2* modulation in SF impacts their myeloid-supporting phenotype. (A–C) Relative gene expression levels of *G0S2*, *CCL2*, and *GM-CSF* before and after rosiglitazone treatment. All donors were in passage 4, (A) $n = 6$ per group (B, C) $n = 4$ per group. (D, E) Relative gene expression levels of *CCL2* and *GM-CSF* before and after adipogenesis stimulation. All donors were in passage 4, $n = 6$ per group. Data are presented as median + IQR. Statistical differences were assessed using a Kruskal-Wallis test with Dunn's multiple comparisons for unpaired data. Paired comparisons were analysed using either a Wilcoxon matched-pairs signed-rank test (nonnormal distribution) or a paired t test (normal distribution) to analyse the effect of adipogenesis stimulation. (F) Log10 normalised expression levels of *G0S2* in ex vivo synovial tissue biopsies from RA-risk individuals derived from the publicly available microarray dataset (ArrayExpress accession E-MTAB-11874). In the original publication (16), *G0S2* was included in [Supplementary Table S1](#) listing transcripts significantly associated with arthritis development (Cox regression, FDR < 0.05). Normalised expression values are available in Supplementary Multimedia Component 1 of the published study (16). FDR, false discovery rate; RA, rheumatoid arthritis; RA-risk, risk of developing rheumatoid arthritis; SF, synovial fibroblast.

within the cell [58]. They protect cells from lipotoxicity by converting excess free fatty acids (FFAs) into neutral lipids for storage [59]. LD turnover is tightly regulated by the enzyme ATGL (encoded by gene *PNPLA2*), which initiates lipolysis, and by its endogenous inhibitor GOS2, which suppresses ATGL activity [60,61]. Consistent with prior studies in adipocyte cell lines and mouse models, where *GOS2* overexpression promotes LD formation and *GOS2* deficiency increases lipolysis [63,64], our findings indicated that RA and RA-risk SFs exhibit reduced *GOS2* expression and lower LD content at baseline, which can be restored by adipogenic stimulation. This established a potential link between *GOS2* levels and the lipid content in SFs. Moreover, activation of the adipogenesis regulator PPAR γ [48]—necessary for an efficient lipolytic response [50]—significantly increased *GOS2* expression in all groups, suggesting a mechanistic connection between PPAR γ activation, *GOS2* expression, and lipolysis. As experiments inhibiting ATGL demonstrated that SFs across diagnosis retain the ability to form LDs under suppressed lipolytic activity, this suggests that the reduced LD content observed in RA and RA-risk SFs is likely driven by *GOS2* deficiency, leading to enhanced ATGL-driven lipolysis and increased release of FFAs. Importantly, induction of *GOS2* was associated with decreased expression of myeloid cell supporting cytokines, including *CCL2* and *GM-CSF*, highlighting a potential connection between lipid homeostasis and the immune-recruiting phenotype of SFs. Extending these findings to synovial tissue, lower *GOS2* expression in RA-risk biopsies was associated with progression to clinical arthritis, suggesting that *GOS2* deficiency may represent an early molecular event driving synovial pathogenicity at both the cellular and tissue levels.

Apart from LDs, β -oxidation of fatty acids is also essential for preventing the build-up of harmful levels of FFAs within cells [65]. When this metabolic pathway is impaired, excess FFAs accumulate and may result in lipotoxicity, which induces cellular stress, inflammation, and potential cell death [66,67]. Our previous research showed that RA-risk SFs have a significantly reduced β -oxidation activity, which is even more pronounced in SFs from patients with RA [22]. The combined effect of impaired β -oxidation and disrupted lipid metabolism—characterised by excessive LD degradation and altered lipid-related pathways—would then result in the accumulation of FFAs that are not efficiently metabolised. This accumulation triggers lipotoxic stress, a well-established driver of cellular dysfunction (reviewed by [68–70]). In the context of RA, such cellular stress may contribute to the emergence of pathogenic SFs' phenotypes that actively promote disease progression [71,72]. Although direct evidence linking lipotoxicity to SF dysfunction in RA is limited, the observed metabolic alterations in the preclinical stage underscore the potential role of lipid-induced stress in modulating SF activity. Further research is warranted to elucidate the impact of lipotoxicity on SFs' function and its contribution to RA pathogenesis.

Our study has several limitations that should be considered when interpreting the findings. First, the small sample size and high interindividual variability limited the statistical power. This is largely due to the technical challenges of isolating and culturing SFs from small, noninflamed synovial biopsies, particularly in RA-risk individuals. Also, most patients with RA and OA had received treatment, which may have influenced results, whereas all RA-risk individuals were untreated. Another limitation is that biopsies were taken from knee joints, whereas RA typically starts in smaller joints. However, obtaining sufficient noninflamed synovial tissue from small joints is challenging, especially from RA-risk individuals. Nevertheless, given the

systemic nature of RA, findings from knee biopsies likely reflect broader disease processes [73]. Furthermore, the synovial biopsy acquisition method was different between RA-risk individuals and most patients with RA and OA and controls. Although this may introduce variability, we did not observe evidence that the sampling method was a driver of our observed differences.

Overall, this study builds on our previous findings linking reduced lipid metabolism to RA progression [16] and provides novel insights by characterising the transcriptomic profile of cultured RA-risk SFs alongside RA and control SFs. Although earlier evidence of altered lipid metabolism in preclinical RA was primarily based on serum analyses [74–76] and synovial biopsies [16], this study reveals cell-intrinsic metabolic dysregulation in SFs. These findings underscore the relevance of lipid metabolism in SFs in RA pathogenesis and highlight it as a potential therapeutic target. Expanding research in larger RA-risk cohorts will further clarify whether these early metabolic alterations can serve as predictors of disease and actively drive its initiation.

Competing interests

All authors declare they have no competing interests.

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Contributors

MJ-M and TAdJ contributed to the conception, design, acquisition of samples, experimentation, analysis and interpretation of the data, and the realisation of the manuscript, with both authors contributing equally to this work. MH contributed to the analysis and interpretation of data and the reviewing and editing of the submitted manuscript. JFS and MGHvdS contributed to the acquisition of samples and the reviewing and editing of the submitted manuscript. NZ contributed to the partial design and the reviewing and editing of the submitted manuscript. WD contributed to supervision and the reviewing and editing of the submitted manuscript, and LGMvB contributed to the conception and design of the study, acquisition of funding, supervision, and the reviewing and editing of the submitted manuscript. All authors read and approved the final submitted manuscript.

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Patient consent for publication

Written informed consent was obtained from all participants prior to inclusion.

Ethics approval

The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Institutional Review Board of Amsterdam UMC.

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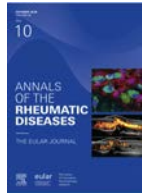
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Axial spondyloarthritis

Enhancing diagnostic accuracy in axial spondyloarthritis through centralised clinical and imaging evaluation: results from a prospective multicentre project

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ABSTRACT

Objectives: The objective of this study was to evaluate the impact of centralised clinical and imaging case evaluation on the diagnostic assessment of patients with suspected axial spondyloarthritis (axSpA).

Methods: We established a central telemedicine platform that allowed the collection of clinical and imaging information from patients who presented to rheumatologists and orthopaedists with suspicion of axSpA. Collected information included the suspected diagnosis, demographic, clinical, laboratory, and imaging data relevant for the diagnosis. Remote central expert evaluation comprised a detailed review of the submitted clinical and imaging data, followed by an expert diagnostic conclusion on the presence or absence of axSpA.

Results: A total 969 cases of suspected axSpA were evaluated. Based on the local assessment, 264 of 969 patients (27.2%) were diagnosed with axSpA. After central review, axSpA was considered present in 238 (24.6%) cases overall. Among patients with a local diagnosis of axSpA, 142 (53.8%) were considered axSpA also by central evaluation; however, in 89 (33.7%), an alternative cause of back pain—most commonly degenerative or mechanical disorders—was considered more likely. In comparison, among 319 patients locally assessed as not having axSpA, 265 (83.1%) were considered as no axSpA by central reviewers. One of the main drivers of the discrepancy between local and central diagnostic assessment was the interpretation of imaging findings as indicative of axSpA or not.

Conclusions: Remote evaluation of clinical and imaging information by assessors with expertise in axSpA may support the diagnostic process in patients with suspected axSpA and help reduce the risk of misdiagnosis.

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WHAT IS ALREADY KNOWN ON THIS TOPIC

- The diagnosis of axial spondyloarthritis (axSpA) is challenging because chronic back pain is common and no single diagnostic test alone is sufficient to confirm or exclude the disease.
- Imaging plays a key role in the diagnostic work-up of axSpA but requires considerable expertise because imaging abnormalities may also occur in mechanical or degenerative conditions.
- Although diagnostic delay in axSpA is well recognised, the extent of misdiagnosis or overdiagnosis in routine clinical practice remains insufficiently studied.

WHAT THIS STUDY ADDS

- In a large prospective multicentre cohort of patients with suspected axSpA, only about half of locally diagnosed cases were considered as axSpA after central expert evaluation.
- About one-third of locally diagnosed axSpA cases were evaluated as non-axSpA, most commonly due to misinterpretation of imaging findings related to mechanical or degenerative changes.
- To our knowledge, this work provides the first large-scale evidence indicating the magnitude of the problem of axSpA overdiagnosis and the factors associated with it.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- The findings indicate that overdiagnosis and misdiagnosis represent important challenges in the diagnostic evaluation of axSpA in routine practice.
- Improved training in the interpretation of axial imaging, use of standardised magnetic resonance imaging (MRI) protocols, and better communication between clinicians and radiologists may help improve diagnostic accuracy.
- Structured expert consultation, including telemedicine-based evaluation, may support clinicians in complex diagnostic situations.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease that predominantly affects the sacroiliac joints and the spine [1,2]. Early and accurate diagnosis of axSpA is important for appropriate management of this condition. Recent data from an inception cohort indicate that early diagnosis and treatment initiation in axSpA—within the first 2 years after symptom onset—are associated with better long-term clinical outcomes [3]. However, early diagnosis remains a significant challenge due to the nonspecific nature of initial symptoms and the complexity of distinguishing axSpA from other causes of chronic back pain.

The initial manifestation of axSpA often includes low back pain, a symptom prevalent in the general population and associated with a variety of conditions. This nonspecificity contributes to substantial diagnostic delays. Studies indicate an average delay of 5.7 years between the onset of symptoms and the diagnosis of axSpA in Germany [4], a pattern observed globally [5–7].

The diagnostic process for axSpA involves a thorough evaluation of clinical features, laboratory tests, and imaging findings [2,8]. In addition to back pain, clinical indicators include the presence of peripheral manifestations (peripheral arthritis, enthesitis, and dactylitis), extramusculoskeletal manifestations (eg, uveitis, inflammatory bowel disease, and psoriasis), response to nonsteroidal anti-inflammatory drugs (NSAIDs),

family history of SpA, and the HLA-B27 genotype, as well as elevated acute phase reactants, such as C-reactive protein (CRP) [2,8]. Imaging, particularly magnetic resonance imaging (MRI) of the sacroiliac joints, plays a central role in the diagnostic approach as the method of objective detection of inflammatory affection of the axial skeleton. MRI can detect early signs of inflammation and structural changes, which are critical for diagnosing axSpA. However, interpretation of MRI findings requires significant expertise due to the potential for false positives from noninflammatory causes of bone marrow oedema (BMO) [9,10]. Dedicated studies have shown that BMO in the sacroiliac joints can be found in postpartum women, athletes, patients with degenerative disorders including osteitis condensans ilii (OCI) and even in healthy subjects [11–14]. Relying solely on the presence of BMO in the sacroiliac joints—and, to a lesser extent, in the spine—may lead to overdiagnosis or misdiagnosis of axSpA, as well as the inappropriate use of advanced treatment options that are likely to fail in the absence of true immune-mediated inflammation [15]. Recent advances in differentiating the imaging characteristics of immune-mediated and mechanically induced conditions involving axial skeleton [9] combined with the use of telemedicine, offer promising solutions to the diagnostic challenges in axSpA.

The aim of the Improve-axSpA project, a prospective multicentre international telemedicine initiative, was to evaluate whether centralised expert evaluation of clinical and imaging data could enhance diagnostic accuracy in patients presenting with suspected axSpA in routine care.

METHODS

Design of the project

The Improve-axSpA project is a prospective, multicentre, international project conducted across Germany and Austria. The project implemented a central telemedicine platform to enable standardised collection and remote evaluation of clinical and imaging data from patients presenting with suspected axSpA to rheumatologists and orthopaedists.

Platform and data security

All project data were collected and transmitted using the axSpAnet platform (<http://axspanet.net>), developed by VISUS Health IT GmbH. The system ensured secure, encrypted communication, data storage, and processing in compliance with the General Data Protection Regulation (GDPR) and national data protection laws. The platform enabled seamless interaction between local investigators and central assessors, including the upload of standardised clinical information in structured electronic case report forms and images in digital imaging and communications in medicine (DICOM) format, and ensured a 2-way communication for review and feedback. Each case was pseudonymised prior to upload, and only participating investigators could access identifiable data at their site.

Participating centres and patient population

A total of 63 centres from Germany (n = 47) and Austria (n = 16) participated in the project, comprising academic and nonacademic rheumatology and orthopaedic clinics as well as private practices. Centres were encouraged to recruit

consecutive patients who presented with chronic back pain lasting ≥ 3 months with onset before the age of 45 years, in whom axSpA was clinically suspected by the treating physician. Exclusion criteria were limited to the absence of sufficient clinical or imaging information to permit central evaluation.

Data collection

Local physicians systematically collected clinical and laboratory information. The information included demographic data, such as age, sex, body mass index (BMI), and duration of symptoms (back pain); laboratory results, such as CRP levels and HLA-B27 status; clinical characteristics, such as localisation and character of back pain, response to NSAIDs, history of peripheral arthritis, enthesitis, or dactylitis; extramusculoskeletal manifestations (EMMs), such as uveitis, psoriasis, and inflammatory bowel disease; family history, such as the presence of SpA-related conditions in first-degree relatives; and mechanical stress factors, such as physically demanding work, sports, pregnancy, and childbirth history. Routinely obtained imaging, including conventional radiographs (CRs) and MRI of the sacroiliac joints and spine (further imaging modalities, such as computed tomography, could be provided as well), were uploaded via the platform. The need for imaging as well as the choice of imaging modalities used for the initial diagnostic evaluation were determined by the local physician according to routine clinical practice. Local physicians provided their interpretation of the imaging findings (presence or absence of SpA-typical changes for each modality) and their overall diagnostic impression (suspected presence or absence of axSpA). This assessment was based on the totality of information available to the treating physician in routine care, including clinical and laboratory data, imaging findings, and local radiology reports.

Central evaluation

All submitted cases were reviewed centrally by 1 of 2 central assessors with expertise in clinical and imaging aspects of axSpA (DP or XB). Case allocation was performed randomly, with 50:50 distribution of all cases. The central assessors had access to the structured clinical and laboratory information submitted through the platform, as well as to the uploaded imaging studies, including DICOM images. Local radiology reports were not available to the central assessors and were not included in the central evaluation.

The central evaluation included a detailed review of both clinical and imaging data, with particular emphasis on the presence or absence of SpA-compatible active inflammatory and structural changes in the sacroiliac joints and/or spine. Each case was assigned a final diagnostic conclusion (axSpA present or not present or information was insufficient for the definite diagnosis). In the no axSpA cases, the most likely alternative explanation of symptoms was provided. For unclear or incomplete cases, central assessors provided recommendations for further diagnostic workup (eg, laboratory testing and imaging optimisation). Where relevant, structured feedback on image quality and suggestions for technical improvement (eg, MRI sequence optimisation and slice orientation) were communicated back to the submitting centre through the platform.

Outcome measures and statistical analysis

The primary outcome was the agreement between the local diagnostic assessment and the central expert evaluation

regarding the presence of axSpA. For the purpose of this project, the central expert evaluation was used as the reference standard for comparison.

Secondary analyses included the description of demographic, clinical, imaging, and laboratory characteristics of patients with concordant and discrepant diagnoses. Descriptive statistics were used to summarise key patient characteristics. Categorical variables were expressed as absolute and relative frequencies, and continuous variables as means $\pm$ SD.

Exploratory subgroup analyses were performed according to the nature of the participating centre and the primary specialty of the submitting physician. Centres were categorised as hospital-based centres or community/office-based practices. Submitting physicians were categorised according to primary specialty as rheumatologists or orthopaedists.

Additional exploratory logistic regression analyses were performed to identify factors associated with diagnostic discordance. These analyses focused on patients with a local diagnosis of axSpA and assessed factors associated with a central diagnostic conclusion of no axSpA compared with central confirmation of axSpA. The opposite discordant pathway, namely local no axSpA followed by central axSpA, was not analysed because of the small number of cases. Candidate variables were selected based on clinical plausibility and included age, sex, BMI, CRP levels, HLA-B27 status, and discordant imaging findings. Discordant imaging findings were defined as lesions considered present by the local physician but not confirmed by central assessment. Because imaging modalities were not available in all patients and because of the limited number of events for some imaging domains, separate multivariable models were constructed for MRI of the sacroiliac joints, MRI of the spine, radiographs of the sacroiliac joints, and radiographs of the spine. Results are presented as odds ratios with 95% CIs.

Patient and public involvement

Patients and members of the public were not formally involved in the planning, design, or conduct of this project.

RESULTS

Overall diagnostic agreement and disagreement

A total of 969 unique cases of suspected axSpA (831 from Germany and 138 from Austria) were included across the participating centres between March 2020 and February 2025. Across all included patients, local physicians diagnosed 264 patients (27.3%) as axSpA, 319 patients (32.9%) as not having axSpA, and 386 patients (39.8%) as unclear. Central evaluation concluded axSpA in 238 patients (24.6%), no axSpA in 641 patients (66.2%), and unclear (ie, requiring further diagnostic workup, in most of the cases due to insufficient imaging finding) in 90 patients (9.3%; Fig 1). Central evaluation led to notable change of diagnoses compared with the initial local assessment. Among the 264 patients locally labelled as axSpA, 89 (33.7%) were re-assigned to the no axSpA category after central review; all these cases (as opposed to those, which remained unclear and warranted further evaluation) showed clear indicators against axSpA upon central assessment. In contrast, patients initially assessed as not having axSpA rarely changed categories. Of the 319 locally negative referrals, only 27 patients (8.5%) were assessed as axSpA by the central reviewers, illustrating that missed diagnoses were uncommon in this group. The largest referral category consisted of patients with unclear local

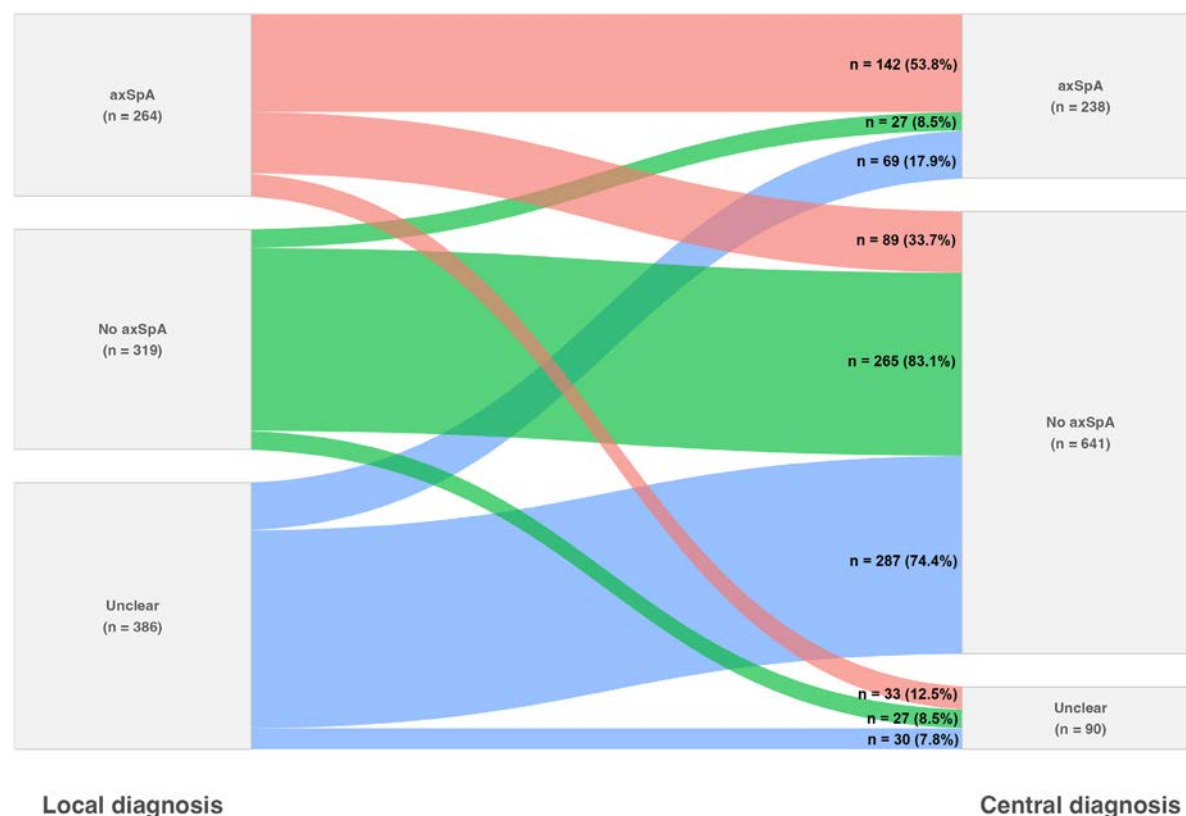


Figure 1. Flow from local diagnostic assessment to central expert diagnostic conclusion in patients with suspected axial spondyloarthritis. Sankey diagram showing the flow from the local diagnostic assessment to the central expert diagnostic conclusion in patients with suspected axial spondyloarthritis. Local and central assessments included 3 categories: axSpA, no axSpA, and unclear diagnostic status. Numbers and percentages indicate patients in each diagnostic pathway.

axSpA, axial spondyloarthritis.

diagnosis (n = 386), highlighting the diagnostic uncertainty frequently encountered in routine practice. Within this group, central assessors identified 69 patients (17.9%) with axSpA. However, the majority were labelled as no axSpA (n = 287), and a small subset remained inconclusive (n = 30; Fig 1).

Exploratory analyses stratified by practice setting and by primary specialty of the submitting physician are shown in [Supplementary Figures S1 and S2](#), respectively. Among 103 cases submitted from hospital-based centres, 22 had a local diagnosis of axSpA; of these, central assessment considered axSpA present in 45% and no axSpA in 55% of them. Among 866 cases submitted from community/office-based practices, 242 had a local diagnosis of axSpA; of these, axSpA was considered present centrally in 55% and no axSpA in 32% of the cases. When stratified by specialty, 874 cases were submitted by rheumatologists and 95 by orthopaedists. Among patients locally diagnosed with axSpA by rheumatologists, central assessment considered axSpA present in 55% and no axSpA in 33% of the cases. Among patients locally diagnosed with axSpA by orthopaedists, central assessment considered axSpA present in 45% and no axSpA in 41% of the cases, whereas some cases remained unclear.

Clinical and imaging characteristics of patient subgroups with concordant and discordant diagnostic evaluation

Clinical characteristics of all patients, as well as of subgroups stratified according to the local and central diagnosis, are presented in [Table 1](#). Imaging assessments performed by the referring physicians are shown in [Table 2](#), whereas the central evaluation of imaging findings is provided in [Table 3](#).

When comparing patients with concordant local and central diagnosis of axSpA (axSpA/axSpA) to those diagnosed with axSpA locally but in whom the diagnosis was excluded by central assessment (axSpA/no axSpA), clear differences were observed across demographic, clinical, laboratory, and imaging domains. Patients in the axSpA/axSpA group (n = 142) were younger (mean age 36.3 years) and predominantly men (73%), whereas patients in the axSpA/no axSpA group were older overall and included a higher proportion of women and a higher BMI. HLA-B27 positivity was substantially more frequent in the axSpA/axSpA group (82%) compared with 47% in the axSpA/no axSpA group. Objective inflammatory activity also differed, with elevated CRP levels observed in 44% of patients with axSpA/axSpA versus 19% in patients with axSpA/no axSpA. Inflammatory back pain was reported by 93% of patients with axSpA/axSpA and by 95% of those with axSpA/no axSpA, indicating limited discriminatory value of this symptom alone. A good response to NSAIDs was frequent in both subgroups as well: 73% in the axSpA/axSpA group and 67% in the axSpA/no axSpA group.

Imaging findings represented the most pronounced point of divergence between these 2 groups. In the axSpA/axSpA group, imaging consistently demonstrated active inflammatory and/or structural lesions across modalities, supporting the diagnosis. In contrast, although radiographs in the axSpA/no axSpA group were frequently interpreted as abnormal locally—27% considered to show definite sacroiliitis and 12% spinal changes compatible with axSpA—central assessment did not confirm definite sacroiliitis or axSpA-compatible spinal changes in any available sacroiliac or spine radiographs. Even larger discrepancy was

Table 1

Demographic, occupational, clinical and laboratory characteristics of all included patients and patients stratified according to the local/central diagnostic assessment

Variable	All patients (N = 969)	axSpA/axSpA (n = 142)	axSpA/no axSpA (n = 89)	No axSpA/axSpA (n = 27)	No axSpA/no axSpA (n = 265)	axSpA/Unclear (n = 33)	No axSpA/unclear (n = 27)	Unclear/axSpA (n = 69)	Unclear/no axSpA (n = 287)	Unclear/unclear (n = 30)
Age, y (mean $\pm$ SD)	43.8 $\pm$ 13.0	36.3 $\pm$ 11.9	44.8 $\pm$ 10.4	42.4 $\pm$ 15.9	46.3 $\pm$ 12.7	41.6 $\pm$ 11.8	46.4 $\pm$ 14.4	42.8 $\pm$ 13.2	45.6 $\pm$ 12.6	41.6 $\pm$ 14.8
Male sex, n/N (%)	376/969 (39%)	104/142 (73%)	33/89 (37%)	11/27 (41%)	86/265 (32%)	12/33 (36%)	10/27 (37%)	40/69 (58%)	69/287 (24%)	11/30 (37%)
BMI, kg/m ² (mean $\pm$ SD)	26.2 $\pm$ 5.7	25.0 $\pm$ 4.9	26.5 $\pm$ 5.7	27.1 $\pm$ 10.4	26.3 $\pm$ 5.4	25.2 $\pm$ 3.8	26.5 $\pm$ 6.4	25.5 $\pm$ 4.7	26.8 $\pm$ 5.8	27.0 $\pm$ 6.0
History of pregnancy, n/N (%)	366/592 (62%)	15/38 (39%)	37/56 (66%)	8/16 (50%)	116/179 (65%)	15/21 (71%)	10/17 (59%)	14/29 (48%)	144/217 (66%)	7/19 (37%)
Number of births ^a (mean $\pm$ SD)	1.9 $\pm$ 0.9	1.5 $\pm$ 0.8	2.1 $\pm$ 0.8	1.4 $\pm$ 0.7	1.9 $\pm$ 0.8	1.9 $\pm$ 1.0	1.7 $\pm$ 0.8	1.6 $\pm$ 0.9	1.9 $\pm$ 0.9	1.9 $\pm$ 0.7
Occupational activity	310/969 (32%)	41/142 (29%)	36/89 (40%)	10/27 (37%)	71/265 (27%)	7/33 (21%)	4/27 (15%)	19/69 (28%)	112/287 (39%)	10/30 (33%)
Equally mental/physical, n/N (%)										
Predominantly mental, n/N (%)	460/969 (47%)	57/142 (40%)	39/89 (44%)	16/27 (59%)	133/265 (50%)	19/33 (58%)	17/27 (63%)	38/69 (55%)	127/287 (44%)	14/30 (47%)
Predominantly physical, n/N (%)	199/969 (21%)	44/142 (31%)	14/89 (16%)	1/27 (3.7%)	61/265 (23%)	7/33 (21%)	6/27 (22%)	12/69 (17%)	48/287 (17%)	6/30 (20%)
Regular sport activity, n/N (%)	503/969 (52%)	90/142 (63%)	43/89 (48%)	13/27 (48%)	132/265 (50%)	13/33 (39%)	8/27 (30%)	34/69 (49%)	153/287 (53%)	17/30 (57%)
Sport activity, hours/week (mean $\pm$ SD)	4.0 $\pm$ 4.6	3.8 $\pm$ 2.7	3.8 $\pm$ 2.4	3.7 $\pm$ 2.7	4.0 $\pm$ 5.6	5.7 $\pm$ 10.4	2.4 $\pm$ 1.3	6.4 $\pm$ 8.6	3.6 $\pm$ 2.8	4.1 $\pm$ 2.0
Back pain duration, years (mean $\pm$ SD)	7.1 $\pm$ 8.4	6.5 $\pm$ 7.5	7.4 $\pm$ 7.3	6.3 $\pm$ 8.1	4.4 $\pm$ 6.1	5.0 $\pm$ 6.0	1.3 $\pm$ 1.2	8.1 $\pm$ 12.2	9.6 $\pm$ 9.1	3.6 $\pm$ 6.4
Inflammatory back pain, n/N (%)	390/670 (58%)	114/122 (93%)	71/75 (95%)	2/23 (8.7%)	42/202 (21%)	28/29 (97%)	1/24 (4.2%)	19/33 (58%)	104/145 (72%)	9/17 (53%)
Good response to NSAIDs, n/N (%)	588/969 (61%)	103/142 (73%)	60/89 (67%)	12/27 (44%)	133/265 (50%)	21/33 (64%)	11/27 (41%)	41/69 (59%)	188/287 (66%)	19/30 (63%)
Back pain localisation:	195/969 (20%)	23/142 (16%)	24/89 (27%)	4/27 (15%)	40/265 (15%)	2/33 (6.1%)	4/27 (15%)	16/69 (23%)	76/287 (26%)	6/30 (20%)
Cervical spine, n/N (%)										
Thoracic spine, n/N (%)	272/969 (28%)	44/142 (31%)	29/89 (33%)	9/27 (33%)	56/265 (21%)	8/33 (24%)	7/27 (26%)	25/69 (36%)	88/287 (31%)	6/30 (20%)
Lumbar spine, n/N (%)	757/969 (78%)	104/142 (73%)	74/89 (83%)	20/27 (74%)	213/265 (80%)	24/33 (73%)	21/27 (78%)	48/69 (70%)	230/287 (80%)	23/30 (77%)
Sacroiliac joints/buttocks, n/N (%)	783/969 (81%)	125/142 (88%)	75/89 (84%)	19/27 (70%)	203/265 (77%)	24/33 (73%)	18/27 (67%)	54/69 (78%)	244/287 (85%)	21/30 (70%)
Back pain intensity in the last week, 0–10 NRS (mean $\pm$ SD)	5.7 $\pm$ 2.3	5.4 $\pm$ 2.4	6.0 $\pm$ 2.2	5.7 $\pm$ 2.3	5.6 $\pm$ 2.3	5.5 $\pm$ 2.5	5.4 $\pm$ 2.6	5.8 $\pm$ 2.2	5.8 $\pm$ 2.2	5.3 $\pm$ 2.2
Peripheral arthritis	67/969 (6.9%)	3/142 (2.1%)	13/89 (15%)	0/27 (0%)	14/265 (5.3%)	2/33 (6.1%)	1/27 (3.7%)	5/69 (7.2%)	26/287 (9.1%)	3/30 (10%)
Current, n/N (%)										
Past, n/N (%)	124/969 (13%)	14/142 (9.9%)	19/89 (21%)	0/27 (0%)	29/265 (11%)	4/33 (12%)	3/27 (11%)	5/69 (7.2%)	46/287 (16%)	4/30 (13%)
Enthesitis	91/969 (9.4%)	7/142 (4.9%)	13/89 (15%)	0/27 (0%)	18/265 (6.8%)	0/33 (0%)	0/27 (0%)	5/69 (7.2%)	45/287 (16%)	3/30 (10%)
Current, n/N (%)										
Past, n/N (%)	74/969 (7.6%)	6/142 (4.2%)	16/89 (18%)	0/27 (0%)	20/265 (7.5%)	3/33 (9.1%)	0/27 (0%)	1/69 (1.4%)	25/287 (8.7%)	3/30 (10%)
Dactylitis	26/969 (2.7%)	1/142 (0.7%)	5/89 (5.6%)	0/27 (0%)	3/265 (1.1%)	1/33 (3.0%)	0/27 (0%)	2/69 (2.9%)	14/287 (4.9%)	0/30 (0%)
Current, n/N (%)										
Past, n/N (%)	30/969 (3.1%)	2/142 (1.4%)	3/89 (3.4%)	0/27 (0%)	5/265 (1.9%)	2/33 (6.1%)	0/27 (0%)	2/69 (2.9%)	14/287 (4.9%)	2/30 (6.7%)
Uveitis	12/969 (1.2%)	3/142 (2.1%)	1/89 (1.1%)	0/27 (0%)	1/265 (0.4%)	0/33 (0%)	0/27 (0%)	3/69 (4.3%)	4/287 (1.4%)	0/30 (0%)
Current, n/N (%)										
Past, n/N (%)	38/969 (3.9%)	16/142 (11%)	0/89 (0%)	1/27 (3.7%)	5/265 (1.9%)	4/33 (12%)	2/27 (7.4%)	3/69 (4.3%)	7/287 (2.4%)	0/30 (0%)
Inflammatory bowel disease	25/969 (2.6%)	5/142 (3.5%)	5/89 (5.6%)	0/27 (0%)	2/265 (0.8%)	0/33 (0%)	1/27 (3.7%)	4/69 (5.8%)	8/287 (2.8%)	0/30 (0%)
Current, n/N (%)										
Past, n/N (%)	12/969 (1.2%)	4/142 (2.8%)	1/89 (1.1%)	1/27 (3.7%)	2/265 (0.8%)	0/33 (0%)	0/27 (0%)	1/69 (1.4%)	3/287 (1.0%)	0/30 (0%)
Psoriasis	65/969 (6.7%)	7/142 (4.9%)	5/89 (5.6%)	0/27 (0%)	22/265 (8.3%)	3/33 (9.1%)	4/27 (15%)	4/69 (5.8%)	19/287 (6.6%)	1/30 (3.3%)
Current, n/N (%)										
Past, n/N (%)	43/969 (4.4%)	4/142 (2.8%)	7/89 (7.9%)	2/27 (7.4%)	15/265 (5.7%)	1/33 (3.0%)	0/27 (0%)	2/69 (2.9%)	10/287 (3.5%)	2/30 (6.7%)
Family history of axSpA, n/N (%)	103/770 (13%)	23/115 (20%)	15/68 (22%)	3/25 (12%)	17/216 (7.9%)	4/25 (16%)	1/19 (5.3%)	7/55 (13%)	28/222 (13%)	5/25 (20%)
Other SpA and related conditions ^b , n/N (%)	140/722 (19%)	16/108 (15%)	16/72 (22%)	7/26 (27%)	30/199 (15%)	5/26 (19%)	2/21 (9.5%)	9/50 (18%)	52/196 (27%)	3/24 (13%)
HLA-B27 positivity, n/N (%)	370/878 (42%)	113/138 (82%)	37/79 (47%)	12/20 (60%)	57/244 (23%)	20/30 (67%)	6/23 (26%)	37/61 (61%)	76/256 (30%)	12/27 (44%)
CRP, mg/L (mean $\pm$ SD)	6.8 $\pm$ 25.3	13.7 $\pm$ 55.1	4.9 $\pm$ 11.3	11.5 $\pm$ 27.3	3.8 $\pm$ 6.2	8.8 $\pm$ 13.4	2.7 $\pm$ 2.3	10.9 $\pm$ 27.9	4.8 $\pm$ 11.4	7.2 $\pm$ 9.6
Elevated CRP (>5 mg/L), n/N (%)	219/822 (27%)	58/131 (44%)	14/73 (19%)	7/25 (28%)	47/231 (20%)	12/26 (46%)	3/19 (16%)	23/59 (39%)	45/231 (19%)	10/27 (37%)
ESR, mm/h (mean $\pm$ SD)	10.9 $\pm$ 12.3	13.7 $\pm$ 14.5	8.6 $\pm$ 8.1	13.2 $\pm$ 14.2	10.0 $\pm$ 12.6	17.0 $\pm$ 19.0	15.4 $\pm$ 13.5	15.4 $\pm$ 17.9	8.9 $\pm$ 8.1	9.1 $\pm$ 7.1

^a In women with a history of pregnancies^b Including reactive arthritis, psoriasis, uveitis, and inflammatory bowel disease

axSpA, axial spondyloarthritis; BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; NSAIDs, nonsteroidal anti-inflammatory drugs; IBD, inflammatory bowel disease; NRS, numeric rating scale; SD, standard deviation; SpA, spondyloarthritis.

Table 2
Imaging characteristics according to the local evaluation of all included patients and patients stratified according to the diagnostic assessment

Variable	All patients (N = 969)	axSpA/axSpA (n = 142)	axSpA/no axSpA (n = 89)	No axSpA/axSpA (n = 27)	No axSpA/no axSpA (n = 265)	axSpA/Unclear (n = 33)	No axSpA/unclear (n = 27)	Unclear/axSpA (n = 69)	Unclear/no axSpA (n = 287)	Unclear/unclear (n = 30)
Radiographs of sacroiliac joints available, n/N (%)	185/969 (19%)	32/142 (23%)	15/89 (17%)	4/27 (15%)	40/265 (15%)	6/33 (18%)	5/27 (19%)	17/69 (25%)	55/287 (19%)	11/30 (37%)
Presence of definite radiographic sacroiliitis ^a , n/N (%)	41/185 (22%)	23/32 (72%)	4/15 (27%)	2/4 (50%)	2/40 (5.0%)	2/6 (33%)	1/5 (20%)	1/17 (5.9%)	6/55 (11%)	0/11 (0%)
Radiographs of spine available, n/N (%)	223/969 (23%)	36/142 (25%)	17/89 (19%)	6/27 (22%)	50/265 (19%)	9/33 (27%)	4/27 (15%)	23/69 (33%)	68/287 (24%)	10/30 (33%)
Presence of radiographic spinal changes indicative of axSpA, n/N (%)	24/223 (11%)	10/36 (28%)	2/17 (12%)	0/6 (0%)	2/50 (4.0%)	2/9 (22%)	0/4 (0%)	1/23 (4.3%)	7/68 (10%)	0/10 (0%)
MRI of sacroiliac joints available, n/N (%)	842/969 (87%)	128/142 (90%)	80/89 (90%)	23/27 (85%)	225/265 (85%)	26/33 (79%)	18/27 (67%)	60/69 (87%)	258/287 (90%)	24/30 (80%)
Presence of active inflammatory lesions indicative of axSpA, n/N (%)	293/842 (35%)	92/128 (72%)	43/80 (54%)	7/23 (30%)	36/225 (16%)	15/26 (58%)	4/18 (22%)	25/60 (42%)	61/258 (24%)	10/24 (42%)
Presence of structural lesions indicative of axSpA, n/N (%)	163/842 (19%)	58/128 (45%)	28/80 (35%)	3/23 (13%)	20/225 (8.9%)	7/26 (27%)	1/18 (5.6%)	9/60 (15%)	36/258 (14%)	1/24 (4.2%)
MRI of spine available, n/N (%)	707/969 (73%)	99/142 (70%)	64/89 (72%)	16/27 (59%)	198/265 (75%)	26/33 (79%)	23/27 (85%)	53/69 (77%)	205/287 (71%)	23/30 (77%)
Presence of active inflammatory lesions indicative of axSpA, n/N (%)	95/707 (13%)	32/99 (32%)	12/64 (19%)	0/16 (0%)	9/198 (4.5%)	9/26 (35%)	3/23 (13%)	8/53 (15%)	16/205 (7.8%)	6/23 (26%)
Presence of structural lesions indicative of axSpA, n/N (%)	58/707 (8.2%)	21/99 (21%)	7/64 (11%)	1/16 (6.3%)	6/198 (3.0%)	3/26 (12%)	2/23 (8.7%)	5/53 (9.4%)	11/205 (5.4%)	2/23 (8.7%)

^a According to the radiographic system of the modified New York criteria. axSpA, axial spondyloarthritis; MRI, magnetic resonance imaging.

observed for MRI within this group. Locally, active inflammatory lesions of the sacroiliac joints were reported in 54% and structural lesions in 35%, and 19% and 11% of spinal MRIs were interpreted as showing active spinal inflammation and structural lesions, respectively, whereas central evaluation identified no active inflammatory or structural changes indicative of SpA and instead revealed a high frequency of nonspecific findings, particularly BMO in the sacroiliac joints.

A comparable pattern was observed when comparing patients with an unclear local diagnosis who were subsequently considered as axSpA by central assessment (unclear/axSpA) to those with an unclear local diagnosis in whom axSpA was excluded centrally (unclear/no axSpA). Patients in the unclear/axSpA group (n = 69) as compared to unclear/no axSpA group (n = 287) were slightly younger (mean age 42.8 years vs 45.6 years), more frequently men (58% vs 24%), and more often HLA-B27 positive (61% vs 30%). Elevated CRP levels were present in 39% patients with unclear/axSpA, but only in 19% patients with unclear/no axSpA. Inflammatory back pain was reported by 58% patients with unclear/axSpA, whereas this symptom was more frequently (72%) reported in patients with unclear/no axSpA, again limiting its discriminatory value. Imaging was again the key distinguishing factor. In patients with unclear/axSpA, typical axSpA-compatible changes on imaging supported the central diagnosis. By contrast, patients with unclear diagnosis/no axSpA showed no centrally confirmed active inflammatory or structural changes indicative of SpA, with imaging instead dominated by nonspecific findings, mirroring the pattern observed in the axSpA/no axSpA group.

Exploratory logistic regression analyses assessing factors associated with diagnostic discordance are shown in Table 4. These analyses focused on patients locally diagnosed with axSpA and evaluated factors associated with a central diagnostic conclusion of no axSpA. Older age and higher BMI were associated with a higher probability of diagnostic change from local axSpA to central no axSpA, whereas male sex, HLA-B27 positivity, and higher CRP levels were associated with a lower probability of such change. Among imaging variables, discordant MRI findings of the sacroiliac joints—defined as active inflammatory or structural lesions reported locally but not confirmed centrally—showed the strongest association with diagnostic change. Associations for spinal MRI and radiographic findings were less robust and should be interpreted cautiously because of smaller numbers and wider CIs.

Alternative diagnoses in patients with low level of suspicion of axSpA after central evaluation

In patients in whom axSpA was considered unlikely (no axSpA after central assessment), the most frequent alternative explanations were degenerative spinal disease (n = 172), degenerative sacroiliac joint changes or OCI (n = 200), and a combination of degenerative changes in the sacroiliac joints and spine (n = 123). Less common alternative diagnoses included hip joint pathology (n = 2), trochanteric enthesitis (n = 1), a sacral fracture (n = 2), and conditions within the chronic noninfectious osteomyelitis/SAPHO spectrum (n = 2). Two cases raised concern for infectious spondylodiscitis and required extended diagnostic evaluation, and one case showed imaging features suspicious for bone metastases. In 136 cases, imaging showed no clear inflammatory or structural correlate to explain symptoms. Cases remained unclear mostly due to insufficient imaging information, including limited image quality, incomplete anatomic coverage, and the use of older MRI protocols

Table 3

Imaging characteristics according to the central evaluation of all included patients and patients stratified according to the diagnostic assessment

Variable	All patients (N = 969)	axSpA/axSpA (n = 142)	axSpA/no axSpA (n = 89)	No axSpA/axSpA (n = 27)	No axSpA/no axSpA (n = 265)	axSpA/Unclear (n = 33)	No axSpA/ unclear (n = 27)	Unclear/axSpA (n = 69)	Unclear/no axSpA (n = 287)	Unclear/unclear (n = 30)
Radiographs of sacroiliac joints available, n/N (%)	142/969 (15%)	29/142 (20%)	11/89 (12%)	3/27 (11%)	30/265 (11%)	6/33 (18%)	2/27 (7.4%)	13/69 (19%)	42/287 (15%)	6/30 (20%)
Presence of definite radiographic sacroiliitis ^a , n/N (%)	27/142 (19%)	19/29 (66%)	0/11 (0%)	2/3 (67%)	0/30 (0%)	1/6 (17%)	0/2 (0%)	3/13 (23%)	0/42 (0%)	2/6 (33%)
Radiographs of spine available, n/N (%)	138/969 (14%)	24/142 (17%)	9/89 (10%)	3/27 (11%)	30/265 (11%)	8/33 (24%)	3/27 (11%)	14/69 (20%)	40/287 (14%)	7/30 (23%)
Presence of radiographic spinal changes indicative of axSpA, n/N (%)	17/138 (12%)	9/24 (38%)	0/9 (0%)	1/3 (33%)	0/30 (0%)	1/8 (13%)	1/3 (33%)	3/14 (21%)	1/40 (2.5%)	1/7 (14%)
MRI of sacroiliac joints available, n/N (%)	911/969 (94%)	135/142 (95%)	88/89 (99%)	25/27 (93%)	250/265 (94%)	26/33 (79%)	25/27 (93%)	64/69 (93%)	272/287 (95%)	26/30 (87%)
Presence of any active inflammatory lesions, n/N (%)	538/911 (59%)	113/135 (84%)	51/88 (58%)	17/25 (68%)	124/250 (50%)	14/26 (54%)	9/25 (36%)	55/64 (86%)	144/272 (53%)	11/26 (42%)
Presence of active inflammatory lesions indicative of axSpA, n/N (%)	192/911 (21%)	110/135 (81%)	0/88 (0%)	17/25 (68%)	1/250 (0.4%)	4/26 (15%)	5/25 (20%)	51/64 (80%)	1/272 (0.4%)	3/26 (12%)
BMO fulfilling the ASAS definition of a positive MRI, n/N (%)	189/192 (98%)	110/110 (100%)	0/0 (NA%)	17/17 (100%)	0/1 (0%)	4/4 (100%)	5/5 (100%)	49/51 (96%)	1/1 (100%)	3/3 (100%)
Capsulitis, n/N (%)	33/192 (17%)	22/110 (20%)	0/0 (NA%)	3/17 (18%)	0/1 (0%)	1/4 (25%)	0/5 (0%)	7/51 (14%)	0/1 (0%)	0/3 (0%)
Inflammatory signal in the joint space, n/N (%)	58/192 (30%)	39/110 (35%)	0/0 (NA%)	4/17 (24%)	0/1 (0%)	0/4 (0%)	0/5 (0%)	14/51 (27%)	0/1 (0%)	1/3 (33%)
Inflammation at the site of erosion, n/N (%)	76/192 (40%)	47/110 (43%)	0/0 (NA%)	5/17 (29%)	0/1 (0%)	1/4 (25%)	2/5 (40%)	19/51 (37%)	0/1 (0%)	2/3 (67%)
Enthesitis, n/N (%)	36/192 (19%)	16/110 (15%)	0/0 (NA%)	4/17 (24%)	0/1 (0%)	1/4 (25%)	0/5 (0%)	14/51 (27%)	0/1 (0%)	1/3 (33%)
Joint space fluid, n/N (%)	42/192 (22%)	28/110 (25%)	0/0 (NA%)	5/17 (29%)	0/1 (0%)	1/4 (25%)	1/5 (20%)	6/51 (12%)	0/1 (0%)	1/3 (33%)
Presence of structural lesions indicative of axSpA, n/N (%)	158/911 (17%)	92/135 (68%)	1/88 (1.1%)	15/25 (60%)	1/250 (0.4%)	2/26 (7.7%)	2/25 (8.0%)	43/64 (67%)	0/272 (0%)	2/26 (7.7%)
Erosions, n/N (%)	133/158 (84%)	78/92 (85%)	0/1 (0%)	11/15 (73%)	0/1 (0%)	2/2 (100%)	1/2 (50%)	39/43 (91%)	0/0 (NA%)	2/2 (100%)
Fat lesions, n/N (%)	116/158 (73%)	73/92 (79%)	1/1 (100%)	9/15 (60%)	1/1 (100%)	0/2 (0%)	1/2 (50%)	30/43 (70%)	0/0 (NA%)	1/2 (50%)
Backfill, n/N (%)	54/158 (34%)	33/92 (36%)	0/1 (0%)	5/15 (33%)	0/1 (0%)	0/2 (0%)	0/2 (0%)	16/43 (37%)	0/0 (NA%)	0/2 (0%)
Sclerosis, n/N (%)	93/158 (59%)	56/92 (61%)	1/1 (100%)	9/15 (60%)	0/1 (0%)	1/2 (50%)	1/2 (50%)	24/43 (56%)	0/0 (NA%)	1/2 (50%)
Ankylosis, n/N (%)	20/158 (13%)	11/92 (12%)	0/1 (0%)	3/15 (20%)	0/1 (0%)	0/2 (0%)	1/2 (50%)	5/43 (12%)	0/0 (NA%)	0/2 (0%)
Bone buds, n/N (%)	21/158 (13%)	12/92 (13%)	0/1 (0%)	2/15 (13%)	0/1 (0%)	0/2 (0%)	0/2 (0%)	7/43 (16%)	0/0 (NA%)	0/2 (0%)
MRI of spine available, n/N (%)	712/969 (73%)	98/142 (69%)	64/89 (72%)	18/27 (67%)	200/265 (75%)	27/33 (82%)	22/27 (81%)	52/69 (75%)	204/287 (71%)	27/30 (90%)
Presence of active inflammatory lesions indicative of axSpA, n/N (%)	101/712 (14%)	50/98 (51%)	0/64 (0%)	9/18 (50%)	3/200 (1.5%)	4/27 (15%)	2/22 (9.1%)	25/52 (48%)	2/204 (1.0%)	6/27 (22%)
Presence of structural lesions indicative of axSpA, n/N (%)	81/712 (11%)	39/98 (40%)	0/64 (0%)	4/18 (22%)	2/200 (1.0%)	0/27 (0%)	2/22 (9.1%)	24/52 (46%)	5/204 (2.5%)	5/27 (19%)
Another relevant imaging modality available, n/N (%)	46/969 (4.7%)	4/142 (2.8%)	5/89 (5.6%)	0/27 (0%)	14/265 (5.3%)	2/33 (6.1%)	1/27 (3.7%)	5/69 (7.2%)	13/287 (4.5%)	2/30 (6.7%)
Presence of lesions typical of SpA, n/N (%)	11/46 (24%)	4/4 (100%)	0/5 (0%)	0/0 (NA%)	0/14 (0%)	1/2 (50%)	1/1 (100%)	4/5 (80%)	0/13 (0%)	1/2 (50%)

^a According to the radiographic system of the modified New York criteria.

ASAS, Assessment of SpondyloArthritis International Society; axSpA, axial spondyloarthritis; BMO, bone marrow oedema; MRI, magnetic resonance imaging.

Table 4
Factors associated with central nonconfirmation of a local diagnosis of axial spondyloarthritis

Characteristic	N	Univariable OR (95% CI)	Multivariable Model: MRI SIJ OR (95% CI)	Multivariable Model: MRI Spine OR (95% CI)	Multivariable Model: SIJ Radiographs OR (95% CI)	Multivariable Model: Spine Radiographs OR (95% CI)
Age, y	261	1.06 (1.03-1.08)	1.05 (1.01-1.09)	1.04 (1.00-1.08)	1.17 (1.00-1.50)	1.16 (1.00-1.48)
Male sex	264	0.30 (0.17-0.51)	0.29 (0.11-0.74)	0.19 (0.07-0.46)	0.34 (<0.01-11.3)	2.80 (0.17-99.2)
BMI, kg/m ²	264	1.06 (1.01-1.11)	1.12 (1.00-1.25)	1.09 (1.01-1.20)	1.36 (0.87-2.47)	1.16 (0.77-1.93)
CRP, mg/L	230	0.97 (0.92-1.00)	0.88 (0.81-0.95)	0.97 (0.93-1.00)	1.02 (0.95-1.29)	0.99 (0.95-1.01)
HLA-B27 positivity	247	0.23 (0.13-0.41)	0.20 (0.08-0.50)	0.29 (0.12-0.69)	0.05 (<0.01-0.80)	0.06 (<0.01-0.80)
Active inflammatory lesions on MRI SIJ, local present/central absent	228	18.6 (8.64-44.0)	10.3 (3.54-34.5)	-	-	-
Structural lesions on MRI SIJ, local present/central absent	228	17.8 (6.56-62.4)	6.56 (1.64-32.2)	-	-	-
Active inflammatory lesions on MRI spine, local present/central absent	174	2.90 (1.08-8.04)	-	2.51 (0.43-13.7)	-	-
Structural lesions on MRI spine, local present/central absent	174	3.14 (0.96-11.0)	-	0.79 (0.09-7.79)	-	-
Definite radiographic sacroiliitis, local present/central absent	31	1.70 (0.28-9.38)	-	-	2.07 (0.10-74.9)	-
Radiographic spinal changes indicative of axSpA, local present/central absent	36	4.67 (0.17-130)	-	-	-	11.4 (0.03-not estimable)

N indicates the number of patients with available data for the respective variable.

axSpA, axial spondyloarthritis; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; MRI, magnetic resonance imaging; OR, odds ratio; SIJ, sacroiliac joints.

that did not allow reliable evaluation of active inflammatory or structural lesions.

Agreement and disagreement between the local and central assessments of imaging

Figure 2 and Supplementary Tables S1 to S6 present the agreement and disagreement between the local and central assessments of imaging modalities for signs indicative of axSpA. For radiographic sacroiliitis, concordance on the presence of definite sacroiliitis was limited, with fewer than half of locally positive cases confirmed centrally, while most locally negative

radiographs were also judged negative by the central assessors. A similar pattern was observed for radiographic spinal changes, where locally suspected abnormalities were confirmed in only a minority of cases, whereas central assessors confirmed the absence of lesions in the majority of locally negative evaluations. For MRI of the sacroiliac joints, discrepancies were even more pronounced. When local assessors reported active inflammatory lesions, these were confirmed centrally in less than half of cases, and more than half of locally positive assessments were considered negative by the central panel. Structural sacroiliac joint lesions showed a similar discrepancy, with a substantial proportion of locally positive readings not being reproduced

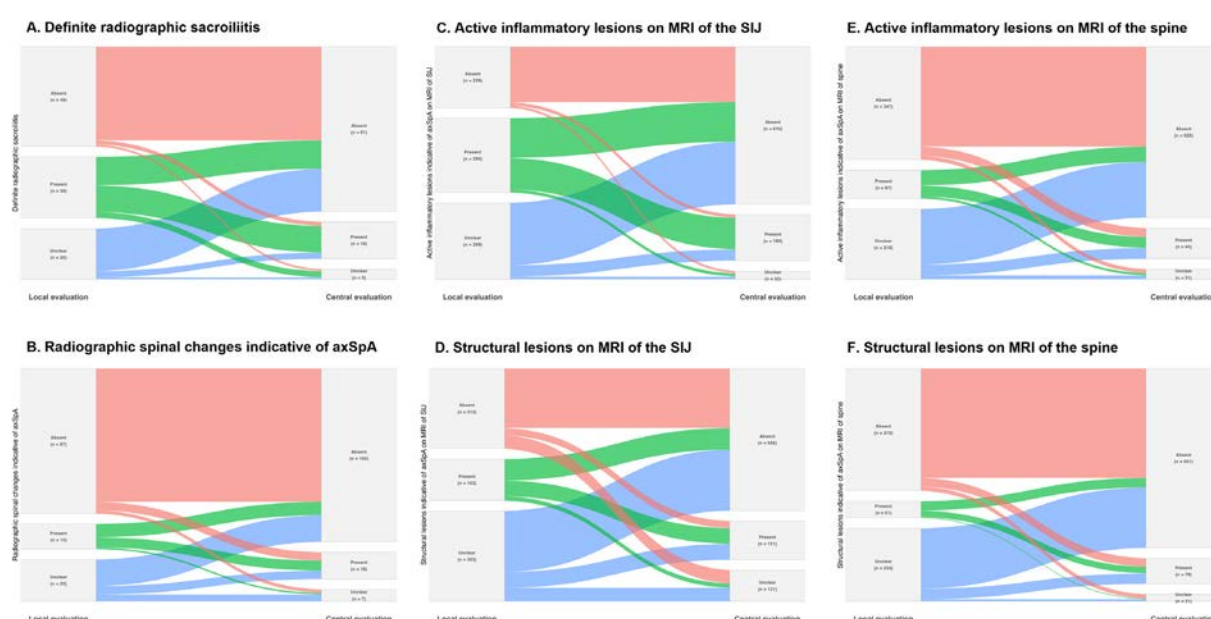


Figure 2. Local vs central assessment of imaging changes indicative of axial spondyloarthritis on radiographs and MRI of the sacroiliac joints and spine. Sankey diagrams show the agreement and disagreement between local and central assessment of imaging findings across 6 imaging domains: definite radiographic sacroiliitis (A), radiographic spinal changes indicative of axSpA (B), active inflammatory lesions on MRI of the sacroiliac joints (C), structural lesions on MRI of the sacroiliac joints (D), active inflammatory lesions on MRI of the spine (E), and structural lesions on MRI of the spine (F). For each imaging domain, local and central evaluations were categorised as present, absent, or unclear.

axSpA, axial spondyloarthritis; MRI, magnetic resonance imaging; SIJ, sacroiliac joints.

centrally, whereas local absence of lesions was confirmed in the majority of cases. For MRI of the spine, the discrepancy pattern remained unchanged. Active inflammatory lesions reported locally were verified centrally in fewer than half of the cases, with the remainder evaluated as negative. Structural spinal lesions demonstrated comparable disagreement, with only a minority of local positive assessments confirmed centrally, whereas local negative assessments showed high concordance with the central reading.

Quality aspects of imaging performed routinely for the assessment of suspected axSpA

We also had the opportunity to examine the quality of axial skeletal imaging that rheumatologists work with in routine practice. Among the pelvic radiographs available for central assessment ($n = 142$), image quality was rated as optimal or sufficient in 136 cases (98%). Quality issues were uncommon; when present, they were mainly related to artefacts or motion blur, suboptimal positioning, or incomplete coverage of the region of interest. Among the patients in whom spine radiographs were available for central assessment ($n = 138$), image quality was rated as optimal or sufficient in 137 cases (99%), whereas only 1 radiograph (0.7%) was considered not sufficient for diagnostic evaluation. Quality issues were infrequent and, when present, most commonly related to artefacts and anatomic coverage. MRI of the sacroiliac joints was available for central assessment in 911 patients. Image quality was rated as optimal or sufficient for the assessment in 877 examinations (96%), whereas 34 scans (3.7%) were considered not sufficient for diagnostic evaluation. Among the examinations with documented limitations, the most frequent problem was missing sequences (most frequently missing erosion-sensitive sequence) or inadequate slice planning, seen in 311 cases (64%). Incomplete depiction of the region of interest as well as artefacts appeared in several constellations and contributed to reduced evaluability in a smaller proportion of scans. MRI of the spine was available for central assessment in 712 patients. Image quality was rated as optimal or sufficient in 706 examinations (99%), whereas 6 scans (0.8%) were considered not sufficient for diagnostic evaluation. Among the examinations with documented limitations, the most frequent issue was missing sequences or inadequate slice planning (most commonly, an inflammation-sensitive sequence was missing in the sagittal plane), observed in 207 cases (90%). Problems related to incomplete depiction of the region of interest occurred in several patterns and accounted for a smaller proportion of limitations.

DISCUSSION

In this first large, prospective, multicentre project evaluating centralised evaluation of patients referred with suspected axSpA, several important observations emerged highlighting substantial challenges in current diagnostic practice. The main findings demonstrate considerable diagnostic discordance between the local and central evaluation. Only about half of the locally diagnosed axSpA cases were considered as axSpA by the central assessment, whereas more than one-third were evaluated as no axSpA. Our data show that axSpA overcall in our data was driven by both clinical context and imaging interpretation. Patients in whom a local axSpA diagnosis was not confirmed centrally had a profile less typical of axSpA, including older age, higher BMI, lower frequencies of male sex and HLA-B27 positivity, and lower inflammatory activity. Importantly, discordant

MRI findings of the sacroiliac joints were the strongest imaging-related factors associated with diagnostic disagreement, reinforcing the central role of expert interpretation of sacroiliac joint MRI in avoiding misdiagnosis.

In contrast, missed diagnoses were relatively uncommon, with only a small proportion of patients initially labelled as no axSpA switching to the axSpA category after central assessment. These findings illustrate a diagnostic landscape dominated more by overdiagnosis and misdiagnosis than by missed diagnoses, which represents a shift from traditional concerns that have historically focused on the problem of delayed diagnosis [16].

The exploratory subgroup analyses by practice setting and by primary specialty of the submitting physician indicate that disagreement between local and central assessment was not confined to one specific clinical setting. Diagnostic discrepancies were observed among cases submitted from both hospital-based centres and community/office-based practices, as well as among cases submitted by rheumatologists and by orthopaedists. Numerically, local axSpA diagnoses submitted by nonrheumatologists and by hospital-based centres were less frequently confirmed by central assessment, but these findings should be interpreted cautiously because of the relatively small number of cases in these subgroups.

It is important to emphasise that the present work did not evaluate the performance of classification criteria, nor did it aim to classify patients according to a predefined set of criteria. The categories used in the local and central assessments reflected diagnostic conclusions in individual patients with suspected axSpA. This distinction is relevant because classification criteria are primarily developed to define relatively homogeneous patient groups for research, whereas diagnosis in clinical practice requires integration of the full clinical context, laboratory findings, imaging results, pretest probability, and alternative explanations for symptoms and imaging abnormalities [8]. In the present project, the central evaluation therefore did not consist of applying classification criteria to the submitted data, but of reassessing the probability of axSpA based on the same clinical and imaging information available through the platform. Accordingly, the observed discordance between local and central assessment should be interpreted as disagreement in diagnostic judgement, largely driven by differences in the interpretation and contextualisation of imaging findings.

The issues and uncertainties surrounding the correct diagnosis of axSpA have been recognised in the recent years [8,15,17]; however, until now, evidence documenting the scale of the problem has largely remained scarce. This report is the first to provide robust, large-scale data demonstrating that misinterpretation of imaging findings, particularly MRI abnormalities of the sacroiliac joints, contributes substantially to diagnostic inaccuracy. The risk of misdiagnosis is particularly high in patients with nonspecific back pain and imaging findings that reflect mechanical stress or degenerative changes rather than immune-mediated inflammation. BMO in the sacroiliac joints, although a key feature of axSpA when present in the appropriate pattern, is not specific for inflammatory disease and can arise in numerous other contexts, including postpartum changes, recreational and professional athletic activity, mechanical overload, and conditions such as OCI [11–14,18,19]. The most recent and largest population-based study to date showed that nonspecific sacroiliac joint BMO is common in the general population, affecting nearly one-third of adults, and is strongly associated with mechanical and load-related factors rather than inflammatory disease [20]. The tendency to interpret any presence of BMO as indicative of axSpA appears to be a major driver of

overdiagnosis in routine practice, as supported by the consistently high frequencies of local MRI positivity that could not be confirmed centrally.

The potential reasons for diagnostic uncertainty are multifactorial. AxSpA lacks a single decisive diagnostic marker, and most clinical features, including inflammatory back pain, elevated CRP levels, and HLA-B27 positivity, are neither highly sensitive nor specific for the disease. In this context, imaging has become central to diagnosis, but its interpretation is complex and highly dependent on expertise. The present data clearly show that imaging interpretation represents a major challenge for routine practice, with substantial challenges affecting interpretation of radiographs and MRI of sacroiliac joints and spine. The majority of locally positive MRI evaluations of the sacroiliac joints and spine were assessed as negative or nonspecific after central evaluation. Suboptimal image quality, including the widespread use of outdated or incomplete MRI protocols, further exacerbates the problem. Missing fluid-sensitive sequences, lack of erosion-sensitive sequences for MRI of sacroiliac joints, suboptimal planes and incomplete coverage of the region of interest were frequent issues that limited the ability to make accurate assessments.

Several strategies may help to overcome these challenges. Training of both rheumatologists and radiologists remains critical, particularly in the interpretation of MRI of the axial skeleton. Over the past decade, substantial progress has been made in differentiating SpA-specific inflammatory and structural lesions from nonspecific or mechanically induced abnormalities. When modern lesion definitions and pattern recognition criteria are applied, most cases can be evaluated with high confidence. Standardisation of imaging requests, including the provision of relevant clinical information to radiologists, may further improve diagnostic accuracy. The recent Assessment of Spondyloarthritis International Society (ASAS) recommendations on requesting [21] and reporting [22] imaging in patients with suspected axSpA represent an important step towards harmonising practice. Similarly, adherence to modern MRI protocols, including the use of erosion-sensitive sequences for sacroiliac joint imaging as recommended jointly by ASAS and the SpondyloArthritis Research and Treatment Network (SPARTAN) [23], is essential to ensure adequate visualisation of active and structural lesions.

The telemedicine-based central support model used in this work also demonstrated the potential benefit of evaluation by physicians with expertise in axSpA including imaging for improving diagnostic accuracy. Central assessment helped to identify cases of misinterpreted imaging, to contextualise clinical and imaging findings appropriately, and in many cases to prevent an erroneous diagnosis of axSpA. In this research project, the central evaluation was advisory and did not replace the responsibility or clinical judgement of the treating physician, who remained free to decide whether and how to incorporate the central opinion into clinical care. Implementation outside a research setting would require a clear legal and organisational framework, including patient consent, secure data transfer, defined responsibilities, and, ideally, integration into structured expert-consultation networks or multidisciplinary rheumatology–radiology boards. This type of structured input may complement routine clinical decision making, especially in settings with limited radiologic expertise in musculoskeletal imaging.

This project has several limitations. For the purpose of the project and the present analysis, the central expert evaluation was used as the reference standard, or operational gold standard, for comparison with the local diagnostic assessment. In

addition, the central evaluation was performed remotely, using the clinical information and imaging studies uploaded through the platform, without direct patient examination, face-to-face history taking, or longitudinal follow-up. Each case was usually reviewed by one of 2 central experts rather than by a formal consensus panel; therefore, inter-reader variability and the potential added value of collegial adjudication could not be fully assessed in the present analysis. We did not collect information on the level of confidence in the imaging evaluation or in the final diagnostic conclusion at either the local or central level. Some clinical and imaging information were incomplete or of insufficient quality in a subset of cases, reflecting the real-world nature of the project. Because imaging modalities were selected by the local physician according to clinical need, we did not formally assess the effect of having complete imaging of all predefined regions and modalities on diagnostic outcome. Because longitudinal follow-up was not available, we could not assess how central recommendations influenced patient management, subsequent diagnostic trajectories, or long-term clinical outcomes. The project was also not designed to quantify the socioeconomic consequences of misdiagnosis, overdiagnosis, or potentially unnecessary treatment. Furthermore, although the telemedicine setting reflects the intended model of remote expert support, it may not capture all elements of routine clinical care. Finally, while we were able to perform an analysis stratified by clinical setting and physician specialty, more granular data on local expertise were not available. Specifically, we did not collect information on duration of practice, individual experience with axSpA, specific training in musculoskeletal imaging, etc.

In summary, this large multicentre telemedicine project demonstrates that misdiagnosis and overdiagnosis—driven mainly by misinterpretation of nonspecific or mechanically induced MRI findings—represent a major challenge in the diagnostic workup of patients with suspected axSpA. Improving training in imaging interpretation, standardising imaging acquisition and reporting, applying modern MRI protocols, and developing telemedicine consultation pathways are essential steps toward enhancing diagnostic accuracy in routine care.

Looking ahead, technological developments may further enhance diagnostic accuracy. Artificial intelligence-based interpretation of radiographs and MRI is emerging as a promising tool to support clinicians in distinguishing SpA-related imaging abnormalities from nonspecific findings [24]. Molecular imaging targeting specific, inflammation-related molecules (such as relevant cytokines) may also contribute to differentiating immune-mediated inflammation from mechanical or degenerative conditions in the future [9]. These technologies, when combined with structured training and standardised imaging protocols as well as expert support in challenging cases, may provide a more robust framework for diagnostic decision making in axSpA.

Competing interests

DP has received research support from AbbVie, Bristol Myers Squibb, Eli Lilly, Johnson and Johnson, Novartis, Pfizer, and UCB; consulting fees from AbbVie, Eli Lilly, GlaxoSmithKline, Greywolf Therapeutics, Johnson and Johnson, Merck, Moonlake, Novartis, Pfizer, and UCB; and speaker fees from AbbVie, Canon, Celltrion, Eli Lilly, Globemed, Johnson and Johnson, Medscape, Novartis, Peervoice, Pfizer, and UCB. He serves as a member of executive committee of ASAS. M Kämmerer is an employee of VISUS Health IT GmbH. DG is an employee of Novartis. Xenofon

Baraliakos declares research support from AbbVie, Celltrion, Janssen, Moonlake, and Novartis as well as consulting fees from AbbVie, Advanz, Alexion, Alphasigma, Amgen, AstraZeneca, BMS, CESAS, Celltrion, Clarivate, Dr. Reddy's, Galapagos, Greywolfe, Globemed, J&J, Lilly, Moonlake, Novartis, Peervoice, Pfizer, Roche, Sandoz, Springer, STADA, Takeda, UCB, and Zuelig. All other authors declare they have no competing interests

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Contributors

DP and XB planned the project, supervised it during conduction, and generated central evaluation data; DP, IR and XB performed the data analysis and drafted the manuscript. All authors contributed to the interpretation of the results and revising the manuscript for important intellectual content. All authors have approved the final version of the manuscript.

Ethics approval

This work was conducted in accordance with the Declaration of Helsinki and approved by the respective institutional review boards or ethics committees of participating centres. All participants provided written informed consent prior to inclusion. Data collection, transfer, and storage were fully complied with GDPR and national regulations governing medical research and telemedicine.

Data availability statement

The original data of this work is not publicly available. Specific research requests can be considered and should be addressed to the authors.

Supplementary materials

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.ard.2026.06.019](https://doi.org/10.1016/j.ard.2026.06.019).

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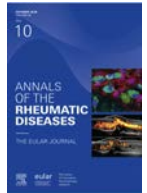
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Axial spondyloarthritis

EXTL3 dysfunction identified as a driver of aberrant bone development in severe familial ankylosing spondylitis

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ABSTRACT

Objectives: Pathological ossification leading to spinal ankylosis is a hallmark of ankylosing spondylitis (AS), yet its molecular mechanisms remain poorly understood. We investigated the genetic basis and functional consequences of severe familial AS to identify pathways driving structural progression.

Methods: Genome-wide linkage analysis and whole-exome sequencing were performed in a multiplex family with severe AS. A heterozygous knock-in mouse carrying the identified EXTL3 variant was generated and characterized using microCT, histology, transcriptomics, primary osteoblast and chondrocyte cultures, and biochemical analyses of heparan sulfate (HS) metabolism.

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Results: A rare missense variant in *EXTL3*, a key enzyme involved in HS biosynthesis, was identified in affected family members. *Extl3^{mut/+}* mice developed sacroiliac structural abnormalities, osteoid accumulation and early joint bridging, together with impaired trabecular bone architecture. Mechanistically, the mutation accelerated chondrocyte hypertrophy while delaying osteoblast maturation and mineralization. These changes were associated with activation of Wnt signalling, increased HS accumulation, enhanced heparanase expression and altered glycosaminoglycan homeostasis. Collectively, these findings identify *EXTL3*-mediated HS dysregulation as a novel mechanism linking abnormal bone remodelling to pathological ossification.

Conclusions: Our study identifies *EXTL3* as a genetic modifier of structural severity in AS and reveals a previously unrecognised role for HS metabolism in pathological ossification. These findings support a model in which HLA-B27 primarily confers disease susceptibility, whereas rare variants such as *EXTL3* contribute to structural progression, highlighting HS-related pathways as potential therapeutic targets.

What is already known on this topic

- Ankylosing spondylitis (AS) is characterised by pathological new bone formation, leading to spinal ankylosis.
- Structural progression may occur despite effective control of inflammation.
- HLA-B27 explains disease susceptibility but not the severity of structural damage.
- The molecular mechanisms driving pathological ossification remain poorly understood.

What this study adds

- We identified a rare *EXTL3* variant in a family with severe AS.
- The results showed that *EXTL3* acts as a genetic modifier of structural severity, independent of HLA-B27.
- We further demonstrated that heparan sulfate dysregulation could drive abnormal ossification.
- Functional data showed uncoupled bone remodelling, with increased chondrocyte hypertrophy and impaired osteoblast mineralisation.

How this study might affect research, practice or policy

- This study identifies heparan sulfate metabolism as a potential therapeutic target in AS.
- It supports the concept that structural progression requires specific treatment strategies beyond controlling inflammation.
- Our study suggests that genetic factors beyond HLA-B27 may help stratify patients at risk of severe structural damage.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease of young adults that can cause major disability through structural changes of the sacroiliac joints (SIJs) and spine [1]. In its most severe form, ankylosing spondylitis (AS), progressive ossification leads to complete spinal ankylosis and the characteristic ‘bamboo spine’. Although biologic therapies targeting tumour necrosis factor (TNF) or interleukin (IL)-17 have transformed disease management [2], a subset of patients still experience severe structural progression despite optimal inflammatory control. In the SURPASS trial, ~8% of patients developed radiographic progression under biologic therapy [3], suggesting that ossification may, at least in part, proceed through pathways not directly dependent on TNF or IL-17. Thus, although inflammation remains the dominant driver of disease, additional mechanisms are likely to contribute specifically to structural progression. Current models implicate dysregulated endochondral ossification as the basis for new bone formation, yet the molecular drivers remain poorly understood [4].

In this study, we studied a family with multiple cases of severe AS marked by SIJ fusion and advanced spinal ankylosis. Genetic analyses identified a rare heterozygous variant in *EXTL3*, a glycosyltransferase involved in heparan sulfate (HS) biosynthesis. Notably, related family members *EXT1* and *EXT2* are mutated in hereditary multiple exostoses [5], a genetic disorder marked by aberrant bone growth. Moreover, additional data support a potential role for *EXTL3* in skeletal remodelling [6–9]. To explore the functional consequences of this variant, we generated an *EXTL3* knock-in (*Extl3^{mut/+}*) mouse model and performed comprehensive phenotypic and molecular analyses. We uncovered dual and opposing abnormalities in chondrocytes and osteoblasts, consistent with impaired HS metabolism and disrupted coordination of bone remodelling. Together, our findings reveal a previously unrecognised mechanism driving severe ossification in AS and establish the concept that rare variants in glycosaminoglycan (GAG) biosynthesis genes can function as genetic modifiers, distinguishing susceptibility from structural disease severity in axSpA.

METHODS

A detailed description of the methods and materials is available in the Supplementary Methods.

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

RESULTS

Rare *EXTL3* variant associated with severe AS in an HLA-B27–positive family

We investigated a multiplex family displaying striking clustering of severe AS, with bamboo spine in several siblings in order to elucidate genetic determinants of structural disease severity (Fig 1A,B, and Supplementary Fig S1). All family members underwent systematic clinical evaluation and standardised radiographic imaging of the pelvis and spine. This evaluation demonstrated a pattern of ossifying involvement characteristic of AS, including the presence of bridging syndesmophytes (patients II-3, II-4, and II-6); ossification of the posterior spinal elements, including the spinal facet joints and/or spinal ligament ankylosis (patients II-1, II-3, II-4, and II-6); ossification of the interspinous ligament (patients II-3 and II-4); and complete fusion of the SIJs (patients II-1, II-3, II-4, and II-6). In contrast, patient II-2 presented with erosive sacroiliitis without vertebral involvement or SIJ fusion. HLA-B27 testing revealed that all siblings carried the allele, inherited from their

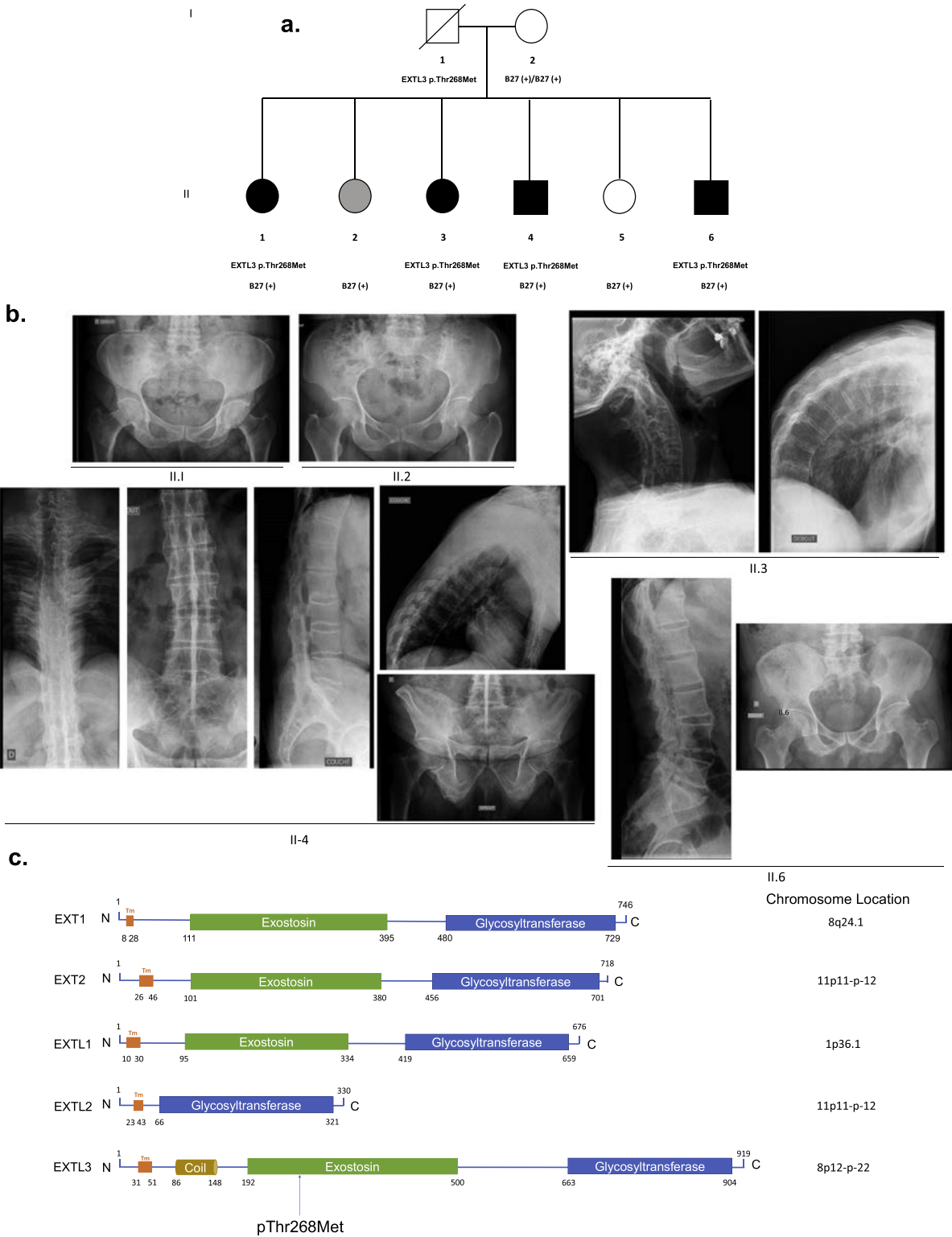


Figure 1. Identification of a new variant in *EXT3*. A, Family pedigree. Black-filled symbols indicate family members with ankylosing spondylitis and a bamboo spine phenotype. Grey-filled symbols indicate family members with axial spondyloarthritis. B, X-rays of a patient in the family showing a bamboo spine phenotype. C, Exostosin gene family.

homozygous mother (patient I.2). The parents were not consanguineous; the mother was of Armenian descent, and the father, of Iranian origin, had died at age 41 years of an unidentified kidney disease.

Familial clustering of severe spondyloarthritis (SpA) with a bamboo spine is rare. Although the pedigree initially suggested possible maternal transmission, this hypothesis was not supported clinically, as the mother, despite being homozygous for HLA-B27, remained entirely unaffected. This observation argued

against a dominant maternally transmitted effect and reinforced that HLA-B27 alone does not determine structural severity. Instead, the data supported a dual-component model in which HLA-B27 provided baseline susceptibility (as illustrated by patient II.2), whereas an additional nonmaternal genetic factor appeared necessary to drive the severe structural phenotype observed in the other siblings (Fig 1A).

To identify a locus underlying increased structural severity, we performed genome-wide single-nucleotide polymorphism

(SNP) genotyping followed by multipoint linkage analysis. The absence of parental consanguinity ruled out a recessive inheritance model. Combined with the mother's unaffected status despite homozygous HLA-B27 carriage, the findings supported a model in which a paternally inherited variant, acting on an HLA-B27 background, contributed to the marked structural disease.

Genetic analysis proceeded through 2 complementary strategies: linkage analysis in 2 siblings with a bamboo spine (patients II.3 and II.4) and whole-exome sequencing of the most severely affected sibling (patient II.3) (Fig 1A). Comparative integration of the datasets identified 7 heterozygous candidate variants. Five were inherited from the mother and, in light of the genetic and clinical arguments outlined above, were deprioritised. Two paternally inherited variants remained: 1 in *EXTL3*, a member of the exostosin gene family [10], and 1 in *GRB7*, a gene involved in epidermal growth factor receptor (EGFR)-related signalling pathways [11].

Although both variants met standard filtering criteria, *GRB7* was not considered a strong biological candidate, as it has no known role in osteoblast or chondrocyte physiology, and its documented functions relate largely to tumorigenesis, cell migration, and growth factor signalling rather than skeletal development. In contrast, *EXTL3* presented a stronger functional rationale, as 2 related family members—*EXT1* and *EXT2*—are well-established causal genes for hereditary multiple exostoses, a disorder characterised by abnormal endochondral ossification and ectopic bone formation. This genetic and functional convergence strongly supported *EXTL3* as the most plausible driver of the severe structural phenotype and justified its prioritisation for downstream mechanistic investigation.

We identified a rare missense variant in *EXTL3* (minor allele frequency of 0.00025 in the Exome Aggregation Consortium [ExAC] database), consistent with the family's inheritance pattern. *EXTL3* has not previously been implicated in AS or related disorders. The gene spans 60 kb on chromosome 8p12-p22 (Fig 1C) and lies outside regions identified in prior linkage analyses of AS. It has also remained undetected in genome-wide association studies, most likely due to the extremely low frequency of the identified variant. The missense substitution (c.803C>T; p.Thr268Met; rs200615501) is predicted to impair protein function and is classified as deleterious by Sorting Intolerant From Tolerant (SIFT) [12] and possibly damaging by PolyPhen-2 [13]. Notably, the affected threonine residue is highly conserved across mammalian species, including chimpanzee, mouse, rabbit, pig, dog, cat, and horse, underscoring its likely functional importance (Ensembl Genome Browser, rs200615501) [14].

Taken together, the consistent presence of this variant in family members with severe AS, combined with its predicted deleterious effect and strong evolutionary conservation, supports a potential role for *EXTL3* in driving the severe structural manifestations of the disease.

Having established a potential role for a rare paternally inherited variant in *EXTL3*, we next sought to determine its functional consequences *in vivo*.

Generation of a mouse model harbouring a point mutation in the *Extl3* gene

To further investigate the functional consequences of the *EXTL3* rare variant identified in the family with severe AS, we developed a mouse model carrying an identical point mutation in the murine *Extl3* gene using homologous recombination

(Supplementary Fig S2). Mice heterozygous for the rare *Extl3* variant are hereafter referred to as *Extl3*^{mut/+} throughout the manuscript.

Histological and micro-computed tomography (microCT) analysis reveals SIJ defects in *Extl3*^{mut/+} mice

To assess whether the rare *Extl3* variant alters SIJ architecture over time, we performed longitudinal *in vivo* microCT imaging of *Extl3*^{mut/+} and wild-type (WT) littermates (*Extl3*^{+/+}) at 6 and 12 months of age (Fig 2A). A segmentation-based approach defined distinct SIJ compartments, including trabecular bone, cortical margins, joint space, and central islets, thereby allowing precise assessment of cortical integrity and mineralisation (Fig 2B).

At 6 months, *Extl3*^{mut/+} mice displayed defective mineralisation of the sacral slope and cortical erosions of the iliac slope, as confirmed by Von Kossa staining (Fig 2C,D). SIJ scoring identified significant abnormalities in both sacral and iliac cortical bone (Fig 2E). At 12 months, global SIJ scores no longer differed significantly between groups (Supplementary Fig S3A,B), but cortical osteoid inclusions were prominent in *Extl3*^{mut/+} mice. These inclusions were significantly larger by microCT (Fig 2F) and corresponded histologically to osteoid tissue (Fig 2G,H).

At this stage, articular bridges were also observed in the SIJs of *Extl3*^{mut/+} mice: 7/21 (33.3%) compared with 2 of 18 (11.1%) in WT mice. Although bridges in WT mice were confined to mechanically loaded anterior extremities, *Extl3*^{mut/+} mice developed more extensive bridging (Fig 2D). Histological confirmation was obtained in 2 of 6 SIJs, with no bridges at 6 months.

Trabecular bone quality was significantly impaired in *Extl3*^{mut/+} mice at 6 months (Fig 2I–M) and remained altered at 12 months (Supplementary Fig S3D–H). These abnormalities were associated with progressive peritrabecular and subchondral accumulation of osteoid tissue, as reflected by a significantly increased osteoid-to-mineralised tissue ratio at both time points (Supplementary Fig S4A,B).

Taken together, these findings indicate that the *Extl3* variant compromises skeletal integrity of the SIJs. We therefore next investigated the underlying cellular mechanisms, focusing on cartilage and chondrocyte differentiation.

Cartilage disorganisation and accelerated endochondral ossification in *Extl3*^{mut/+} mice

After characterising bone abnormalities in *Extl3*^{mut/+} mice, we next investigated potential alterations in cartilage structure within the SIJs. The joint lesions observed in *Extl3*^{mut/+} mice were strongly indicative of enhanced endochondral ossification.

Lillie's trichrome and Safranin-O staining showed a disorganisation of chondrocyte columns (Fig 3A) and more intense Alcian blue staining (Fig 3B), consistent with early differentiation of chondrocytes into hypertrophic chondrocytes. The cartilage thickness at the sacral slope of the SIJs was significantly increased compared with *Extl3*^{+/+} mice (Fig 3C). Alkaline phosphatase (ALP) staining confirmed the mineralisation capacity of these hypertrophic chondrocytes, a hallmark of endochondral ossification (Fig 3D,E). Further, Safranin-O staining revealed deeper penetration of chondrocyte columns into the subchondral bone, reinforcing evidence of enhanced endochondral ossification (Fig 3B). This altered cartilage architecture corresponded to a significant disruption in cartilage composition. Immunohistochemical analysis showed increased

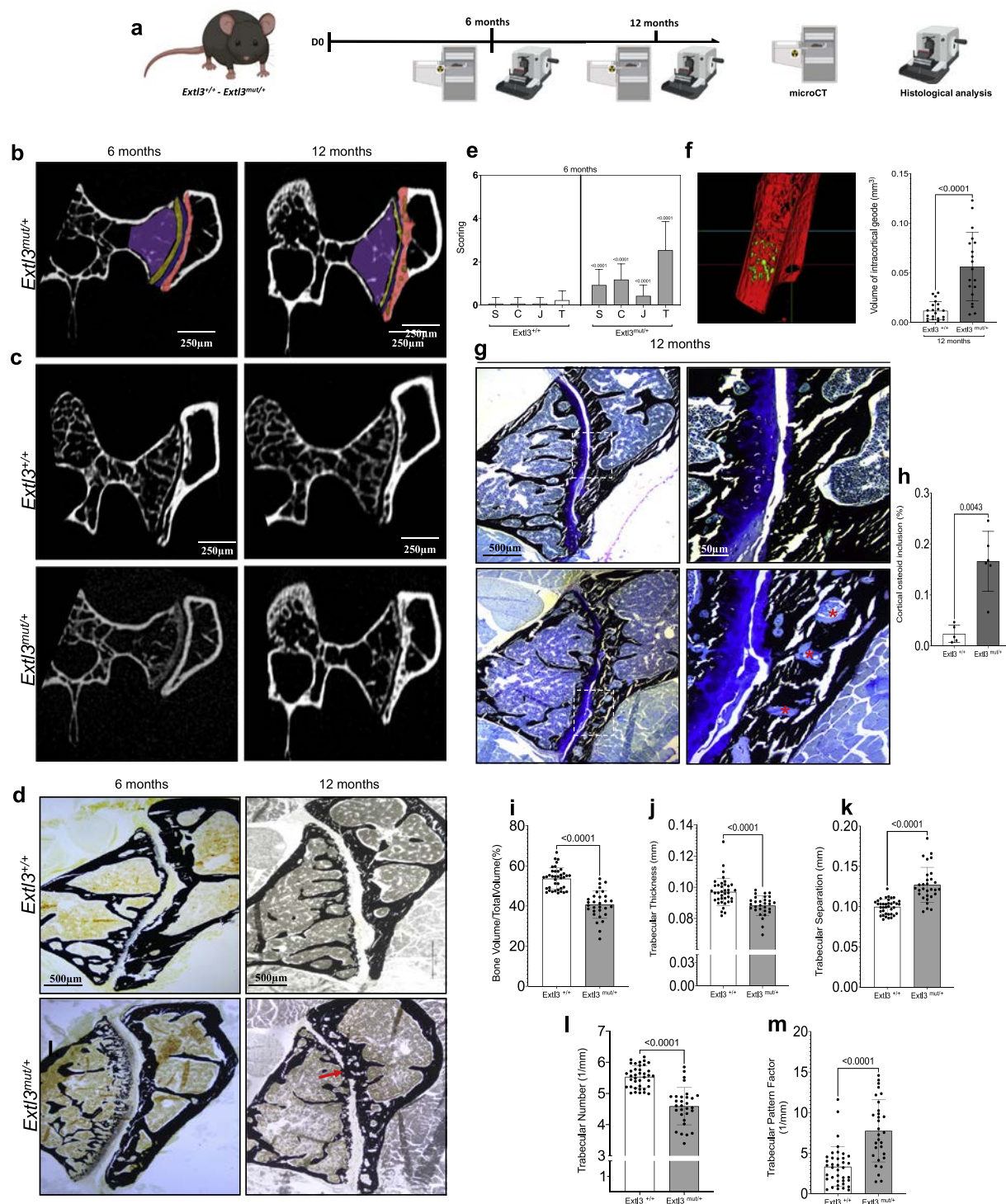


Figure 2. Histological and microCT (micro-computed tomography) analyses reveal sacroiliac joint (SIJ) defects in *EXTL3* knock-in (*Extl3*^{mut/+}) mice. A, Schematic overview of the experimental design of the *Extl3*^{mut/+} mouse model. B, MicroCT segmentation of the different compartments of the SIJ: sacral trabecular bone (purple), sacral cortical bone (yellow), joint space (blue), iliac cortical bone (pink), and intracortical inclusions of the iliac bone (green). C, Representative microCT sections of *Extl3*^{mut/+} and *Extl3*^{+/+} (wild-type littermates) SIJs at 6 and 12 months. D, Von Kossa staining of *Extl3*^{mut/+} and *Extl3*^{+/+} SIJs at 6 and 12 months. Red arrow indicates a SIJ bridge. E, Composite scoring of osteoarticular SIJ lesions in *Extl3*^{mut/+} mice (n = 7) compared with *Extl3*^{+/+} mice (n = 7) at 6 months of age, assessing the sacral slope (S), iliac cortical slope (C), joint space (J), and total score (T). Data are mean ± SD; significance was determined by a 2-tailed Mann-Whitney U test ($p < .05$). F, Three-dimensional reconstruction of intracortical islets in the iliac slope of the SIJs and volumetric quantification of SIJ lesions in *Extl3*^{mut/+} (n = 10) and *Extl3*^{+/+} (n = 10) mice at 12 months. Data are mean ± SD; significance was determined by 2-tailed Mann-Whitney U test ($p < .05$). G, Von Kossa-Toluidine blue staining of the SIJs in *Extl3*^{mut/+} and *Extl3*^{+/+} mice at 6 and 12 months, focused on the iliac cortical slope. Red asterisks indicate osteoid inclusions. H, Quantification of intracortical inclusions in the iliac bone in *Extl3*^{mut/+} (n = 6) and *Extl3*^{+/+} (n = 5) mice. Data are mean ± SD; significance was determined by a 2-tailed Mann-Whitney U test ($p < .05$). I–M, Trabecular bone parameters of the sacral slope of the SIJs in *Extl3*^{mut/+} (n = 15) and *Extl3*^{+/+} (n = 19) mice at 6 months: (I) bone volume/total volume, (J) trabecular thickness, (K) trabecular separation, (L) trabecular number, and (M) trabecular pattern factor. Data are mean ± SD; 2-tailed Student's *t*-test ($p < .05$).

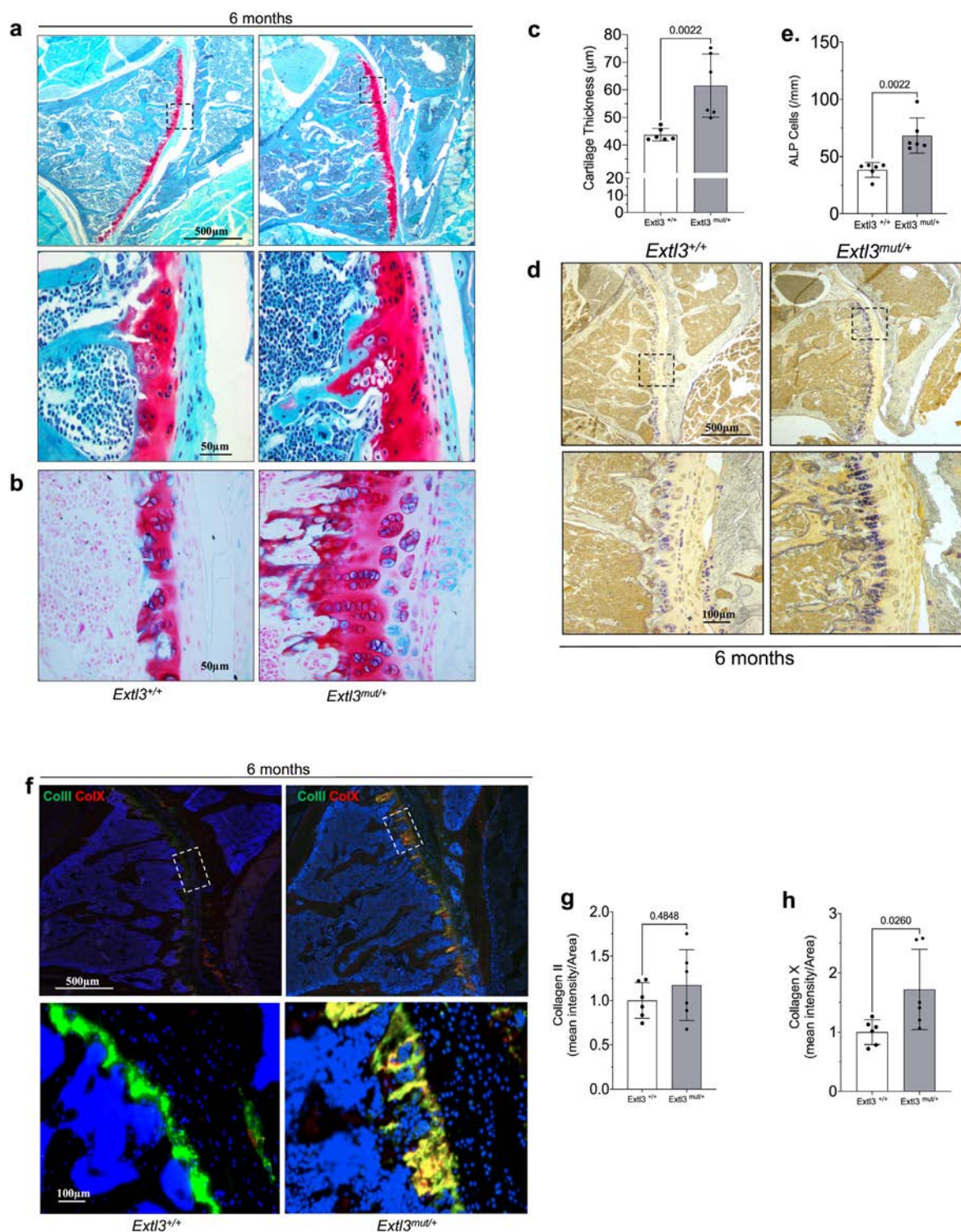


Figure 3. Impaired chondrocyte differentiation in *Extl3* knock-in (*Extl3^{mut/+}*) mice. **A**, Lillie's trichrome and Safranin-O staining of the sacroiliac joints (SIJs) at 6 months in *Extl3^{mut/+}* and *Extl3^{+/+}* (wild-type littermates) mice. **B**, Alcian blue and Safranin-O staining of the SIJs at 6 months in *Extl3^{mut/+}* and *Extl3^{+/+}* mice. **C**, Quantification of hyaline cartilage thickness of the sacral slope of the SIJs at 6 months in *Extl3^{mut/+}* (n = 6) and *Extl3^{+/+}* (n = 6) mice. Data are mean ± SD; 2-tailed Mann-Whitney U test ($p < .05$). **D**, Alkaline phosphatase (ALP) staining of the SIJs in 6-month-old *Extl3^{mut/+}* and *Extl3^{+/+}* mice. **E**, Quantification of ALP-positive chondrocytes (cell number per SIJ length) in the hyaline cartilage of the sacral slope in 6-month-old *Extl3^{mut/+}* (n = 6) and *Extl3^{+/+}* (n = 6) mice. Data are mean ± SD; 2-tailed Mann-Whitney U test ($p < .05$). **F**, Immunohistochemistry of collagen II (green) and collagen X (red) in the SIJs of 6-month-old *Extl3^{mut/+}* and *Extl3^{+/+}* mice. Quantification of **(G)** collagen II and **(H)** collagen X immunofluorescence in the SIJs of 6-month-old *Extl3^{mut/+}* (n = 6) and *Extl3^{+/+}* (n = 6) mice using Fiji software. Data are mean ± SD; 2-tailed Mann-Whitney U test ($p < .05$).

expression of collagen X, a marker of hypertrophic chondrocytes, whereas the levels of collagen II, a classical marker of immature chondrocytes, remained unchanged. Notably, robust colocalisation of collagen II and collagen X was observed,

reflecting disorganisation of the collagenous matrix and suggesting altered chondrocyte differentiation and/or collagen synthesis, leading to a profound alteration in cartilage structure unique to *Extl3^{mut/+}* mice (Fig 3F-H and Supplementary Fig S5A).

Wnt pathway activation underlies premature chondrocyte hypertrophy in *Extl3*^{mut/+} mice

To explore the molecular mechanisms underlying this phenotype, primary cultures of immature articular chondrocytes (IMACs) obtained from *Extl3*^{mut/+} mice and *Extl3*^{+/+} littermates were sequentially differentiated into prehypertrophic and hypertrophic chondrocytes, and RNA sequencing (RNA-Seq) was performed (Fig 4A). Functional enrichment analysis based on Gene Ontology (GO) terms revealed upregulation of pathways related to endochondral ossification and extracellular matrix organisation (Fig 4B) in the prehypertrophic state in mice with mutations. Strikingly, heatmap analysis of differentially expressed genes in *Extl3*^{mut/+} prehypertrophic chondrocytes revealed an accelerated differentiation profile, with hypertrophic markers such as collagen X and MMP13 already expressed at this intermediate stage (Fig 4C).

These findings were further validated by quantitative reverse transcription polymerase chain reaction (qRT-PCR) in 5 to 6 independent primary chondrocyte cultures. Accelerated differentiation was evident by increased messenger RNA (mRNA) expression of prehypertrophic markers (osteomodulin) in IMACs and hypertrophic markers (collagen X [*Col10a1*] and *Bglap*) in IMACs and prehypertrophic chondrocytes (Fig 4D and Supplementary Fig S6A–F). Notably, the hypertrophic stage showed no further increase in marker expression in *Extl3*^{mut/+} cells compared with *Extl3*^{+/+} cells, suggesting that accelerated differentiation is confined to earlier stages. Enhanced collagen X gene expression at the IMACs stage was associated with increased immunohistochemical staining of collagen X (Fig 3F–H).

This acceleration of chondrocyte differentiation was accompanied by Wnt pathway activation from the immature stage. At the hypertrophic stage, RNA-Seq revealed marked activation of the Wnt pathway, evidenced by the downregulation of its natural inhibitors, *Dkk-1* (log2 fold change: −0.68; *p* = .014) and *Sost* (log2 fold change: −1.15; *p* = 9.70×10^{-19}) (Supplementary Fig S7A,B). Immunohistochemistry revealed increased TCF1 staining in the chondrocytes and subchondral areas of the SIJs in *Extl3*^{mut/+} mice, confirming robust Wnt pathway activation during differentiation (Fig 4E). qRT-PCR demonstrated increased expression of canonical Wnt pathway components in *Extl3*^{mut/+} prehypertrophic chondrocytes, including β -catenin (*Ctnnb1*), *Tcf7*, *Wnt4*, *Wnt5a*, and *Wnt5b* (Fig 4F and Supplementary Fig S8A–G). Notably, *Dkk-1* expression fluctuated during differentiation, increasing at the IMAC and prehypertrophic stages but decreasing at the hypertrophic stage, consistent with RNA-Seq data (Supplementary Fig S7A). These fluctuations likely reflect progressive inactivation of the Wnt pathway as differentiation advances.

Accelerated chondrocyte differentiation in *Extl3*^{mut/+} mice was associated with phenotypic alterations beyond the SIJs, including ossification delays in the coxofemoral joint and abnormalities in spinal development (Supplementary Figs S9–S14). Together, these findings provided compelling evidence of dysregulated endochondral ossification driven by altered Wnt signalling. The acceleration of chondrocyte hypertrophy pointed to dysregulated endochondral ossification. To complement this, we next examined osteoblast differentiation and mineralisation.

Delayed osteoblast differentiation

To further investigate alterations in trabecular bone architecture in *Extl3*^{mut/+} mice, we conducted primary osteoblast cultures (Fig 5A) and assessed mineralisation at 2 differentiation

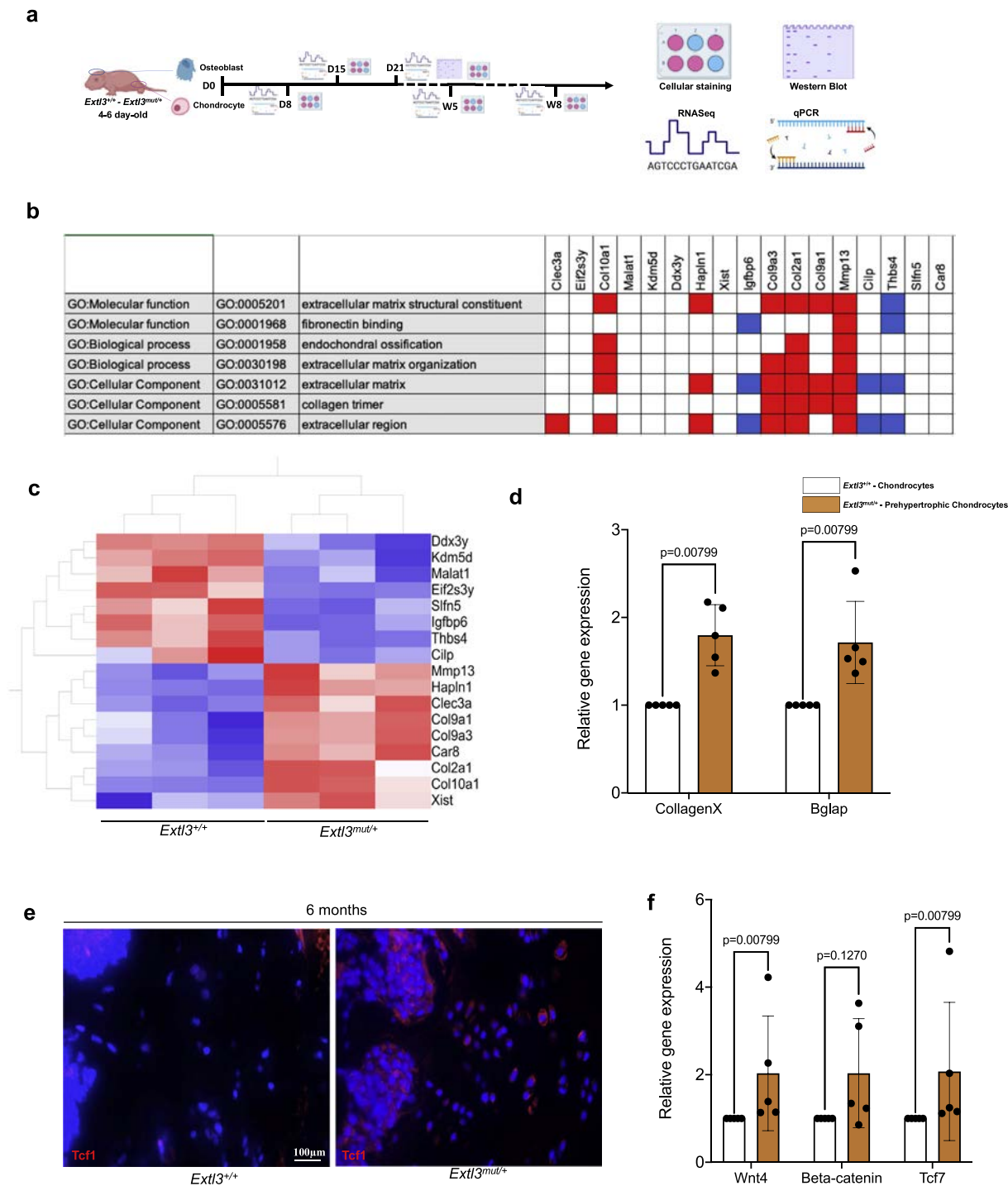
stages: day 15 (D15) and day 21 (D21) (Fig 5A). *Extl3*^{mut/+} osteoblasts demonstrated a significant reduction in mineralisation capacity, as indicated by decreased ALP activity and reduced Alizarin red staining (Fig 5A,B). The mineralisation defect observed in osteoblast cultures was accompanied by a delay in osteoblast differentiation, suggesting a closely linked impairment of both maturation and matrix mineralisation processes. Indeed, exploratory RNA-Seq analyses on D21 revealed marked underexpression of *Bglap*, along with significantly reduced expression of the key endogenous Wnt pathway inhibitors, *Dkk1* (log2 fold change: −4.02; *P* = 8.34×10^{-136}) and *Sost* (log2 fold change: −4.46; *p* = 3.82×10^{-37}) (Fig 5C and Supplementary Fig S15E). Complementary qRT-PCR analyses corroborated these findings, showing significant downregulation of differentiation markers such as *Bglap* and *Alpl*, which encode ALP, on D15 and D21 (Fig 5D and Supplementary Fig S15F–I).

Interestingly, our data pointed to transient activation of the Wnt pathway in *Extl3*^{mut/+} osteoblasts. On D15, we observed upregulation of *Tcf7* expression by qRT-PCR (Fig 5E), but this was not sustained on D21. Consistently, we observed increased TCF1 staining in the subchondral region of the SIJs, possibly related to its higher expression in osteoblastic precursors (Fig 4D). This early activation was accompanied by increased protein levels of TCF1 and β -catenin on D21, as shown by Western blot analysis (Fig 5F–H). The decrease in *Dkk1* expression on D21 further supports a shift in Wnt signalling activity over time. Together, these findings suggest that dysregulated Wnt signalling and delayed osteoblast differentiation contribute to the mineralisation defect observed in osteoblast cultures from *Extl3*^{mut/+} mice. The paradoxical combination of accelerated chondrocyte hypertrophy and delayed osteoblast maturation suggests a common metabolic defect. Given the known role of *EXTL3* in HS biosynthesis, we next investigated GAG metabolism.

Characterisation of HS defects

EXTL3 is essential for HS synthesis, as it encodes a glycosyltransferase that catalyses the addition of the first N-acetylglucosamine (GlcNAc) residue to the tetra-saccharide linkers attached to the core protein of proteoglycans. HSs are the most complex sulfated polysaccharides of the GAG family, which also includes chondroitin sulfate (CS), keratan sulfate, dermatan sulfate, and hyaluronan (HA). Like other GAGs, HS chains are covalently linked to proteoglycans located at the cell membrane or within the extracellular matrix. Owing to their chemically modified and structurally diverse composition, HS chains interact with heparin-binding proteins, including growth factors, cytokines, and enzymes, thereby regulating numerous physiological and pathological processes, including the Wnt signalling pathway. *EXTL3* enzymatic activity plays a critical role in directing GAG biosynthesis towards HS chain formation [9]. Mutations in *EXTL3* are expected to dysregulate HS synthesis and alter the balance of CS chains, thereby disrupting cellular and tissue homeostasis [9]. Our transcriptomic and protein expression analyses revealed no changes in the expression of *Extl3* or other genes involved in HS biosynthesis (Supplementary Fig S16G,H).

To investigate the distribution and quantity of HS, we performed an immunohistochemical analysis of the SIJs. Our findings revealed an increase in HS staining with the 10E4 epitope in 2 distinct anatomical regions: the peritrabecular area and the subchondral bone (Fig 6A,B). Thus, the increased HS labelling may reflect either a higher absolute abundance of HS or alterations in its composition, such as enhanced N-sulfation of HS



chains, a ubiquitous modification of HS. In contrast to HS, CS immunostaining with the IO3H10 epitope in the SIJs showed no genotype-dependent differences (Fig 6C). However, the IO3H10 antibody used for CS staining specifically recognises 6-O-sulfated epitopes and does not allow for absolute quantification of all CS chain types.

HS and osteoid tissue colocalised in peritrabecular and subchondral regions, as confirmed by osteopontin costaining, suggesting osteoblast-derived HS production in *Extl3^{mut/+}* mice (Fig 6D).

In *Extl3^{mut/+}* mice, we observed a slightly increased heparanase (HPSE) labelling, predominantly in the subchondral bone.

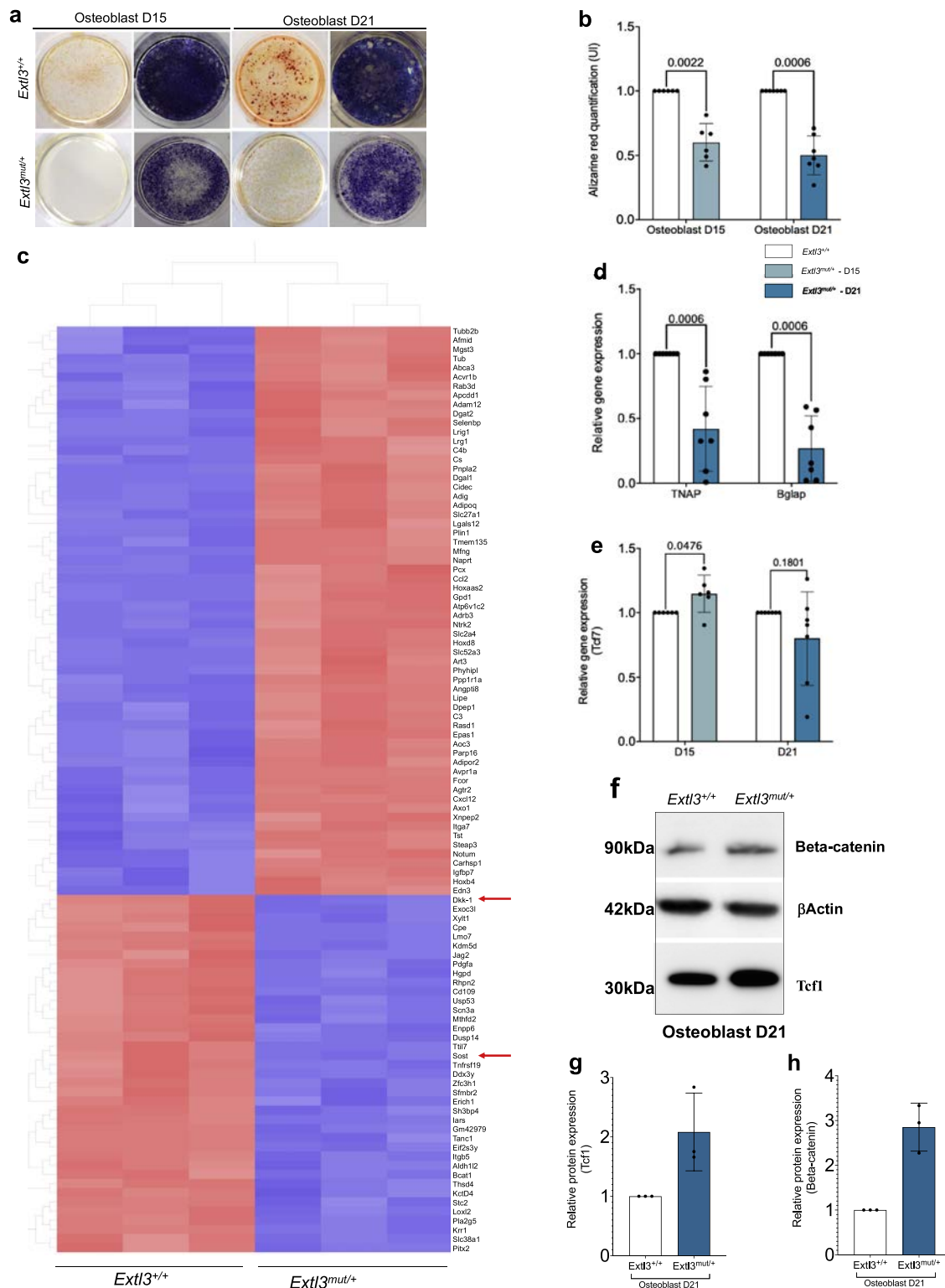


Figure 5. Decreased mineralisation capacity of osteoblast cultures from *EXTL3* knock-in (*Extl3*^{mut/+}) mice. A, Alkaline phosphatase (ALP; left) and Alizarin red (right) staining of primary osteoblast cultures from *Extl3*^{+/+} and *Extl3*^{mut/+} mice on day 15 (D15) and day 21 (D21). B, Spectrophotometric quantification of Alizarin red staining in *Extl3*^{+/+} (wild-type littermates) (n = 6) and *Extl3*^{mut/+} (n = 6) primary osteoblast cultures on D15 and D21. Data are mean $\pm$ SD; 2-tailed Mann-Whitney U test ($p < .05$). C, Heatmap of differentially expressed genes (RNA sequencing [RNA-Seq]) in *Extl3*^{+/+} and *Extl3*^{mut/+} primary osteoblast cultures on D21; arrows indicate the expression of *Dkk1* and *Sost*. D, qRT-PCR (quantitative reverse transcription polymerase chain reaction) of *Alpl* (ALP) and *Bglap* (osteocalcin) in *Extl3*^{+/+} (n = 7) and *Extl3*^{mut/+} (n = 7) D21 osteoblasts. Data are mean $\pm$ SD; 2-tailed Mann-Whitney U test ($p < .05$). E, qRT-PCR of *Tcf7* in *Extl3*^{+/+} (n = 7) and *Extl3*^{mut/+} (n = 7) osteoblasts on D15 and D21. Data are mean $\pm$ SD; 2-tailed Mann-Whitney U test ($p < .05$). F-H, Western blot analysis of (F) β -actin, β -catenin, and TCF1 proteins in total protein lysates from *Extl3*^{+/+} and *Extl3*^{mut/+} osteoblasts on D21, with quantification of (G) TCF1 and (H) β -catenin bands (n = 3) normalised to β -actin. Data are mean $\pm$ SD; no statistical test was performed. kDa, kilodalton.

This pattern, reminiscent of that described in hereditary multiple exostoses [15,16], suggests enhanced HS degradation mediated by heparanase activity, potentially resulting in shorter HS

fragments within the pericellular matrix (Fig 6E,F). These results were confirmed by RNA-Seq data, with overexpression of *Hspe2* at the prehypertrophic stage (log2 fold change: 0.61; $p = .023$)

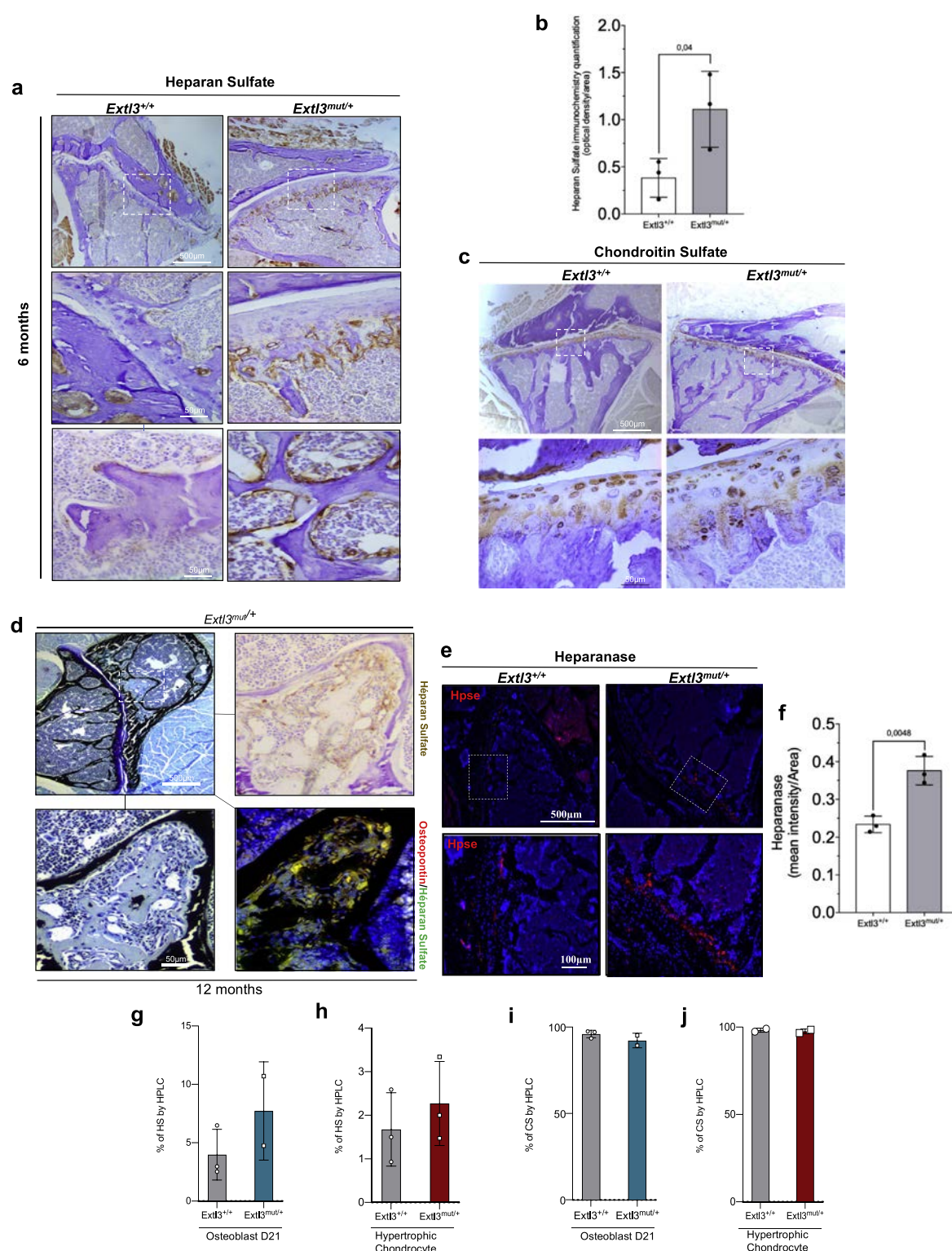


Figure 6. Consequences of the *Extl3* mutation on glycosaminoglycan biosynthesis. A and B, Heparan sulfate (HS) (10E4 antibody) immunohistochemistry in the sacroiliac joints (SIJs) of (A) 6-month-old *Extl3*^{+/+} (wild-type littermates) and *EXTL3* knock-in (*Extl3*^{mut/+}) mice and (B) quantification using Fiji software. Data are mean $\pm$ SD; 2-tailed Mann-Whitney U test ($p < .05$). C, Chondroitin sulfate (CS) (103H10 antibody) immunohistochemistry in the SIJs of 6-month-old *Extl3*^{+/+} and *Extl3*^{mut/+} mice. D, Von Kossa-Toluidine blue staining and coimmunohistochemistry for HS and osteopontin in the SIJs of 12-month-old *Extl3*^{mut/+} mice. E and F, Heparanase (HPSE) immunochemistry in the SIJs of (E) 6-month-old *Extl3*^{+/+} ($n = 3$) and *Extl3*^{mut/+} ($n = 3$) mice and (F) fluorescence quantification using Fiji software. Data are mean $\pm$ SD; 2-tailed Mann-Whitney U test ($p < .05$). G and H, Quantification of HS by (G) high-performance liquid chromatography (HPLC) in *Extl3*^{+/+} ($n = 3$) and *Extl3*^{mut/+} ($n = 2$) day 21 (D21) osteoblasts and (H) in *Extl3*^{+/+} ($n = 3$) and *Extl3*^{mut/+} ($n = 3$) hypertrophic chondrocytes. Data are mean $\pm$ SD; owing to the limited number of biological replicates, no formal statistical analysis was performed, and these results should be considered descriptive. I and J, Indirect quantification of CS by (I) HPLC in *Extl3*^{+/+} ($n = 2$) and *Extl3*^{mut/+} ($n = 2$) D21 osteoblasts and (J) in *Extl3*^{+/+} ($n = 2$) and *Extl3*^{mut/+} ($n = 2$) hypertrophic chondrocytes. Data are mean $\pm$ SD; owing to the limited number of biological replicates, no formal statistical analysis was performed, and these results should be considered descriptive.

and of *Hspe1* at the hypertrophic stage (log2 fold change: 0.57; $p = .045$) (Supplementary Fig S16D).

To verify these findings, we quantified GAGs, specifically HS and CS, in osteoblast and chondrocyte cultures. The dimethyl-methylene blue (DMMB) assay revealed an increase in total sulfated GAG content in *Extl3*^{mut/+} cells vs *Extl3*^{+/+} cells across the 3 stages of chondrocyte (Supplementary Fig S16A–C) and osteoblast (Supplementary Fig S16E–G) differentiation, with no significant differences between the phenotypes. To improve sensitivity of HS detection in these *in vitro* cultures, and to confirm the results previously obtained with DMMB, we used high-performance liquid chromatography (HPLC). This revealed increased HS concentrations in lysates from D21 osteoblast and hypertrophic chondrocyte cultures derived from *Extl3*^{mut/+} mice (Fig 6G,H), consistent with the enhanced immunostaining observed in tissue sections. In contrast, indirect HPLC quantification of CS revealed no significant differences between *Extl3*^{+/+} and *Extl3*^{mut/+} cells (Fig 6I,J), consistent with the results obtained using the DMMB assay (Supplementary Fig S16B). These results point to a dysregulation of HS metabolism contributing to osteoid matrix expansion, providing mechanistic insight into the skeletal abnormalities observed in *Extl3*^{mut/+} mice. Together, these findings reveal that the *Extl3* variant alters both chondrocyte and osteoblast differentiation through HS dysregulation, providing a mechanistic explanation for the severe ossification phenotype observed in the family.

DISCUSSION

Here, we reveal a previously unexplored and conceptually novel mechanism of skeletal ossification in spondyloarthritis, centred on EXTL3-dependent regulation of HS. This translational study identifies a rare missense mutation in the *EXTL3* gene in a multiplex family with severe AS, providing new insights into the genetic basis of disease severity. Using a knock-in mouse model carrying this mutation, we demonstrate that *EXTL3* dysfunction alters the differentiation trajectories of 2 key cell types involved in ossification: chondrocytes and osteoblasts. In chondrocytes, the mutation accelerated differentiation towards the hypertrophic stage, a process for which evidence suggests involvement of early Wnt activation. This shift was associated with disrupted collagen architecture and enhanced expression of hypertrophic markers, such as collagen X and MMP13, consistent with an increase in endochondral ossification. Wnt pathway activation appeared closely linked to HS/CS composition [17,18]. Several studies further indicated that HS shows stronger affinity for Wnt components than CS, pointing to a predominant role of HS in modulating Wnt signalling [19]. In parallel, osteoblasts from *Extl3*^{mut/+} mice showed delayed differentiation and impaired mineralisation capacity, leading to osteoid accumulation and altered trabecular bone microarchitecture in the SIJs and spine.

These findings echo key mechanisms previously implicated in SpA pathogenesis, particularly the central role of endochondral ossification in the development of syndesmophytes and spinal ankylosis. In our model, accelerated chondrocyte hypertrophy, as evidenced by histology and transcriptomics, supports the concept that an aberrant cartilage-to-bone transition is a driver of structural progression. Importantly, Wnt pathway activation emerged as a hallmark of this process, with RNA-Seq and qPCR analyses showing downregulation of natural inhibitors such as *Dkk1* and *Sost*, and upregulation of canonical components like *Tcf7* (confirmed by immunohistochemistry), *Wnt5a/b*, and β -catenin. These findings are in line with previous

mouse models, such as TNF-Tg197 [20,21] and the proteoglycan-induced arthritis (PGIA) model, in which inflammation-induced suppression of DKK1 facilitates pathological bone formation [22]. In these systems, inhibition of DKK1 leads to increased collagen X expression and joint fusion, phenotypes that we also observed, albeit to a lesser extent, in *Extl3*^{mut/+} mice. Human data suggest a prominent role for DKK1 in AS ossification, with altered serum DKK1 levels observed in patients with severe disease [23,24].

In addition to the ossification phenotype, we observed accumulation of unmineralised osteoid tissue and an alteration in trabecular bone structure. This paradoxical combination aligns with the clinical features of axSpA, in which osteoporosis coexists with excessive new bone formation [25,26]. A comparable phenotype has been described in the murine model of X-linked hypophosphatemia, which we previously investigated [27], showing mineralisation defects of the SIJs, with local osteomalacia occurring in a noninflammatory environment and in the absence of immune cell infiltration. This phenotype is particularly misleading, as it closely mimics the radiographic and structural features of spondyloarthritis despite arising from a fundamentally distinct pathogenic mechanism. These findings support a disease-relevant model in which dysregulated endochondral ossification and abnormal mineralisation, rather than inflammation-driven bone destruction, underlie structural progression in severe axSpA.

Our data suggest that osteoid accumulation is secondary to a defect in osteoblast function, specifically a delay in terminal differentiation and mineralisation. Interestingly, this phenotype coincided with increased HS staining, and colocalisation with osteopontin suggests that HS is produced locally by osteoblasts.

This raises the hypothesis that the *EXTL3* mutation induces HS accumulation, which, in turn, impairs osteoblast maturation. Indeed, both the abundance and fine structure of HS, especially its sulfation patterns, are critical for proper osteoblast and chondrocyte function. HS chains regulate a wide range of signalling pathways via their interactions with heparin-binding proteins, including growth factors, bone morphogenetic proteins (BMPs), cytokines, and components of the Wnt pathway [28]. As the initiating enzyme in HS biosynthesis, *EXTL3* plays a unique and nonredundant role in determining HS chain initiation and its balance with CS [29]. Therefore, alterations occurring at this early step of GAG biosynthesis could potentially affect not only HS quantity and bioactivity but also the overall GAG composition of the extracellular matrix, including secondary effects on CS biosynthesis. Such dysregulation may contribute to the altered cartilage-bone interface observed in our model, although further biochemical studies are required to directly establish this mechanism.

We confirmed increased HS levels in *Extl3*^{mut/+} chondrocyte and osteoblast cultures using HPLC quantification, whereas global total GAG and CS content were unchanged, likely reflecting the relatively small proportion of HS in these cell types. These findings align with the enhanced immunostaining and support the hypothesis of increased HS biosynthesis as a primary effect of the mutation. Further characterisation of these HS chains, particularly their sulfation patterns and polysaccharidic chain size—will be essential to understand their functional impact. Notably, our antibody-based detection strategy (10E4 antibody) targets sulfated epitopes of HS and may therefore underestimate or misrepresent certain structural changes in sulfated or acetylated patterns. Functional assays assessing the binding capacity of mutant-derived HS to key ligands, such as DKK1, will help clarify whether HS accumulation leads to

sequestration or inactivation of Wnt inhibitors, providing a direct link between EXTL3 dysfunction and Wnt pathway activation.

Whether the p.Thr268Met mutation results in a gain or loss of EXTL3 function remains unresolved. However, Extl3 gene and protein expression levels were comparable between mutant and WT cells, making a simple quantitative defect unlikely. Instead, our findings support the hypothesis of a qualitative functional alteration in EXTL3, potentially affecting HS biosynthesis or its structure. In this context, the observed HS accumulation may reflect altered HS composition or posttranslational modifications rather than preserved functional activity, as increased abundance does not necessarily equate to normal bioactivity. Further structural HS profiling and ligand-binding assays will therefore be required to determine the functional properties of these GAG chains. Although the present study does not provide direct biochemical evidence of altered EXTL3 enzymatic activity induced by the p.Thr268Met substitution, this important mechanistic question is currently under investigation.

One important limitation of our study is that the ossification phenotype observed in *Extl3*^{mut/+} mice is relatively mild, with few articular bridges. This model does not fully recapitulate the complete clinical picture of AS. However, our findings support the notion that the *EXTL3* mutation may act as a predisposing factor enhancing endochondral ossification potential, which, when combined with an inflammatory environment, may drive progression towards a severe structural phenotype. This is consistent with the human family we studied: only individuals carrying both HLA-B27 and the *EXTL3* variant developed the severe axial phenotype, whereas the HLA-B27-positive relative without the mutation exhibited only erosive sacroiliitis. These observations suggest a possible interaction between HLA-B27 and *EXTL3*, in which inflammation primes the tissue environment and the *EXTL3* mutation amplifies the ossification response. Furthermore, we have not assessed the potential impact of the mutation on other ossification pathways, including intramembranous bone formation. Although some studies suggest that this pathway may contribute to ossification, its involvement seems to be largely context- and site-dependent [30].

Indeed, mice carrying the same *Extl3* genotype displayed incomplete penetrance and variable expressivity [31], ranging from no detectable abnormalities to marked skeletal involvement. Consistently, despite sharing the same genotype, some patients developed early-onset disease (1976 and 1985, respectively) with severe spinal ankylosis, whereas patients II.1 and II.6 exhibited later disease onset (2003 and 2006, respectively) and a milder phenotype. Such phenotypic heterogeneity suggests that disease expression is influenced not only by the causal variant itself but also by modifier factors, including gene dosage, compensatory pathways, epigenetic regulation, and environmental influences.

Another limitation of our study is that, although axial involvement represents the predominant clinical manifestation of AS, we have not performed a systematic phenotypic characterisation of the peripheral joints and limb skeleton in *Extl3*^{mut/+} mice. Since the *Extl3* variant is present in all cell types in this model, we cannot exclude potential effects on nonaxial skeletal sites or peripheral ossification processes. However, the current study was primarily designed to investigate the mechanisms underlying axial skeletal involvement, with a particular focus on the SIJs and spinal ossification, which represent the hallmark features of the human disease. Future comprehensive skeletal phenotyping will be required to determine whether the impact

of the *Extl3* variant is restricted to axial structures or reflects a broader alteration in endochondral ossification.

Furthermore, the observed anomalies may not be exclusive to AS. It is important to note that these alterations, which promote enhanced endochondral ossification, could also be relevant in other pathological contexts characterised by aberrant ossification, including diffuse idiopathic skeletal hyperostosis and osteoarthritis.

Another limitation of our study is that the mechanistic data were generated exclusively in a murine model. Although this knock-in mouse model recapitulates key features of the axial skeletal phenotype observed in the affected family, including SIJ involvement and spinal osteoid accumulation, its relevance to human pathophysiology must be interpreted with caution. Access to axial skeletal tissues in patients with spondyloarthritis is ethically and technically challenging, which currently limits direct validation of our findings in human samples.

We are developing an induced pluripotent stem cell (iPSC)-based platform to model the *EXTL3* mutation and related variants in human chondrocytes and osteoblasts. This ongoing work will help establish genotype–phenotype correlations and identify common dysregulated pathways underlying severe axial ossification.

Future studies are needed to assess the frequency and impact of *EXTL3* and other exostosin gene mutations in larger AS cohorts, particularly in patients with severe structural damage. Genes such as *EXT1* and *EXT2*, previously implicated in hereditary multiple exostoses, may also contribute to excessive bone formation in AS, particularly given the functional specificity of exostosin family members in GAG biosynthesis. Moreover, the dual localisation of EXTL3 in the Golgi apparatus and endoplasmic reticulum (ER) raises the intriguing possibility of an interaction with HLA-B27 in the ER, a compartment already implicated in SpA pathogenesis through protein misfolding and stress responses [32–34]. Consistently, perturbations in ER protein-folding processes are known to disrupt cartilage matrix homeostasis, further supporting a functional connection between ER dynamics and the extracellular matrix [35]. Exploring this axis may yield further insights into the convergence of genetic, cellular, and immunological factors in AS.

In conclusion, our study identifies the *EXTL3* variant as a potential contributor to the severe skeletal phenotype observed in this AS model. The interplay among impaired bone remodeling, dysregulated Wnt signalling, and altered GAG metabolism suggests a potential role for HS-related pathways in the development of abnormal mineralisation and ossification (Supplementary Fig S17). Although the precise biochemical consequences of the *EXTL3* variant on HS biosynthesis remain to be established, our findings provide new insights into the mechanisms underlying severe forms of axSpA. Further studies are warranted to clarify these pathways and determine whether targeting HS metabolism or Wnt signalling may represent a therapeutic opportunity.

Competing interests

The authors declare no competing financial interests.

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Contributors

SH, OF, C Chaussain, CM-R, CB, XH, and PA contributed to the design of the experiments. SH, OF, C Cherifi, MT, KI, CB, XH, PA, and CM-R performed and analysed experiments. SH, OF, CB, and CM-R wrote the manuscript, with contributions from all authors. All authors reviewed and approved the final version of the manuscript. CM-R is the guarantor of the manuscript.

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Patient consent for publication

Not applicable.

Ethics approval

The study was approved by the CPP Île-de-France VII (Protocol PP 14-040) and received authorization from the Comité consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé (CCTIRS) (No. 15.567).

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.2026.06.031.

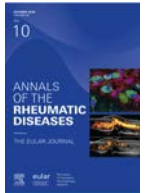
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Systemic lupus erythematosus

Development and validation of quality indicators for lupus nephritis, based on the 2025 EULAR recommendations: adherence trends and impact on clinical outcomes

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ABSTRACT

Objectives: This study aimed to develop a set of quality indicators (QIs) based on the 2025 European Alliance of Associations for Rheumatology (EULAR) recommendations for systemic lupus erythematosus with kidney involvement and explore their association with disease outcomes.

Methods: A modified Research and Development and University of California, Los Angeles appropriateness method was used. In 2 voting rounds, 23 experts rated candidate QIs for validity and feasibility. Median ratings and agreement were calculated, leading to a core QI set. Adherence to recommended care from healthcare providers was assessed at both QI and patient levels in a single tertiary centre contemporary lupus nephritis (LN) cohort (n = 130). High adherence was defined as $\geq 80\%$. Associations between adherence to QIs, damage accrual, and renal flares were explored.

Results: A total of 17 QIs were developed across 4 domains: diagnosis, treatment, monitoring, and pregnancy. High adherence was observed for kidney biopsy, hydroxychloroquine use, maintenance immunosuppression, therapy switching in persistent or relapsing disease, renin–angiotensin system inhibitor use, remission before kidney transplantation, and pregnancy-related QIs. Combination immunosuppressive therapy as initial treatment had low adherence (10.7%), reflecting practice patterns preceding publication of the recommendations. High per-patient adherence was associated with lower odds of damage accrual (increase in Systemic Lupus International Collaborating Clinic Damage Index; odds ratio: 0.29, 95% CI: 0.13–0.62) and fewer renal flares, although the latter association was not statistically significant.

Conclusions: These QIs may serve as a structured framework for implementing the 2025 EULAR recommendations for LN. Higher adherence was associated with reduced damage accrual in a tertiary centre cohort, but results need validation in diverse clinical settings.

KEY MESSAGES

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Kidney involvement is a major cause of morbidity and long-term organ damage in patients with systemic lupus erythematosus.
- European Alliance of Associations for Rheumatology (EULAR) recommendations provide evidence-based guidance for diagnosing and managing lupus nephritis (LN), but their implementation in real-world practice is not self-evident.
- EULAR has recently proposed the development of quality indicators (QIs) in parallel with every update of its recommendations to facilitate implementation.

WHAT THIS STUDY ADDS

- This study proposed 17 QIs for LN based on the 2025 EULAR recommendations using a structured expert consensus method.
- Testing of these QIs in a real-world single-centre cohort revealed generally higher adherence for most diagnostic, treatment, and reproductive health indicators, and lower for combination (triple) immunosuppressive therapy.

- Adherence to QIs was associated with reduced risk of damage accrual.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- The proposed QIs offer a structured approach for auditing and enhancing the application of EULAR recommendations in the real-world management of LN.
- The observed association between higher adherence and reduced damage accrual highlights the potential value of integrating quality-of-care measures into outcome assessment in LN.
- These results support further evaluation of QIs in multicentre research and may inform future efforts to improve quality and develop strategies for guideline implementation.

INTRODUCTION

Kidney involvement represents a serious manifestation of systemic lupus erythematosus (SLE). Despite therapeutic advances and improved understanding of pathogenesis, disease outcomes

remain variable. Complete renal response rates remain <50%, and 5% to 20% of patients with lupus nephritis (LN) may progress to kidney failure within a decade [1,2]. Although disease outcomes are an area of active research, the quality of care delivered to patients with LN has been under investigation to date [3,4].

Assessing quality of care is essential in medicine, as it enables evidence-based improvements in healthcare delivery [5,6]. Quality of delivered care can be measured in 3 domains: “structure” (physical and human resources, such as facilities, personnel, and equipment), “process” (evidence-based clinical interventions and delivery), and “outcome” (eg, mortality) [7]. Evaluating the “outcome” domain is challenging for most conditions, as outcomes may not develop for many years. In contrast, evaluation of the “process” is more feasible, as it is typically under the control of healthcare providers and can be modified over shorter time periods [7].

Quality indicators (QIs) represent standardised tools to measure the quality of care. They facilitate the application of existing evidence into clinical practice and serve as checklists for healthcare providers. QIs may be derived from available scientific evidence, including systematic reviews and clinical practice guidelines or recommendations. In line with this, the European Alliance of Associations for Rheumatology (EULAR) has recently incorporated a limited set of QIs within each set of recommendations [8]. Notably, important distinctions exist between a guideline and a QI: guidelines are systematically developed statements to assist practitioners and patients’ decisions prospectively for specific clinical circumstances; in essence, what is the “right thing to do.” By contrast, a QI is “a measurable element of practice performance for which there is evidence or consensus that it can be used to assess the quality, and hence change in the quality, of care provided” [9]. QIs typically measure the minimum care that should be delivered and can therefore identify areas requiring intervention. Consequently, effective implementation of these indicators requires key properties, such as acceptability, validity, reliability, feasibility, and sensitivity to change [9,10].

Although QIs have been previously developed for rheumatic diseases, including rheumatoid arthritis [11] and SLE [12–15], there are no validated QIs currently existing for LN. EULAR recommendations for the management of LN have been recently updated, providing 4 overarching principles and 13 recommendations [8]. Based on these updated recommendations, we aimed to develop a set of QIs, with the primary objectives of (i) facilitating their implementation, (ii) bridging the gap between state-of-the-art and real-life clinical practice, (iii) identifying areas that require improvement, and (iv) providing a standardised assessment tool for healthcare providers.

METHODS

We used the modified Research and Development and University of California, Los Angeles (RAND/UCLA) method [16], a validated approach for incorporating expert opinion. The RAND/UCLA Appropriateness Method is a structured consensus approach based on the modified Delphi technique, in which candidate indicators developed by a core research team from available evidence and clinical expertise are evaluated by an expert panel using 9-point Likert ratings. Agreement is determined through predefined statistical criteria, such as median scores and measures of dispersion, rather than requiring unanimous consensus. The procedure typically includes 2 voting rounds, allowing rating revision and qualitative feedback. In the present study, the process involved sequential steps: review of the 2025 EULAR recommendations for SLE with kidney involvement [8], creation of preliminary QIs, performance of 2 rounds of expert voting, and finalisation of the QI set. Last, we sought to apply the QIs within a single-centre LN cohort (Fig 1).

Step 1. Development of the preliminary set of QIs

A first set of QIs was derived from the EULAR recommendations. Of note, a systematic review underpinning these recent recommendations had been performed by members of the study team (AF and MK) [17]. The quality of evidence for each QI was graded according to the grading of recommendations, assessment, development and evaluation system. Emphasis was placed primarily on the actual statements, although relevant textual content accompanying each recommendation was also reviewed. The QIs were formulated using the “IF-THEN” structure, where “IF” refers to eligible patients for a particular QI, and “THEN” indicates the expected care from the healthcare provider. A preliminary set of 24 QIs was established through discussions among principal investigators (EO, MK, GB, AF, and DB) (Supplementary Table S1z).

Step 2. Assembly of an expert panel

A total of 32 experts were initially invited via e-mail. Ultimately, 23 experts from 18 countries completed the voting process, forming the international expert panel, consisting of 15 rheumatologists, 7 nephrologists, and 1 paediatric rheumatologist. The selection process prioritised the inclusion of SLE specialists from geographically dispersed regions to reflect the international diversity in health systems and organisation of care, and the global epidemiology of SLE [18]. The countries represented in the expert panel are shown in Figure 2.

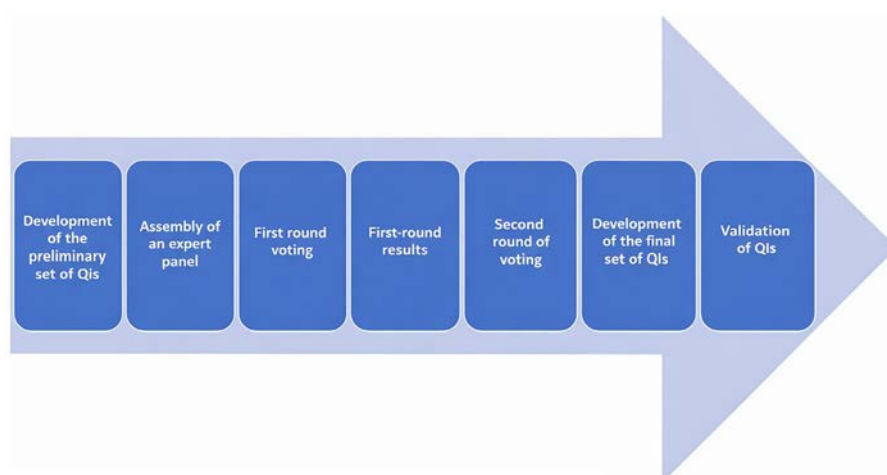


Figure 1. Process of developing quality indicators for patients with SLE with kidney involvement. QI, quality indicator; SLE, systemic lupus erythematosus.

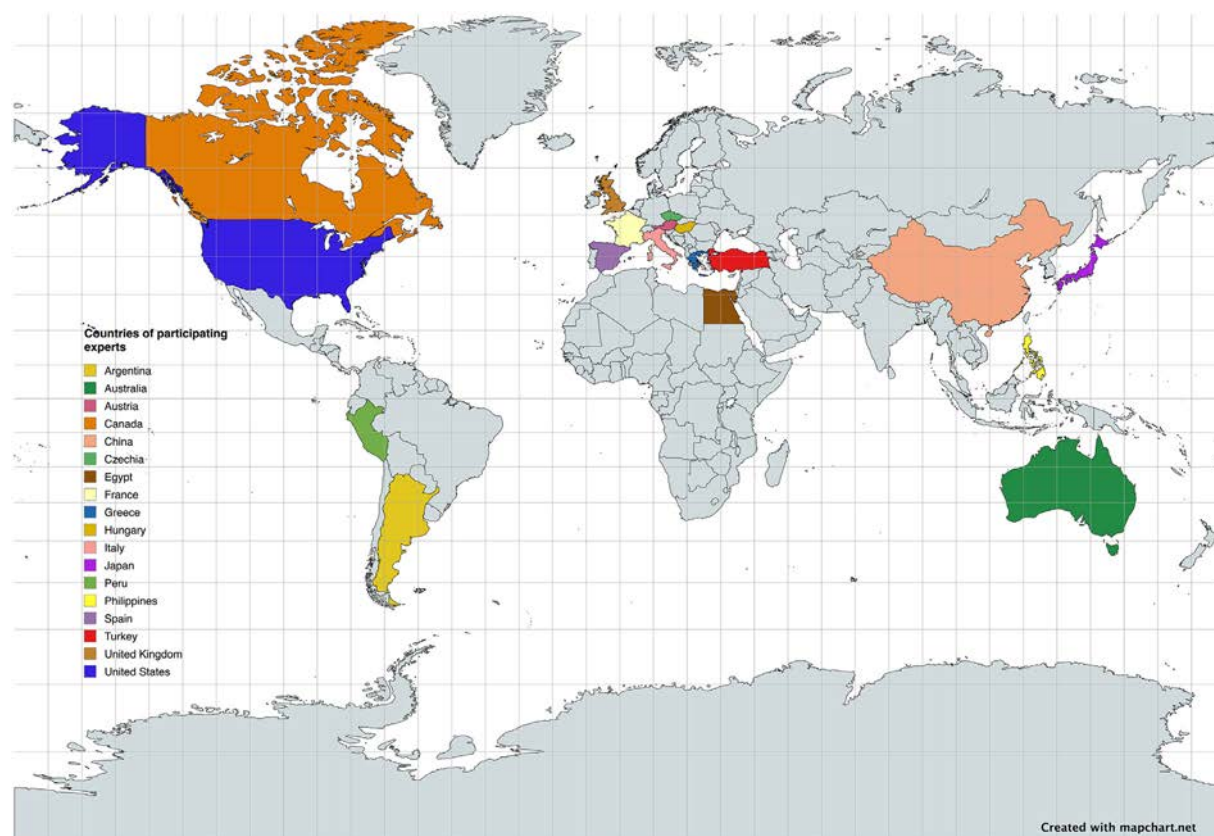


Figure 2. Countries of experts involved in the rating process.

Step 3. First round of voting

Before the first round, experts were informed about the procedure. Instructions regarding the definitions of validity and feasibility for all QIs were provided. Experts were asked to rate each QI for both validity and feasibility on a Likert scale from 1 to 9 (1 meaning ‘definitely not valid/feasible’ and 9 meaning ‘definitely valid/feasible’). In this round, experts were permitted to provide comments. The definitions of validity and feasibility are summarised in [Supplementary Table S2](#) [14].

Step 4. Analysis of first round results and resulting modification of QIs

The modified RAND/UCLA method [16] was applied to evaluate the voting outcomes. Although all QIs passed the prespecified threshold for agreement (see agreement process in statistical analysis section) ([Supplementary Table S3](#)), some disagreements among experts were noted for certain QIs, leading to respective modifications or even removal of particular QIs. The primary concerns driving these modifications included the limited strength of available evidence (eg, performing kidney biopsy in isolated glomerular haematuria), variability in clinical approaches among physicians for specific QIs (eg, administration of intravenous pulse glucocorticoids [GCs]), uncertainties in operational definitions (eg, suggestions to avoid broad intervals, such as ‘3–6 months’), and concerns about feasibility (eg, evaluation of thrombotic microangiopathy [TMA] in a kidney biopsy specimen). Consequently, 7 of the 24 preliminary QIs (numbers 3, 12, 15, 17, 22, 23, and 24) were removed, and 11 QIs (numbers 1, 2, 5, 7, 8, 9, 11, 14, 16, 18, and 20) were revised accordingly.

Step 5. Second round of voting and development of the final set

Experts were asked to re-evaluate the modified QIs for validity and feasibility, using the same definitions, but without the option to provide comments. As no principal disagreement existed on any QIs in the first round, a face-to-face meeting, often organised to address divergent opinions or clarify misunderstandings, was not deemed necessary. Instead, a detailed explanation for each QI was provided before the second voting round, based on previous comments by the experts. Furthermore, an anonymised summary of the voting analysis results was provided to each expert, including the median scores for all QIs and their individual ratings.

The same process of voting was followed, wherein the 23 experts voted on the total 17 QIs for the second round. As a result, all QIs reached consensus on validity and feasibility ([Table 1](#)), and these 17 QIs formed the final set ([Table 2](#)).

Step 6. Application of QIs in an LN cohort

The prevalent LN cohort of the Attikon University Hospital (part of the larger ‘Attikon SLE cohort’, in which expert rheumatologists are the primary responsible physicians) was used to perform an initial evaluation of the final set of QIs. For the purpose of this study, we considered patients with lupus (2019 EULAR/American College of Rheumatology and/or 2012 Systemic Lupus International Collaborating Clinics [SLICC] Classification Criteria [19,20]) who had high clinical suspicion of LN or confirmed LN through kidney biopsy, regardless of whether the biopsy was performed at Attikon Hospital or other centres. All QIs were audited through electronic medical record review, except preconception counselling, which additionally relied on patient interviews and recall. Each QI had specific eligibility

Table 1
Validity and feasibility ratings of the final quality indicators for SLE with kidney involvement

Indicator	Validity ^a		Feasibility ^b	
	Median	Mean absolute deviation	Median	Mean absolute deviation
<i>(1) Diagnosis QIs</i>				
1. IF a patient with SLE presents with persistent proteinuria (≥ 0.5 g/24 h or UPCR ≥ 500 mg/g), and/or an unexplained decrease in glomerular filtration rate ^c THEN a kidney biopsy should be performed, unless contraindications are noted.	9	0.04	9	0.34
2. IF a patient with SLE undergoes a kidney biopsy THEN the classification according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 system, including assessment of activity and chronicity, should be reported.	9	0.17	9	0.30
<i>(2) Treatment QIs</i>				
3. IF a patient has lupus nephritis THEN like all individuals with SLE, hydroxychloroquine should be administered, unless contraindications are noted.	9	0.04	9	0.04
4. IF a patient has active proliferative or mixed (class III/IV + V) forms of lupus nephritis THEN intravenous pulse methylprednisolone should be administered.	8	0.69	9	0.34
5. IF a patient has active lupus nephritis THEN following pulse steroid therapy, oral prednisone should be initiated at a dose not exceeding 0.7 mg/kg/d.	8	0.69	9	0.39
6. IF a patient has active lupus nephritis THEN combination treatment [mycophenolate /low-dose intravenous CYC with belimumab or mycophenolate with a calcineurin inhibitor (voclosporin or tacrolimus), or mycophenolate with obinutuzumab] should be initiated, based on drug availability. ^d	9	0.65	9	0.82
7. IF a patient with lupus nephritis has improved after initial therapy THEN oral glucocorticoids should be tapered to ≤ 5 mg/d prednisone-equivalent by 6 mo.	9	0.26	9	0.52
8. IF a patient with lupus nephritis has responded to therapy with mycophenolate—either alone or in combination with belimumab, a calcineurin inhibitor, or Obinutuzumab THEN treatment with the same regimen should be continued for at least 3 y. ^e	9	0.78	9	0.69
9. IF a patient with lupus nephritis has responded to therapy with CYC—either alone or in combination with belimumab THEN CYC should be replaced with mycophenolate or azathioprine, and belimumab should be continued. ^f	9	0.39	9	0.47
10. IF a patient has lupus nephritis THEN complete withdrawal of glucocorticoids should be attempted by 12 mo in the presence of inactive disease.	9	0.60	9	0.86
11. IF a patient with lupus nephritis has persistently active or relapsing disease THEN a switch among different immunosuppressive and/or biologic drugs should be made, after excluding drug nonadherence.	9	0.34	9	0.52
12. IF a patient with lupus nephritis has persistent proteinuria or arterial hypertension THEN an angiotensin converting enzyme inhibitor or angiotensin II receptor blocker should be initiated.	9	0.13	9	0.13
<i>(3) Monitoring</i>				
13. IF a patient has lupus nephritis THEN serum creatinine and/or eGFR, serum albumin, urine analysis, UPCR and/or 24-h urine protein, anti-dsDNA, and C3/C4 should be measured at baseline and at least every 6mo. ^g	9	0.04	9	0.39
14. IF a patient with SLE develops kidney failure and kidney transplantation is planned THEN clinically inactive disease (renal and extrarenal) for at least 6 mo should be achieved before transplantation.	9	0.47	9	0.52
<i>(4) Reproductive health (Pregnancy and Fertility)</i>				
15. IF a patient with lupus nephritis is of reproductive age THEN preconception counselling should be provided.	9	0.08	9	0.56
16. IF a patient with lupus nephritis is considering pregnancy THEN pregnancy should be planned following at least 6 mo of clinically inactive disease, while on pregnancy-compatible medications.	9	0.60	9	0.82
17. IF a patient with lupus nephritis is pregnant THEN low-dose aspirin should be initiated.	8	0.56	9	0.21

CYC, cyclophosphamide; eGFR, estimated glomerular filtration rate; IV, intravenous; QIs, quality indicators; SLE, systemic lupus erythematosus; UPCR, urine protein creatinine ratio.

^a Validity is established when an indicator is evidence-based, improves patient health outcomes, distinguishes higher-quality physicians through greater adherence, and is largely under physician control.

^b Feasibility is achieved when adherence can be reliably and unbiasedly assessed using routinely documented information in standard medical records, with poor documentation itself indicating lower quality.

^c Abnormal eGFR that is below the expected level based on age and clinical history, or decreasing eGFR, with no attributable cause other than systemic lupus erythematosus.

^d Alternatively, single-agent immunosuppressive regimens—such as mycophenolate mofetil or low-dose intravenous cyclophosphamide—can be used in cases of mild disease or when access to standard therapies is limited. Moreover, in patients with rapidly progressing glomerulonephritis, a short course of high-dose intravenous cyclophosphamide (6–7 monthly pulses) can also be considered.

^e In situations where access to medications is limited, continuing treatment with a single therapeutic agent is considered acceptable. Regarding the combination with tacrolimus, data beyond 2 years are lacking. Obinutuzumab was administered for a maximum of 1 y (52 wk) in the REGENCY trial; longer courses are pending further evaluation.

^f Preferably mycophenolate, unless pregnancy is being considered. In situations where access to medications is limited, continuing treatment with a single therapeutic agent is considered acceptable.

^g Frequency of visit should be individualised and may range from every 2 to 4 wk in active LN to 6 mo in stable chronic disease.

Table 2
Final set of quality indicators for SLE with kidney involvement

(1) Diagnosis QIs
1. IF a patient with SLE presents with persistent proteinuria (≥ 0.5 g/24 h or UPCR ≥ 500 mg/g) (B), and/or an unexplained decrease in glomerular filtration rate (C) ^a THEN a kidney biopsy should be performed, unless contraindications are noted.
2. IF a patient with SLE undergoes a kidney biopsy THEN the classification according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 system (B), including assessment of activity and chronicity (A), should be reported.
(2) Treatment QIs
3. IF a patient has lupus nephritis THEN like all individuals with SLE, hydroxychloroquine should be administered (A), unless contraindications are noted.
4. IF a patient has active proliferative or mixed (class III/IV + V) forms of lupus nephritis THEN intravenous pulse methylprednisolone should be administered (C).
5. IF a patient has active lupus nephritis THEN following pulse steroid therapy, oral prednisone should be initiated at a dose not exceeding 0.7 mg/kg/d (C).
6. IF a patient has active lupus nephritis THEN combination treatment [mycophenolate/low-dose intravenous CYC with belimumab (A) or mycophenolate with a calcineurin inhibitor (voclosporin or tacrolimus) (A), or mycophenolate with obinutuzumab (A)] should be initiated, based on drug availability. ^b
7. IF a patient with lupus nephritis has improved after initial therapy THEN oral glucocorticoids should be tapered to ≤ 5 mg/d prednisone-equivalent by 6 mo (C).
8. IF a patient with lupus nephritis has responded to therapy with mycophenolate—either alone or in combination with belimumab, a calcineurin inhibitor, or obinutuzumab THEN treatment with the same regimen (A) should be continued for at least 3 y (B). ^c
9. IF a patient with lupus nephritis has responded to therapy with CYC—either alone or in combination with belimumab THEN CYC should be replaced with mycophenolate (A) or azathioprine (A), and belimumab should be continued. ^d
10. IF a patient has lupus nephritis THEN complete withdrawal of glucocorticoids should be attempted by 12 mo (D), in the presence of inactive disease.
11. IF a patient with lupus nephritis has persistently active or relapsing disease THEN a switch among different immunosuppressive and/or biologic drugs should be made (B), after excluding drug nonadherence.
12. IF a patient with lupus nephritis has persistent proteinuria or arterial hypertension THEN an angiotensin converting enzyme inhibitor or angiotensin II receptor blocker should be initiated (D).
(3) Monitoring
13. IF a patient has lupus nephritis THEN serum creatinine and/or eGFR, serum albumin, urine analysis, UPCR and/or 24-h urine protein (B), anti-ds DNA, and C3/C4 (C) should be measured at baseline and at least every 6 mo. ^e
14. IF a patient with SLE develops kidney failure and kidney transplantation is planned THEN clinically inactive disease (renal and extrarenal) for at least 6 mo should be achieved before transplantation (C).
(4) Reproductive health (pregnancy and fertility)
15. IF a patient with lupus nephritis is of reproductive age THEN preconception counselling should be provided (A).
16. IF a patient with lupus nephritis is considering pregnancy THEN pregnancy should be planned following at least 6 mo of clinically inactive disease (A), while on pregnancy-compatible medications.
17. IF a patient with lupus nephritis is pregnant THEN low-dose aspirin should be initiated (C).

CYC, cyclophosphamide; eGFR, estimated glomerular filtration rate; GRADE, grading of recommendations, assessment, development and evaluation; IV, intravenous; QIs, quality indicators; SLE, systemic lupus erythematosus; UPCR, urine protein creatinine ratio.

A to D denote levels of evidence according to the GRADE system.

^a Abnormal eGFR that is below the expected level based on age and clinical history, or decreasing eGFR, with no attributable cause other than systemic lupus erythematosus

^b Alternatively, single-agent immunosuppressive regimens—such as mycophenolate mofetil or low-dose intravenous cyclophosphamide—can be used in cases of mild disease or when access to standard therapies is limited. Moreover, in patients with rapidly progressing glomerulonephritis, a short course of high-dose intravenous cyclophosphamide (6–7 monthly pulses) can also be considered.

^c In situations where access to medications is limited, continuing treatment with a single therapeutic agent is considered acceptable. Regarding the combination with tacrolimus, data beyond 2 y are lacking. Obinutuzumab was administered for a maximum of 1 y (52 wk) in the REGENCY trial; longer courses are pending further evaluation.

^d Preferably mycophenolate, unless pregnancy is being considered. In situations where access to medications is limited, continuing treatment with a single therapeutic agent is considered acceptable.

^e Frequency of visit should be individualised and may range from every 2 to 4 wk in active LN to 6 mo in stable chronic disease.

criteria; therefore, eligible populations and missing data varied across indicators. Only patients with available data for a given QI were included in the corresponding adherence analysis (Supplementary Table S4).

Statistical analysis

Descriptive data are presented as percentages, mean (SD), or median (IQR), depending on data distribution. Follow-up duration spanned from the LN diagnosis to the final assessment visit.

Median scores for validity and feasibility served as the primary criteria for the exclusion of QIs (median score < 7), whereas the disagreement index [16] informed discussions and

guided consensus before the second round of voting (details shown in Appendix S1). This methodology was applied consistently in the analysis of the second-round voting [14,16].

In the LN cohort, adherence was defined at the healthcare provider level and considered fulfilled when the recommended intervention was appropriately prescribed or initiated, regardless of subsequent patient compliance. Eligibility was calculated separately for each QI, with the denominator representing eligible patients with available data and the numerator those receiving the recommended care. For instance, for QI#4 (use of intravenous pulse methylprednisolone in patients with active proliferative or mixed LN), the denominator was eligible patients with these forms of LN, for whom data on intravenous

pulse GC use were available. The numerator consisted of patients who actually received this treatment. Per-QI adherence was calculated as numerator/denominator, whereas per-patient adherence was defined as the proportion of adhered QIs among all eligible QIs for each patient.

The Youden index was used to determine the optimal cutoff for adherence, regarding the subsequent increase of the SLICC Damage Index (SDI), based on the area under the receiver operating characteristic curve. Sensitivity analysis supporting this threshold is provided in [Supplementary Table S5](#) and [Supplementary Figure S1](#). To compare mean adherence rates between the period before 2016 (year of establishment of the Attikon cohort) and after, a linear regression was conducted. Multivariable logistic regression was employed to evaluate whether adherence to QIs is associated with irreversible damage accrual (measured by the SDI), renal flares, and estimated glomerular filtration rate (eGFR) slope. The primary exposure was per-patient adherence ($\geq 80\%$ vs $< 80\%$), with adjustments for age at LN, sex, SLE–LN interval for damage, and activity index for flares. In addition, we investigated the relationship between QI-level adherence and SDI > 0 .

RESULTS

The final set included 17 QIs, divided into 4 domains: diagnosis, treatment, monitoring of LN, and reproductive health. The final set was accepted with a high level of agreement; median validity and feasibility scores for all QIs were ≥ 8 ([Table 1](#)). Modifications leading to the final set of QIs are summarised below.

Diagnosis of LN

From the initial 3 QIs, 1 was eliminated: evaluation and treatment of TMA in kidney biopsy was excluded due to its relative rarity and the absence of standardised diagnostic approaches, coupled with underreporting in many centres.

The remaining 2 QIs in this domain were pertinent to performing a kidney biopsy and optimal reporting of its result. Practical challenges associated with performing a kidney biopsy, such as procedural difficulty, patient refusal, and relative contraindications, were discussed among experts. Nevertheless, given that kidney biopsy is integral for the management of LN, and that identification of potential gaps between clinical practice and recommendations is a major objective of the QIs, biopsy-related QIs were retained. Notably, the limited evidence supporting biopsy in cases of isolated haematuria prompted the panel to conclude that evaluating patients presenting solely with haematuria might not be a matter of quality of care; instead, individualised clinical decision-making remains central in these scenarios.

Treatment of LN

From the initially proposed 12 QIs, 2 were removed, resulting in 10 treatment-related QIs: 1 concerning hydroxychloroquine (HCQ) use, 4 related to GC therapy (initiation, tapering, and withdrawal), 4 related to initial and subsequent immunosuppressive therapies, and 1 addressing nonimmune therapy, namely renin–angiotensin–aldosterone system (RAAS) blockade. The QI related to sodium-glucose transporter 2 inhibitor therapy was removed due to concerns raised by the expert panel about the insufficient evidence. Also, the section addressing the dose of intravenous GC pulse was removed owing to variability in clinical practice regarding appropriate dosing, while the indicator itself was retained as a QI in the final set. A modification

was decided, stating that GC pulses may be more appropriate for specific patient subgroups, namely, patients with proliferative or mixed LN. Finally, concerns about access to combination therapy (as defined in QI#6) for all patients led to the inclusion of a clarifying footnote for this QI, acknowledging variations in drug access across settings.

Monitoring of LN

From the initial set, 1 of 3 QIs was excluded, resulting in 2 QIs in this domain: 1 related to routine laboratory follow-up (eg, serum creatinine, urinalysis, anti-dsDNA antibodies, complement C3/C4 levels) and the other to the management of patients with kidney failure before transplantation. By contrast, the QI on targets of therapy was excluded because of comments from the expert panel indicating that these outcomes depend on a variety of factors that are outside the control of the treating physician (thus, not specifically measuring quality of care).

Reproductive health (pregnancy and fertility)

This domain initially included 3 QIs, all of which were retained in the final set: preconception counselling, attainment of remission before pursuing pregnancy, and the use of aspirin during gestation. Although preconception counselling is of utmost importance, concerns were raised regarding the reliability of its assessment through chart review, mainly due to documentation issues. Nevertheless, the panel agreed that recognising this gap in routine care is important.

Outside the 4 domains mentioned above, the QIs addressing management of comorbidities—cardiovascular disease, osteoporosis, and vaccination—were also removed. The absence of standardised care for these conditions in SLE, combined with the influence of factors for vaccination, such as patient refusal, age, and timing, would limit the meaningful evaluation of quality of care in this domain.

Application of QIs in a real-world tertiary centre LN cohort

We analysed 130 patients with prevalent LN (83.8% females, mean [SD] age 45.6 [15.2] years; median [IQR] follow-up duration 74.5 [31.5–157.2] months). Detailed characteristics of the cohort are summarised in [Table 3](#).

All eligible patients were retrieved from the LN cohort, with the exception of QI#1. For the latter, 124 of 378 patients with SLE qualified for a kidney biopsy, based on recommended indications; of these, 93.5% did undergo a kidney biopsy, with 1 individual refusing. All biopsy reports used the 2003 International Society of Nephrology/Renal Pathology Society system, whereas biopsies performed before 2003 were evaluated using the WHO criteria [21]. Activity and chronicity indices were assigned to proliferative cases (class III/IV, with or without class V); these indices were reported in 86.7% of eligible patients ([Table 4](#)).

For treatment of LN, almost all eligible patients (98.4%) received HCQ during follow-up. Among patients with proliferative LN, intravenous pulse GC was administered in 79.5% as part of the initial treatment. In addition, 72.5% of patients received GC regimen at a dose not exceeding 0.7 mg/kg/d after pulse GC. However, only 10.7% of patients received combination treatment as initial therapy for active LN, possibly reflecting practice patterns preceding publication of the recommendations. GC tapering to ≤ 5 mg/d by 6 months was achieved in 50%, whereas complete GC withdrawal within 12 months was

Table 3
Characteristics of patients with SLE with kidney involvement in the cohort

Demographics	n = 130
Female, n (%)	109 (83.8)
Caucasians, n (%)	129 (99.2)
Follow-up, mo, median (IQR)	74.5 (31.5–157.2)
Current age, mean (SD)	45.6 (15.2)
Age at SLE diagnosis, mean (SD)	30.2 (15.7)
Age at LN diagnosis, mean (SD)	33.8 (15.3)
System involvements, ever, n (%)	
Photosensitivity	93 (71.5)
Malar Rash	36 (27.7)
Alopecia	54 (41.5)
Arthritis	120 (92.5)
Serositis	30 (23.1)
Neuropsychiatric	13 (10)
Anaemia	11 (8.5)
Leucopenia	54 (41.5)
Thrombocytopenia	31 (23.8)
LN histology, n (%)	
Class II	10 (7.7)
Class III	30 (23.1)
Class IV	24 (18.5)
Class V	29 (22.3)
Mix (III/IV + V)	25 (19.2)
Others, mean ± SD	
Activity index	8.5 ± 4.4
Chronicity index	2.8 ± 1.9
SLEDAI at biopsy	15.5 ± 5.0
Lab at biopsy	
Proteinuria, mg/24 h, median (IQR)	1800 (950–3100)
Haematuria, n (%)	88 (67.7)
RBC casts, n (%)	22 (16.9)
eGFR, mL/min/1.73 m ² , median (IQR)	101 (56–120)
Serology at biopsy, n (%)	
ANA	125 (96.2)
Anti-dsDNA	93 (71.5)
Low C3/C4	101 (77.7)
Treatment, induction, n (%)	
Mycophenolate mofetil	50 (38.5)
Cyclophosphamide	54 (41.5)
Belimumab	14 (10.8)
Calcineurin inhibitor	6 (4.6)
Treatment, maintenance, n (%)	
Mycophenolate mofetil	79 (60.8)
Azathioprine	30 (23.1)
Belimumab	29 (22.3)
Outcomes, n (%)	
Complete remission	91 (70)
Partial remission	24 (18.5)
CKD	27 (20.8)
ESRD	9 (6.9)
SDI >0 at last visit	70 (53.8)
Flare	34 (26.1)

ANA, antinuclear antibody; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; LN, lupus nephritis; RBC, red blood cell; SDI, SLICC Damage Index; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

accomplished in 79.4% of eligible patients. Recommended maintenance therapy after mycophenolate mofetil or cyclophosphamide-based regimens was initiated in 85.4% and 87.8% of patients, respectively. In all patients with persistently active or relapsing LN (defined by the judgement of the treating physician), a drug switch was performed accordingly. RAAS blockade utilisation was documented in 81.0% of patients (Table 4).

The recommended laboratory monitoring (defined in QI#13) was conducted in 59% of eligible patients. In 5 patients in whom kidney transplantation was scheduled, remission was appropriately achieved before the procedure. Finally, preconception

counselling was offered to 82.5% of patients (based on patient recall). Remission before conception was achieved in all ongoing pregnancies, and all pregnant women received aspirin during pregnancy (Table 4).

When comparing adherence rates before and after 2016, mean adherence improved, particularly for diagnosis (74.1%–93.8%, $P = .008$) and monitoring QIs (40.6%–76.3%, $P < .001$) (Table 5). Notably, reporting of activity and chronicity indices became more common after 2016 (74.1%–93.8%, $P = .008$). In addition, laboratory monitoring was higher after 2016 (40.5%–71.4%, $P = .001$) (Supplementary Table S6).

Per-patient adherence was high in 33.1% of patients, based on an 80% threshold. Importantly, patients with high adherence to QIs experienced a lower risk of damage accrual (increased SDI) at the last visit, even after adjusting for age at LN onset, sex, and disease duration between SLE and LN diagnosis (odds ratio [OR]: 0.29, 95% CI: 0.13–0.62) (Table 6). However, per-patient adherence did not serve as an independent predictor for renal flares, although a respective trend was evident (OR: 0.67, 95% CI: 0.20–2.13) (Table 6). In sensitivity analyses using per-patient adherence as a continuous variable, each 10% increase in adherence was associated with lower odds of SDI >0 (OR: 0.78, 95% CI: 0.62–0.98; $P = .032$). In contrast, adherence was not significantly associated with renal flares when analysed continuously (OR: 1.09, 95% CI: 0.84–1.41; $P = .50$) (Supplementary Table S7 and Supplementary Fig S2). High per-patient adherence was not significantly associated with eGFR slope (Supplementary Fig S3), with mean annualised slopes of 2.64 vs 4.84 mL/min/1.73m²/y ($P = .455$).

A sensitivity analysis of individual QI adherence was performed to assess associations with damage accrual. Adherence to QI#13 (laboratory monitoring) was significantly associated with lower damage accrual; 64.5% of adherent patients had an SDI of 0, compared with 18.6% of nonadherent patients (OR: 7.52, 95% CI: 3.03–18.64; $P < .001$) (Supplementary Fig S4).

DISCUSSION

The proposed set of QIs specifically for patients with SLE with kidney involvement was developed based on the EULAR 2025 recommendations [8], using the modified RAND/UCLA method to integrate evidence and expert opinion. The primary objective was to operationalise recommendations into measurable indicators to support the assessment and implementation of care quality in clinical practice. As a proof-of-concept study, we evaluated their applicability in a real-world LN cohort, demonstrating generally high adherence across most domains, although improvement is still needed, particularly in the uptake of combination immunosuppressive therapy. Given the use of historical data, these findings should partly be interpreted as retrospective benchmarking rather than contemporary auditing, primarily highlighting existing gaps and areas for improvement.

Providing optimal healthcare is essential to improving outcomes in LN. Although some studies in SLE have failed to find a significant relationship between quality of care and outcomes [22], others have reported that high-quality care correlates with more favourable outcomes, including reduced disease activity, lower damage accrual, and improved quality of life [6,23]. Notably, adherence to recommended standards of care among patients with SLE remains only moderate, with reported rates ranging from 50% to 66.7%, although it appears higher in patients with LN [14,24].

Although no established methodology exists for integrating QIs within recommendations, the ideal scenario is the

Table 4
Adherence to the final QI set in the Attikon lupus nephritis cohort

	Eligibility	Adherence
<i>(1) Diagnosis QIs</i>		
1. IF a patient with SLE presents with persistent proteinuria (≥ 0.5 g/24 h or UPCR ≥ 500 mg/g), and/or an unexplained decrease in glomerular filtration rate ^a THEN a kidney biopsy should be performed, unless contraindications are noted.	124 (32.8) ^b	116 (93.5)
2. IF a patient with SLE undergoes kidney a biopsy THEN the classification according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 system, including assessment of activity and chronicity, should be reported.	75	65 (86.7)
<i>(2) Treatment QIs</i>		
3. IF a patient has lupus nephritis THEN like all individuals with SLE, hydroxychloroquine should be administered, unless contraindications are noted.	129	127 (98.4)
4. IF a patient has active proliferative or mixed (class III/IV + V) forms of lupus nephritis THEN intravenous pulse methylprednisolone should be administered.	78	62 (79.5)
5. IF a patient has active lupus nephritis THEN following pulse steroid therapy, oral prednisone should be initiated at a dose not exceeding 0.7 mg/kg/d.	69	50 (72.5)
6. IF a patient has active lupus nephritis THEN combination treatment [mycophenolate /low-dose intravenous CYC with belimumab or mycophenolate with a calcineurin inhibitor (voclosporin or tacrolimus), or mycophenolate with obinutuzumab] should be initiated, based on drug availability. ^c	121	13 (10.7)
7. IF a patient with lupus nephritis has improved after initial therapy THEN oral glucocorticoids should be tapered to ≤ 5 mg/d prednisone-equivalent by 6 mo.	66	33 (50.0)
8. IF a patient with lupus nephritis has responded to therapy with mycophenolate—either alone or in combination with belimumab, a calcineurin inhibitor, or obinutuzumab THEN treatment with the same regimen should be continued for at least 3 y. ^d	41	35 (85.4)
9. IF a patient with lupus nephritis has responded to therapy with cyclophosphamide—either alone or in combination with belimumab THEN cyclophosphamide should be replaced with mycophenolate or azathioprine, and belimumab should be continued. ^e	49	43 (87.8)
10. IF a patient has lupus nephritis THEN complete withdrawal of glucocorticoids should be attempted by 12 mo in the presence of inactive disease.	63	50 (79.4)
11. IF a patient with lupus nephritis has persistently active or relapsing disease THEN a switch among different immunosuppressive and/or biologic drugs should be made, after excluding drug nonadherence.	40	40 (100)
12. IF a patient with lupus nephritis has persistent proteinuria or arterial hypertension THEN an angiotensin converting enzyme inhibitor or angiotensin II receptor blocker should be initiated.	79	64 (81.0)
<i>3. Monitoring</i>		
13. IF a patient has lupus nephritis THEN serum creatinine and/or eGFR, serum albumin, urine analysis, UPCR and/or 24-h urine protein, anti-dsDNA, and C3/C4 should be measured at baseline and at least every 6 mo. ^f	105	62 (59.0)
14. IF a patient with SLE develops kidney failure and kidney transplantation is planned THEN clinically inactive disease (renal and extrarenal) for at least 6 mo should be achieved before transplantation.	5	5 (100.0)
<i>4. Reproductive health (pregnancy and fertility)</i>		
15. IF a patient with lupus nephritis is of reproductive age THEN preconception counselling should be provided.	40	33 (82.5)
16. IF a patient with lupus nephritis is considering pregnancy THEN pregnancy should be planned following at least 6 mo of clinically inactive disease, while on pregnancy-compatible medications.	11	11 (100)
17. IF a patient with lupus nephritis is pregnant THEN low-dose aspirin should be initiated.	6	6 (100)

CYC, cyclophosphamide; eGFR, estimated glomerular filtration rate; IV, intravenous; QIs, quality indicators; SLE, systemic lupus erythematosus; UPCR, urine protein creatinine ratio.

Eligibility was defined among patients who met the 'IF' criteria and had sufficient data available to permit assessment of the indicator. For example, for QI#5, eligible patients included those with active lupus nephritis who received pulse glucocorticoid therapy and for whom both body weight and initial glucocorticoid dose were documented, allowing evaluation of dose appropriateness.

^a Abnormal eGFR that is below the expected level based on age and clinical history, or decreasing eGFR, with no attributable cause other than systemic lupus erythematosus.

^b For QI#1, eligibility was identified in 378 patients with SLE.

^c Alternatively, single-agent immunosuppressive regimens—such as mycophenolate mofetil or low-dose intravenous cyclophosphamide—can be used in cases of mild disease or when access to standard therapies is limited. Moreover, in patients with rapidly progressing glomerulonephritis, a short course of high-dose intravenous cyclophosphamide (6–7 monthly pulses) can also be considered.

^d In situations where access to medications is limited, continuing treatment with a single therapeutic agent is considered acceptable. Regarding the combination with tacrolimus, data beyond 2 years are lacking. Obinutuzumab was administered for a maximum of 1 y (52 wk) in the REGENCY trial; longer courses are pending further evaluation.

^e Preferably mycophenolate, unless pregnancy is being considered. In situations where access to medications is limited, continuing treatment with a single therapeutic agent is considered acceptable.

^f Frequency of visit should be individualised and may range from every 2 to 4 wk in active LN to 6 mo in stable chronic disease.

Table 5
Comparison of QI adherence in each category before and after 2016 in the LN cohort

	<2016 Mean adherence, %	≥2016 Mean adherence, %	P value
Diagnosis	74.1	93.8	.008
Treatment	68.9	70.7	.587
Monitoring	40.6	76.3	<.001
Reproductive health	82.4	84.6	.849

LN, lupus nephritis; QI, quality indicator.
The significance threshold is $p < 0.05$.

concurrent development of QIs as part of the recommendation process to create an immediately implementable metric [25]. Acknowledging this, EULAR has recently advocated for the development of indicative QIs within its sets of recommendations. To this end, the 2025 EULAR recommendations for LN already included 3 proposed QIs, based on the respective recommendations with the best balance between feasibility and impact on quality of care [8]. Herein, we capitalised on this previous work by constructing a full set of QIs that covers the whole spectrum of LN diagnosis and management. This process adopted a robust methodology, including 2 rounds of voting by a geographically diverse expert panel.

Application of QIs in clinical practice is crucial because auditing presents significant challenges. Previous reports indicate that approximately 10% to 20% of proposed QIs ultimately prove to be nonmeasurable [26]. Therefore, considerable effort in the current work was directed towards refining the phrasing of each QI and incorporating detailed feedback from the expert panel to enhance clarity, precision, and practical applicability.

A cutoff of $\geq 80\%$ was employed to denote high adherence to the proposed care [6,24]. This level of adherence was achieved for several domains, including diagnosis, maintenance therapy, appropriate switching of immunosuppressive agents, use of RAAS blockade, attainment of remission before kidney transplantation, and pregnancy-related management. As expected, the current combination treatment showed a low rate of compliance because our LN cohort is a prevalent cohort with many older cases, predating the current evidence base on combination therapy. Consequently, a trend towards combination therapies was nevertheless evident with their higher adoption observed in more recent years, during the chronic treatment phase.

Data on GC use also aligned well with current targets: half of the patients successfully reduced their prednisone dose to ≤ 5 mg/d by 6 months, and 79.4% discontinued GC entirely within 12 months. Among those who continued doses above 5 mg/d, the majority (78.7%) remained on ≤ 10 mg/d. In addition, adherence rates in diagnosis and monitoring categories

showed notable improvement after 2016; however, the observed improvements over time should be interpreted with caution, as they may reflect factors beyond changes in clinical practice alone.

Importantly, we further examined the association between quality of care, as reflected by per-patient adherence, and clinical outcomes, including damage accrual and renal flares. Our explanatory analysis revealed that patients with higher adherence rates experienced a reduced burden of cumulative organ damage. Yazdany et al. [6] have also reported that provision of high-quality care was associated with reduced damage accrual in patients with SLE, although no significant association with disease activity was observed. Kidney involvement itself is a well-established predictor of damage accrual in SLE [27–29]. Adherence to the current QIs—such as low-dose GC regimens after intravenous GCs, early tapering and discontinuation of GCs, and proper monitoring and treatment adjustments—may partly account for the reduced damage observed in these patients. This finding should be interpreted cautiously, given the observational design and the potential influence of unmeasured confounders. However, adherence was not significantly associated with the occurrence of renal relapses. Because active histopathological lesions on kidney biopsy are a major predictor of renal flares [30], along with delayed achievement of remission after initial therapy [31] and persistent serological activity [32], these factors may not be captured by QIs that focus specifically on care delivery.

An additional consideration is variability in auditability across the proposed QIs despite high feasibility agreement. Some indicators were based on objective, routinely documented data, whereas others relied on physician judgement or patient-reported information. Several QIs were readily audit-ready, including those related to kidney biopsy, HCQ use, GC management, maintenance therapy, RAAS blockade, and monitoring. In contrast, preconception counselling and identification of persistently active or relapsing disease were more difficult to operationalise due to reliance on recall or detailed documentation. Further, although blood pressure management is important for LN management, it was not retained as a separate QI because it was removed from the initial list, along with other comorbidities. Elements of hypertension management are only partly captured through QI#12, which recommends initiation of RAAS blockade in patients with persistent proteinuria or arterial hypertension.

Our study is subject to some limitations. First, the modest cohort size constrained the analysis of specific interventions. Second, a scarcity of longitudinal data—particularly for patients with long-standing disease—limited our ability to establish robust predictive outcomes. In addition, the possibility of recall

Table 6
Logistic regression analysis identifying predictors of SDI and flares in the LN cohort

	SDI			Flares		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
Per-patient adherence ≥ 80	0.29	0.13–0.62	.002	0.67	0.20–2.13	.500
Age at LN, years	1.01	0.98–1.03	.500	0.96	0.91–1.00	.064
Female sex	0.59	0.20–1.61	.300	2.56	0.36–51.7	.400
Disease duration, months ^a	1.08	1.00–1.18	.080	—	—	—
Activity index (0–24)	—	—	—	0.99	0.87–1.12	.900

LN, lupus nephritis; SDI, SLICC Damage Index; SLICC, Systemic Lupus International Collaborating Clinics; SLE, systemic lupus erythematosus.

The significance threshold is $p < 0.05$.

^a Duration between the diagnosis of SLE and LN.

bias due to the interview for preconception counselling QI cannot be excluded.

Notably, the validation cohort was derived from a specialised tertiary lupus centre, where adherence to recommended care may be higher than in routine practice, limiting generalisability; therefore, these findings should be regarded as proof of concept. In addition, the absence of direct involvement of patients and nonphysician stakeholders may have limited incorporation of patient-centred and economic perspectives, warranting broader stakeholder engagement in future refinements. The latter may also incorporate cost-effectiveness, comorbidity management, and improved applicability across diverse healthcare settings, including adaptation to differences in drug availability and healthcare systems.

In conclusion, a comprehensive set of QIs was created for patients with SLE and kidney involvement. Although overall adherence to these QIs was generally high within this single-centre cohort, some indicators showed significant gaps between recommended care and actual clinical practice. Higher adherence was linked to less progression of organ damage, although a direct causal inference cannot be claimed by our study design. External validation in multicentre and less specialised cohorts is warranted.

CRedit authorship contribution statement

Eda Otman Akat: Writing – original draft, Validation, Data curation. **Myrto Kostopoulou:** Methodology, Formal analysis. **Spyridon Katechis:** Validation, Formal analysis. **Konstantinos Drougkas:** Validation, Data curation. **Katerina Chavatza:** Validation, Data curation. **Andreas Kronbichler:** Writing – review & editing, Validation. **Bernardo A Pons-Estel:** Writing – review & editing, Validation. **Brad H Rovin:** Writing – review & editing, Validation. **György Nagy:** Writing – review & editing, Validation. **Jinoos Yazdany:** Writing – review & editing, Validation. **Lucy Marie Carter:** Writing – review & editing, Validation. **Luis F Quintana:** Writing – review & editing, Validation. **Mandana Nikpour:** Writing – review & editing, Validation. **Manuel Praga:** Writing – review & editing, Validation. **Manuel F Ugarte-Gil:** Writing – review & editing, Validation. **Maria Dall'Era:** Writing – review & editing, Validation. **Maria Trachana:** Writing – review & editing, Validation. **Marta Mosca:** Writing – review & editing, Validation. **Michael M Ward:** Writing – review & editing, Validation. **Ming-Hui Zhao:** Writing – review & editing, Validation. **Murat Inanc:** Writing – review & editing, Validation. **Nobuyuki Yajima:** Writing – review & editing, Validation. **Sandra Navarra:** Writing – review & editing, Validation. **Sophia Lionaki:** Writing – review & editing, Validation. **Tamer A Gheita:** Writing – review & editing, Validation. **Vladimir Tesar:** Writing – review & editing, Validation. **Zahir Amoura:** Writing – review & editing, Validation. **Zahi Touma:** Writing – review & editing, Validation. **George Bertsias:** Writing – review & editing, Methodology, Conceptualization. **Antonis Fanouriakis:** Writing – review & editing, Supervision, Conceptualization. **Dimitrios T Boumpas:** Writing – review & editing, Supervision, Conceptualization.

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Competing interests

DTB serves as an Editorial Board member of *Annals of the Rheumatic Diseases*. All other authors declare they have no competing interests.

Patient consent for publication

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

Ethics approval

The study received approval from the Ethics Committee of ‘Attikon’ University Hospital, Athens (protocol number 103/06-03-2014).

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

The data supporting the findings of this study are available from the corresponding author (DTB) upon reasonable request.

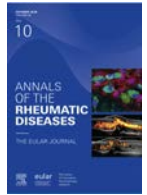
Supplementary materials

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.ard.2026.05.030](https://doi.org/10.1016/j.ard.2026.05.030).

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Systemic lupus erythematosus

A data-driven, weighted flare score that accurately predicts organ damage in systemic lupus erythematosus

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ABSTRACT

Objectives: Flares are key determinants of outcome in systemic lupus erythematosus (SLE), yet the conventional mild/moderate vs severe classification inadequately reflects prognosis. We sought to refine flare classification to improve damage prediction and develop a data-driven flare score.

Methods: We analysed a multicentre longitudinal cohort of 354 patients with active SLE requiring treatment at inclusion (median follow-up 66 months). Flares were adjudicated using a modified Safety of Estrogens in Lupus Erythematosus National Assessment-SLE Disease Activity Index Flare Index (SFI). Random Forests and modified flare definitions assessed the contribution of individual SFI variables to subsequent damage. We derived a flare score using bootstrap-validated mixed-effects models. This was validated in an independent cohort ($n = 110$).

Results: Both mild/moderate and severe SFI flares contributed to elevated 12-month damage risk (incidence rate ratio [IRR]:1.42 and 1.54, respectively). Among SFI subcriteria, major organ involvement requiring high-dose glucocorticoids (GCs) ranked highest for damage prediction

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(weight = 6), followed by the isolated GCs escalation criteria (weights = 1.5–3), whereas minor organ involvement and immunosuppressants/biologics addition carried lower weights (1 each). A flare score based on these subcriteria demonstrated good predictive performance (Brier score = 0.07). A threshold of ≥ 3.5 identified a subset of high-risk flares (40% of all episodes) that displayed the strongest association with subsequent damage (IRR: 2.49, 95% CI: 1.51–4.10) and outperformed the original SFI definition in the validation cohort (area under the curve –receiver operating characteristic: 0.893 vs 0.816). Although both Definitions of Remission in SLE (DORIS) and lupus low disease activity state correlated with lower risk of SFI-defined flares, only sustained DORIS ($>50\%$ of time) reduced high-risk flares.

Conclusions: We defined and validated a novel weighted SLE flare score that better predicts damage accrual and may support treat-to-target implementation and clinical trial design.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Flares are major determinants of organ damage in systemic lupus erythematosus (SLE).
- The Safety of Estrogens in Lupus Erythematosus National Assessment-SLE Disease Activity Index (SELENA-SLEDAI) Flare Index (SFI) distinguishes mild/moderate from severe flares but does not adequately weight their long-term impact on damage.

WHAT THIS STUDY ADDS

- Using longitudinal multicentre data, both mild/moderate and severe SFI events were independently associated with subsequent organ damage.
- Data-driven deconvolution of the SFI identified key subcriteria spanning both mild/moderate and severe flares (new/worsened organ manifestations, glucocorticoid [GC] escalation, treatment intensification) as most strongly associated with damage.
- A weighted flare score (range 1–8) based on these features defined a subgroup of high-risk flares associated with subsequent organ damage—including damage classified as activity-related as well as GC-related—and outperformed conventional SFI flares in an independent cohort.
- Sustained Definition of Remission in SLE remission, but not intermittent low disease activity, was associated with reduced occurrence of such high-risk flares.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

- The new SLE flare definition based on clinical relevance provides a more accurate, outcome-based stratification of flare severity, distinguishing events linked to irreversible organ damage.
- Incorporation of this weighted score may improve treat-to-target monitoring and serve as an endpoint in SLE clinical studies.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder characterised by recurrent flares of disease activity that typically require therapeutic intervention, alongside periods of relative quiescence often sustained by ongoing immunosuppressive treatment [1]. Flares vary in frequency, severity, and organ distribution, reflecting the disease heterogeneity [2]. Their prevention represents a major goal in SLE management, which is reflected by the inclusion of flares as a key secondary endpoint in trials of novel therapeutic agents [3].

The clinical assessment of SLE flares remains challenging. The Safety of Estrogens in Lupus Erythematosus National Assessment-SLE Disease Activity Index Flare Index (SFI) is the most widely applied framework for their definition and stratification

[2,4–6]. However, the index was originally designed for clinical trial standardisation rather than longitudinal risk stratification. Importantly, its dichotomous nature (mild/moderate vs severe) may not accurately reflect the prognostic heterogeneity of disease exacerbations. Furthermore, SFI integrates both clinical and therapeutic criteria without adequately weighting their relative contribution to relevant long-term outcomes, potentially leading to misclassification of flare severity in routine clinical practice. These limitations underscore the need for data-driven approaches capable of distinguishing flares that are clinically relevant and lead to irreversible damage from those with transient or limited clinical impact.

To address these unmet needs, we conducted a retrospective multicentre study exploiting a harmonised, multicentre longitudinal SLE cohort. We sought to dissect the spectrum of flare severity, quantify their temporal patterns and outcomes, and derive a composite flare score based on objective associations with organ damage accrual. We identified score thresholds linked to excess damage accumulation. Importantly, we validated the prognostic significance of these high-risk flares in an independent SLE cohort. By refining the conceptual and operational definition of flares, our study aims to enhance prognostic precision, facilitate treat-to-target implementation, and inform the design of future clinical trials in SLE.

METHODS

Study design, inclusion criteria, and participants

This was a retrospective longitudinal cohort study of patients who were consecutively enrolled and followed at 3 tertiary lupus clinics. All centres maintain harmonised SLE registries [7] and adhere to standardised protocols for clinical and laboratory data collection. Eligible participants were aged ≥ 16 years and fulfilled the 2012 Systemic Lupus International Collaborating Clinics (SLICC) [8] and/or the 2019 European Alliance of Associations for Rheumatology/American College of Rheumatology (EULAR/ACR) [9] classification criteria for SLE. To ensure inclusion of patients with clinically active disease, entry required a SLE disease activity index 2000 (SLEDAI-2K) [10] ≥ 6 and/or a Physician Global Assessment (PGA) [11] ≥ 1.5 , accompanied by treatment intensification ([Supplementary Methods](#)). This strategy was chosen to avoid the mixture of long-standing quiescent and active cases.

Patients with concomitant systemic autoimmune/inflammatory diseases other than SLE were excluded. The baseline visit was defined as the first encounter fulfilling inclusion criteria within the registry period (January 2008–June 2018). Per protocol, follow-up visits were scheduled every 4 to 6 months during the first year and at least annually thereafter. In practice,

observed intervals were even shorter during the first year, yielding a median interval of 3 months (IQR: 3); this aligned with our protocol and reflected the close monitoring required for early, active disease. Data were recorded prospectively using structured electronic forms and retrospectively verified for completeness and accuracy.

Clinical assessment, flares adjudication, and variables collection

Baseline assessments included demographic characteristics, disease duration, prior treatments, and major comorbidities. At each visit, disease activity was evaluated with SLEDAI-2K and PGA (0–3 scale), scored prior to review of serology. Flares were defined using the SELENA-SLEDAI Flare Index (SFI) [12], modified to include contemporary therapies under the definition of severe flare (Supplementary Table S1). The SFI was selected as the primary flare definition because it is a composite instrument integrating SLEDAI changes, organ manifestations (beyond those captured by the SLEDAI), treatment intensification, and physician judgement (PGA), and has been associated with damage accrual in prior cohorts [13,14].

All individual subcriteria from both the mild/moderate and severe SFI definitions were recorded at each visit, irrespective of whether the corresponding flare category was ultimately met. Although the dose-escalation rules for the glucocorticoid (GC) –related severe subcriteria 8 and 9 differ subtly (Supplementary Table S1), in our dataset, all events fulfilling subcriterion 8 also reached ≥ 30 mg/d and would therefore have met the subcriterion 9 dose threshold. To avoid double counting, subcriterion 9 was not scored separately when subcriterion 8 was fulfilled; consequently, the 2 subcriteria differed in practice only by the presence of major organ involvement.

Organ damage was assessed using the SLICC/ACR Damage Index (SDI) [15]. Treatment exposure, including GCs (dose and route), hydroxychloroquine, and other immunosuppressive/biologic agents, was recorded at each visit. Definition of Remission in SLE (DORIS) [16] and lupus low disease activity state (LLDAS) [17] was determined according to established definitions. Laboratory parameters required for SLEDAI-2K scoring were obtained within 30 days of clinical assessment. All definitions and scoring procedures were harmonised across centres and followed the methodology previously described [18,19]. Data were stored in a secure, pseudonymised registry with ongoing quality control and cross-validation between centres.

Construction of the flare score

Random Forest models were trained to evaluate the relative importance of individual SFI subcriteria for SDI progression. Models used an 80/20 train-test split, 5-fold cross-validation, and 1000 trees optimised for the Receiver operating characteristic (ROC) area under the curve (AUC); permutation-based importance quantified the influence of features. In addition, mixed-effects Poisson models with 1000 bootstrap replications were fitted to implement a set of sensitivity analyses in which modified flare definitions were constructed by systematically altering the SFI structure: ‘ignore-one’ models omitted each subcriterion from the full definition; ‘absent-one’ models were restricted to flare episodes that did not fulfil that subcriterion; and ‘present-one’ models were restricted to flare episodes in which the subcriterion was fulfilled. Log-likelihood differences between each modified and the original SFI model were then used to quantify the influence of each subcriterion on the flare-damage association. Top-ranked predictors from both

approaches were combined to construct reduced flare definitions, and retained subcriteria were jointly entered into a final model to derive a unified weighted flare score. Regression coefficients were standardised and converted into integer weights, and model calibration and accuracy were assessed using the Hosmer-Lemeshow test and Brier score.

Statistical analysis

Incidence rates (IRs) of flares were calculated per 100 person-years with exact Poisson 95% CIs, and temporal patterns of flare occurrence were examined using Kaplan-Meier curves, cumulative incidence estimates at specific timepoints and yearly IRs. Visits were classified as flare-free, mild/moderate flare, or severe flare, and the incidence of new damage within the subsequent 12 months was calculated. In addition, mixed-effects Poisson regression with a random intercept for each patient was used to account for repeated observations and to estimate adjusted incidence rate ratios (IRRs) for damage following each flare type. Biological sex (female or male), baseline disease activity, organ damage, and SLE duration were included as covariates. The prognostic performance of high-risk flares was further examined in an independent SLE cohort (Erlangen) using Poisson regression and ROC-AUC. To determine whether early treatment–target attainment protected against future flares, a 12-month landmark design was used: for each patient, the cumulative proportion of time spent in DORIS or LLDAS during the first 12 months was grouped into predefined strata, and Cox models estimated the hazard of severe or high-risk flares occurring beyond 12 months. All analyses were performed using Stata v18.0 (StataCorp LLC, College Station, TX, USA) and R v4.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

Patient and public involvement

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

RESULTS

High flare burden in SLE during long-term follow-up

We included 354 patients (92.5% female) with a median (IQR) age of 45.7 (21.3) years and disease duration of 8.7 (66.4) months (Table 1). At baseline, all patients had active disease with median (IQR) PGA, SLEDAI-2K, and clinical SLEDAI-2K of 2.0 (0.5), 8 (4), and 6 (4), respectively. Over a median follow-up of 66 (IQR: 60) months and excluding the baseline visit, 290 patients (81.9%) experienced at least 1 flare, including 242 (68.4%) with mild/moderate and 210 (59.3%) with severe flares adjudicated according to the SFI (Table 1). The estimated IRs (per 100 person-year) were 47.7 (95% CI: 44.8–50.8), 27.0 (95% CI: 24.8–29.3), and 20.7 (95% CI: 18.9–22.8) for any, mild/moderate and severe flares, respectively.

To characterise the dynamics of flare occurrence, Kaplan-Meier survival curves and IR plots were generated over the entire follow-up period. The cumulative probability of mild/moderate flares was 23.8%, 43.6%, and 55.6% at 12, 24, and 36 months (Fig 1A), respectively, whereas that of severe flares was 18.3%, 28.2%, and 34.6% at the same time points (Fig 1B). The corresponding IR of mild/moderate flares remained relatively stable across follow-up (Fig 1C). By contrast, severe flares occurred most frequently during the first year (45.4 per 100 person-years) and declined thereafter (IRRs: 0.37–0.64 across

Table 1
Demographic and clinical characteristics of patients with SLE at inclusion visit

	N (%) or median (IQR)	
Inclusion visit data		
No. patients	354	
Sex (female)	328 (92.6%)	
Ethnicity (White)	340 (96.1%)	
Age (y)	45.7 (21.3)	
Disease duration (mo)	8.7 (66.4)	
Organ damage (SDI > 0)	94 (26.6%)	
PGA (0–3)	2.0 (0.5)	
SLEDAI-2K	8 (4)	
Clinical SLEDAI-2K	6 (4)	
Follow-up data		
No. visits per patient	12 (9)	
Follow-up, per patient (mo)	66 (60)	
Flare (SFI) events (excl. baseline)		
Any (at least 1; no. events)	290 (81.9%); 3 (4)	
Mild/moderate	242 (68.4%); 1 (3)	
Severe	210 (59.3%); 1 (2)	
Flares incidence rate (per 100 person-year)		
Any	47.7 (95% CI: 44.8–50.8)	
Mild/moderate	27.0 (95% CI: 24.8–29.3)	
Severe	20.7 (95% CI: 18.8–22.8)	
Use of glucocorticoids (inclusion)		
Oral route	219 (61.9%)	
Dose (mg prednisone-equivalent; among users)	15.0 (22.0)	
Intramuscular route	42 (11.9%)	
Dose (mg methylprednisolone acetate; among users)	160 (40)	
Intravenous route	91 (25.7%)	
Dose (mg methylprednisolone; among users)	1000 (500)	
Use of other treatments	At inclusion, n (%)	Ever, n (%)
Hydroxychloroquine	282 (79.7)	319 (90.1)
Methotrexate	80 (22.6)	163 (46.0)
Leflunomide	8 (2.3)	42 (11.9)
Azathioprine	83 (23.4)	169 (47.7)
Calcineurin inhibitor	13 (3.7)	27 (7.6)
Thalidomide	0 (0.0)	5 (1.4)
Mycophenolate	32 (9.0)	108 (30.5)
Cyclophosphamide	21 (5.9)	44 (12.4)
Belimumab	29 (8.2)	112 (31.6)
Rituximab	8 (2.3)	47 (13.3)
IVIg	2 (0.6)	7 (2.0)
Other biologics	0 (0.0)	14 (4.0)

IVIg, intravenous immunoglobulin; PGA, SELENA-SLEDAI Physician Global Assessment; SDI, SLICC/ACR damage index; SFI, Safety of Estrogens in Lupus Erythematosus National Assessment- systemic lupus erythematosus Disease Activity Index Flare Index; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE disease activity index 2000.

subsequent years, all *P*-values < .02) (Fig 1D). Notably, even after 10 years of observation, the IR of severe flares remained notable at 18.2 (95% CI: 13.2–24.6) per 100 person-years. Collectively, these findings demonstrate that the overall flare burden peaked within the first year after inclusion and progressively stabilised over time, particularly for severe flares.

Flares are particularly common in younger and female patients

We next stratified the occurrence of SLE flares according to baseline demographic and disease-related parameters (Supplementary Table S2). Males had significantly fewer mild/moderate flares than females (12.9 vs 28.1 per 100 person-years; IRR: 0.46, 95% CI: 0.29–0.72), whereas the frequency of severe events was comparable between sexes. Compared with younger patients (< 45 lt; 45 years), those aged ≥ 45 years experienced

fewer severe flares (17.2 vs 24.4 per 100 person-years; IRR: 0.71, 95% CI: 0.58–0.85), whereas mild/moderate flare rates did not differ across age groups. Disease duration (> 1 gt; 1 vs ≤ 1 year) showed no significant associations. Although clinical disease activity at inclusion was not strongly related to subsequent flares, active serology (high anti-dsDNA and/or low complement) was linked to reduced flare occurrence, particularly of mild/moderate intensity (IRR: 0.66; 95% CI: 0.55–0.78).

Both mild/moderate and severe SFI flares contribute to damage accrual

Flares are recognised drivers of adverse long-term outcomes in SLE; however, the relative prognostic impact of mild/moderate vs severe events remain uncertain. To address this, we classified all patient visits according to flare status—no flare, mild/moderate flare, or severe flare—and then assessed the incidence of new organ damage occurring within the subsequent 12 months. Following flare-free visits, new damage developed at a rate of 12.9 per 100 person-years (95% CI: 11.3–14.7). This rate increased to 19.3 (95% CI: 14.6–25.0) and 25.4 (95% CI: 19.6–32.4) per 100 person-years after visits with mild/moderate and severe flares, respectively (Supplementary Table S3). In a more robust analysis, mixed-effects Poisson regression was applied to account for repeated observations within individuals. Both mild/moderate and severe SFI flares were associated with increases in SDI documented at subsequent visits, with a tendency towards stronger effects for severe flares (Table 2). Similar results were obtained when both flare types were included simultaneously in the model. By computing a modified SLE-DAS [20] (mSLE-DAS; Supplementary Methods) at each visit and defining flares as an increase of ≥ 1.72 since the previous visit [21], we observed that SFI-defined flares performed at least as well as mSLE-DAS-defined flares in predicting subsequent damage (Supplementary Tables S4 and S5). Collectively, these findings suggest that irrespective of their classification, flares contribute to damage accrual.

Identification and weighting of flare subcriteria with prognostic impact

The SFI is a composite measure integrating disease activity scores, clinical manifestations, and treatment changes. We sought to delineate the relative contribution of its individual subcriteria to the risk of organ damage, as this could facilitate patient risk stratification. Because moderate-to-strong co-segregation was observed among several subcriteria (Supplementary Fig S1), we employed 2 complementary strategies to rank their prognostic importance. First, Random Forest models were applied, as they accommodate possible nonlinear relationships and interdependencies between variables while minimising overfitting. Second, we evaluated the dependency of the original mild/moderate and severe SFI definitions on each subcriterion using 3 sensitivity analyses: (1) ‘ignore-one’ models excluding each subcriterion sequentially from the full definition; (2) ‘absent-one’ models restricted to flare episodes not fulfilling the subcriterion; and (3) ‘present-one’ models restricted to flares in which the subcriterion was present (Supplementary Table S6).

Both approaches yielded convergent results. Among the severe flare components, subcriterion 8 (new/worsened major organ manifestations requiring GCs intensification) and subcriterion 10 (immunosuppressants/biologics addition) emerged as

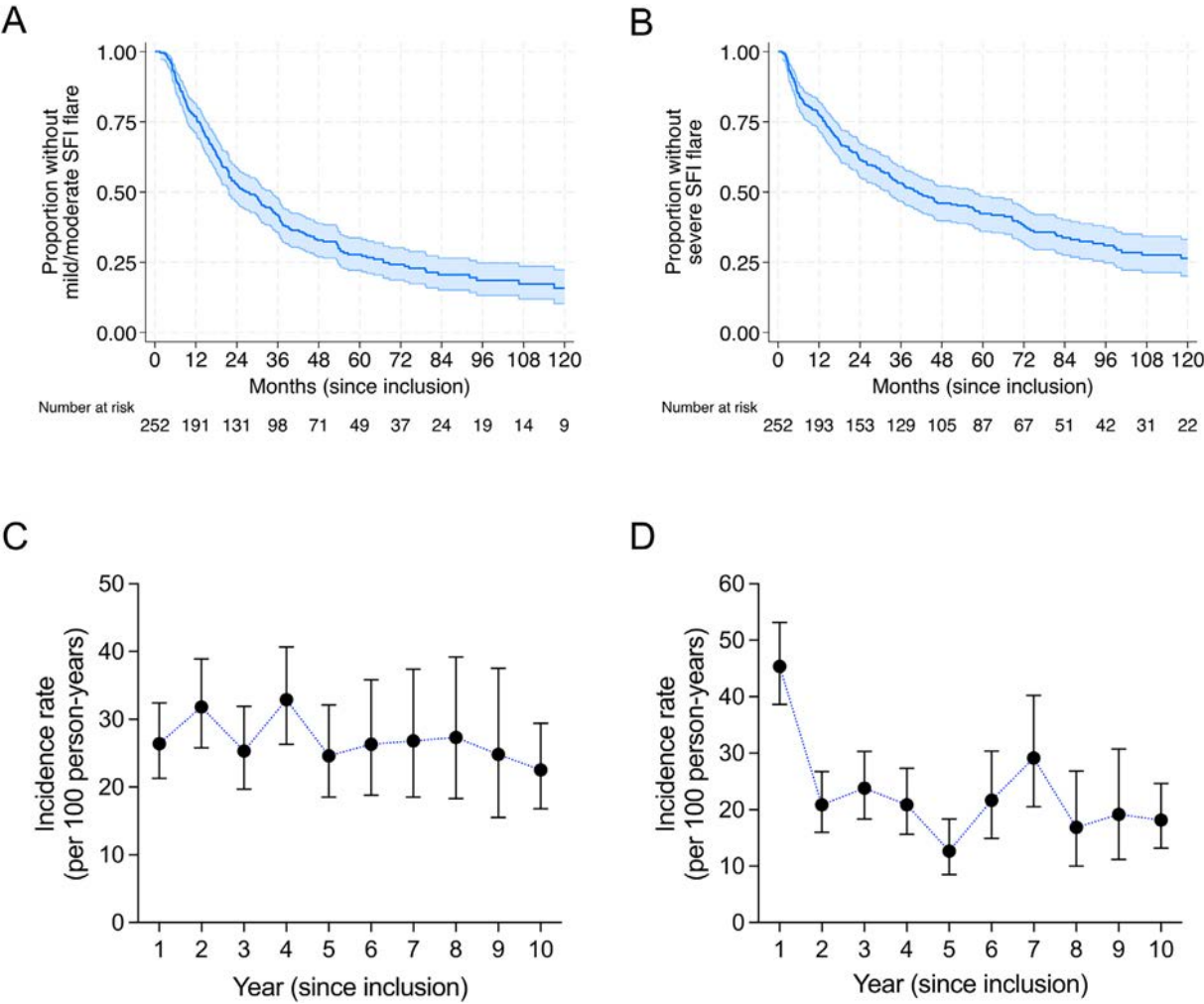


Figure 1. Frequency, incidence, and timing of SLE flares during follow-up. Kaplan-Meier curves show the time to first flare (cumulative probability) of mild/moderate flares (A) and severe flares (B) from study inclusion onwards. Numbers at risk are shown below each panel. Yearly incidence rates (per 100 person-years) of mild/moderate flares (C) and severe flares (D) during follow-up are shown with 95% CIs. Flares were assessed according to the modified SFI. SFI, Safety of Estrogens in Lupus Erythematosus National Assessment—systemic lupus erythematosus Disease Activity Index Flare Index; SLE, systemic lupus erythematosus.

Table 2
Association of mild/moderate and severe SFI flares with the risk for subsequent organ damage accrual in patients with SLE

SDI increase at subsequent visit(s)	Mild/moderate SFI: yes vs no			Severe SFI: yes vs no		
	IRR	95% CI	P value	IRR	95% CI	P value
Within 6 mo	1.39	0.89-2.18	.154	1.74	1.09-2.79	.021
Within 12 mo	1.42	1.07-1.88	.014	1.54	1.10-2.16	.012
Within 18 mo	1.20	0.96-1.52	.114	1.52	1.15-2.01	.004
Within 24 mo	1.20	0.97-1.49	.089	1.51	1.19-1.94	.001

SDI increase at subsequent visit(s)	Mild/moderate SFI vs no-flare visit			Severe SFI vs no-flare visit		
	IRR	95% CI	P value	IRR	95% CI	P value
Within 6 mo	1.52	0.94-2.48	.089	1.89	1.12-3.21	.017
Within 12 mo	1.53	1.13-2.05	.005	1.66	1.15-2.39	.007
Within 18 mo	1.30	1.02-1.65	.033	1.58	1.16-2.14	.003
Within 24 mo	1.30	1.04-1.62	.022	1.55	1.19-2.02	.001

IRR, incidence rate ratio; SDI, SLICC/ACR damage index; SFI, SELENA-SLEDAI Flare Index. Mixed-effect Poisson regression with a random intercept for each patient was used to account for repeated observations and to estimate adjusted IRRs for damage increase within the subsequent 12 months following each flare type. Sex, baseline disease activity, organ damage and SLE duration were included as covariates.

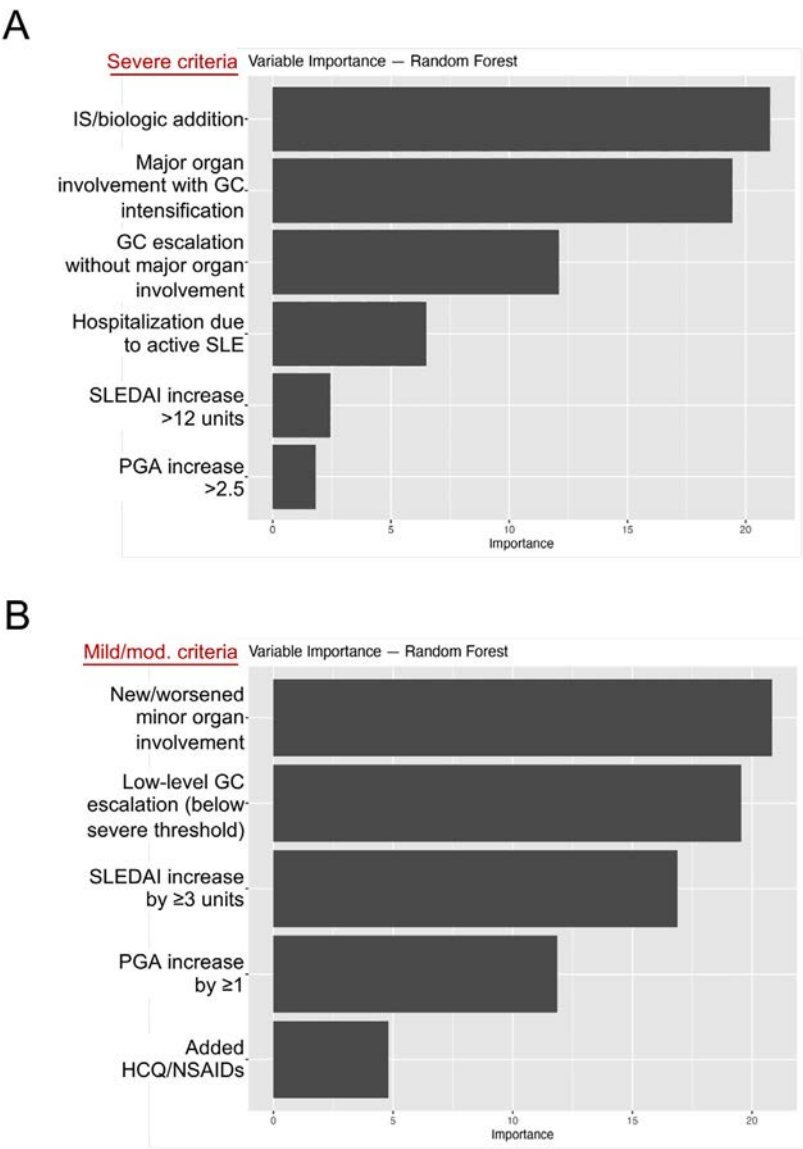


Figure 2. Data-driven prioritisation of SFI subcriteria according to their prognostic relevance for organ damage. Permutation-based variable importance rankings from Random Forest models predicting subsequent organ damage accrual are presented for severe SFI subcriteria (A) and mild/moderate SFI subcriteria (B). Importance reflects the relative influence of each individual subcriterion on subsequent organ damage accrual as determined by permutation-based measures. GC, glucocorticoid; HCQ, hydroxychloroquine; IS, immunosuppressant; NSAIDs, nonsteroidal anti-inflammatory drugs; SFI, Safety of Estrogens in Lupus Erythematosus National Assessment- systemic lupus erythematosus Disease Activity Index Flare Index; SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index PGA; Physician Global Assessment.

the most influential, followed by subcriterion 9 (GCs addition/escalation) and subcriterion 11 (hospitalisation due to active SLE) (Fig 2A, Supplementary Table S6). For mild/moderate flares, subcriterion 3 (new/worse clinical manifestations) and subcriterion 4 (increased or added GCs) ranked highest, followed by subcriterion 1 (increase in SLEDAI) and subcriterion 2 (increase in PGA) (Fig 2B, Supplementary Table S6).

Guided by these findings, we next constructed simplified flare definitions by iteratively combining top-ranked subcriteria (Supplementary Table S7). For severe flares, a Poisson model restricted to subcriteria 8 and 10 retained most of the prognostic information of the original SFI, with slightly higher overall accuracy (83.1% vs 81.0%). Including subcriteria 9 and 11 further improved the model β -coefficient and overall fit, yielding the best balance between sensitivity and specificity (Supplementary Table S7). For mild/moderate flares, a simplified definition comprising subcriteria 3 and 4 retained the prognostic value of the original SFI while modestly improving overall model performance (accuracy 76.2% vs 75.1%). When the remaining subcriteria 1 and 2 were added to this basic model, they showed negative β -coefficients and no improvement in model fit, and were therefore excluded (Supplementary Table S7). Collectively, reduced models containing only 4 of 6 SFI subcriteria for severe

flares and 2 of 5 SFI subcriteria for mild/moderate flares preserve the prognostic performance of the full definitions while improving simplicity.

Development of a weighted SLE flare score

Building on our findings that both mild/moderate and severe flares are associated with an increased likelihood of organ damage accrual, and having identified the most impactful SFI subcriteria underlying this relationship, we sought to develop a unified weighted flare score. Retained subcriteria from both the mild/moderate and severe SFI definitions were jointly entered into a Poisson regression model with 1000 bootstrap replications to ensure coefficient stability (Fig 3A). Subcriterion 11 (hospitalisation due to active SLE) had no measurable effect and was excluded from further weighting. Based on the relative β -coefficients, weighting factors were assigned proportionally (Fig 3B), providing the basis for the new flare score, which ranged from 1 to 8. Decomposition of subcriterion 9 into oral-only and intravenous methylprednisolone (IV-MP)-containing events showed a stronger association with subsequent damage for the former ($\beta = 0.36$ vs 0.17; Supplementary Table S8). However, because 81% of episodes were IV-MP-containing, the overall subcriterion was dominated by this lower-risk

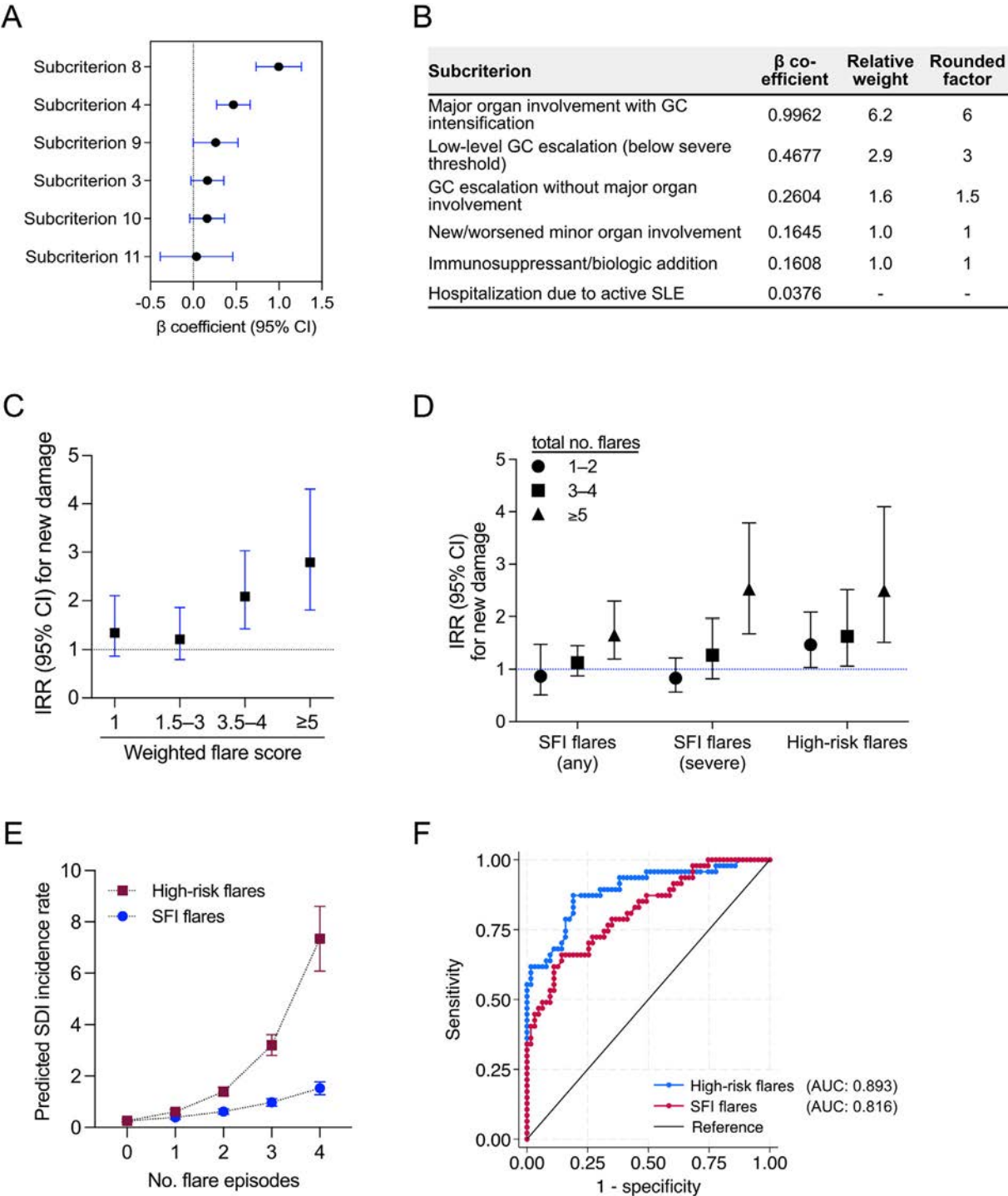


Figure 3. Derivation and validation of the weighted SLE flare score and high-risk flares. Standardised regression coefficients (95% CIs) from the bootstrap-validated mixed-effects Poisson model including the retained SFI subcriteria associated with subsequent damage accrual (A). Relative contribution of each retained SFI subcriterion, expressed as proportional weights and converted into rounded integer factors for construction of the final SLE flare score. Because severe SFI subcriterion 8 (major organ involvement requiring GC intensification) and subcriterion 9 (standalone GC escalation) were mutually exclusive in our dataset, the 2 subcriteria differ only by the presence of major organ involvement (B). Incidence rate ratios (IRRs) for new organ damage across predefined SLE flare score categories (1, 1.5–3, 3.5–4, and ≥ 5) in the derivation cohort (C). IRRs for new organ damage according to the number (1–2, 3–4, or ≥ 5) of any SFI flares, severe SFI flares, and high-risk flares (D). Model-predicted incidence rates of damage accrual (increase in SDI by ≥ 1) according to increasing numbers of high-risk flares compared with severe SFI flares in the external validation cohort (E). Receiver operating characteristic curves (ROC-AUC) in the external validation cohort comparing high-risk flares and severe SFI flares for discrimination of any increase in SDI (≥ 1) (F). GC, glucocorticoid; IRR, incidence rate ratio; ROC-AUC, receiver operating characteristic–area under the curve; SDI, SLICC ACR Damage Index; SFI, Safety of Estrogens in Lupus Erythematosus National Assessment-systemic lupus erythematosus Disease Activity Index Flare Index; SLE, systemic lupus erythematosus.

phenotype. As decomposition did not improve model fit, we retained the original SFI structure for simplicity. The Hosmer-Lemeshow goodness-of-fit test indicated no evidence of model miscalibration ($\chi^2[7] = 4.72$, $P = .69$), and the Brier score was 0.07, well within the acceptable range (0–0.25) and close to 0, indicating good overall predictive accuracy.

Operational use of the SLE flare score and definition of high-risk flares

Examination of the distribution of the new flare score across all longitudinal visits led to the formation of 4 approximately even categories: A (score = 1), B (scores 1.5–3), C (scores 3.5–4), and D (scores ≥ 5). Compared with flare-free intervals, the risk of new organ damage within a rolling window of 12 months increased progressively across higher flare score categories, reaching statistical significance for categories C ($\beta = 0.73$, $P < .001$) and D ($\beta = 1.03$, $P < .001$) (Fig 3C). Overall, 40.6% of flare episodes had a weighted score ≥ 3.5 , hereafter referred to as high-risk flares. These flares could arise from either severe or mild/moderate SFI subcriteria, or from mixed combinations thereof (Supplementary Table S9), suggesting that damage-inducing flare activity is not confined to conventionally defined severe or mild/moderate SFI events. By classifying damage as definitely, possibly, or not related to GC [22], we observed that high-risk flares were associated with increased accrual risk across all 3 categories, with the strongest association for steroid-independent damage (Supplementary Table S10).

At the operational level, using a >0 threshold (any flare) preserved comparable sensitivity with the original SFI model, while marginally improving specificity (73.6% vs 72.9%) (Supplementary Table S11). Raising the threshold to ≥ 3.5 (high-risk flares), further enhanced specificity for predicting future damage (89.6%) and improved overall model fit.

Association of high-risk flares with organ damage and external validation

We assessed the cumulative, patient-level impact of flare burden, testing whether individuals who experienced repeated high-risk flares had greater overall damage accrual during the entire follow-up as compared with those meeting the original SFI definition. As shown in Figure 3D, the risk for damage accumulation rose progressively with increasing number of high-risk flares, becoming significant even after 1 or 2 events (IRR: 1.47, 95% CI: 1.03–2.08) and further increasing for patients with 3 to 4 (IRR: 1.63, 95% CI: 1.06–2.52) or ≥ 5 events (IRR: 2.49, 95% CI: 1.51–4.10). By contrast, SFI flares showed a significant effect only after ≥ 5 episodes (IRR: 2.52, 95% CI: 1.68–3.78 for severe flares).

Using an independent cohort of 110 patients with SLE (Supplementary Table S12) observed over a median (IQR) of 66.3 (34.7) months, high-risk flares were more strongly associated with increases in SDI than SFI flares (IRR: 2.29, 95% CI 1.99–2.64 vs 1.57, 95% CI 1.34–1.84) (Fig 3E). Consistently, the discriminative ability for detecting any increase in SDI (≥ 1 vs 0) was higher for high-risk flares (AUC: 0.89, 95% CI: 0.83–0.96) compared with SFI flares (AUC: 0.82, 95% CI: 0.74–0.90) (Fig 3F). Collectively, these findings reinforce the high discriminatory performance of the high-risk flare definition, capturing the prognostic impact of even a limited number of high-intensity flare episodes.

High-risk flares may be prevented only by sustained attainment of treatment targets

Achieving DORIS or LLDAS has been linked to reduction of severe SFI flares [19]. To determine whether this association extends to high-risk flares, we quantified for each patient in the derivation cohort the cumulative proportion of time spent in either target during the first 12 months after inclusion. Cox-regression models were then used to assess the risk of flares occurring beyond the 12-month landmark, stratified by predefined target duration thresholds. Spending $>25\%$ of the first year in DORIS was associated with a significantly lower hazard of subsequent severe SFI (hazard ratio [HR]: 0.40, 95% CI: 0.23–0.68) and high-risk flares (HR: 0.54, 95% CI: 0.34–0.85), whereas no significant effect was observed for smaller exposure ($> 0\%$ – 25%) (Fig 4A,B). Attainment of LLDAS during the first 12 months showed a graded protective effect against severe SFI flares, with borderline benefit for $> 0\%$ to 25% and $> 25\%$ to 50% exposure, and a significant risk reduction when LLDAS was maintained for $> 50\%$ of the first year (HR: 0.55, 95% CI: 0.35–0.84). In contrast, LLDAS exposure did not meaningfully reduce the hazard of high-risk flares, irrespective of duration, including LLDAS $> 50\%$ of time (HR: 0.85, 95% CI: 0.57–1.27) (Fig 4C,D).

DISCUSSION

In this study, we show that the prognostic impact of SLE flares varies according to the specific SFI subcriteria involved. Weighting subcriteria derived from both mild/moderate and severe SFI classification identified new or worsened organ manifestations, GC escalation, and addition of immunosuppressants/biologics as key features of high-risk flares. These flares were associated with a markedly higher risk of subsequent organ damage, outperforming conventional SFI definitions, and remained prognostically informative already after only 1 or 2 events. Notably, high-risk flares occurred across both mild/moderate and severe SFI-defined categories, indicating that this conventional dichotomy does not fully capture the heterogeneity of flare severity. In this context, our findings suggest that sustained DORIS remission, rather than intermittent low disease activity, may be needed to meaningfully reduce the occurrence of high-risk flares and subsequent organ damage.

Flare occurrence in our SLE population was higher than that reported in other longitudinal studies, which estimated IRs of ~ 0.25 to 0.30 flares per patient-year [23–25]. Direct comparisons across studies, however, are challenging due to differences in patient populations, baseline disease activity, use of treatments, flare definitions, and duration of follow-up. The higher flare frequency observed in our cohort likely reflects the inclusion of patients with active disease at baseline.

Although severe SFI flares exerted a greater prognostic impact, mild/moderate flares also contributed to damage. Consistently, in the Asia-Pacific Lupus Collaboration, mild/moderate SFI flares increased the risk of subsequent damage by 32% and severe flares by 58% [13]. Similar results were reported in the Latin American Group for the Study of Lupus (GLADEL) cohort, in which each flare—irrespective of severity—increased the likelihood of damage accrual by almost 2-fold [14]. Altogether, these data suggest that even mild/moderate flares are clinically relevant contributors to long-term morbidity in SLE. Part of this burden might reflect less intensive treatment approaches for mild/moderate flare episodes in earlier therapeutic eras, compared with contemporary strategies favouring earlier immunosuppressive/biologic escalation [3].

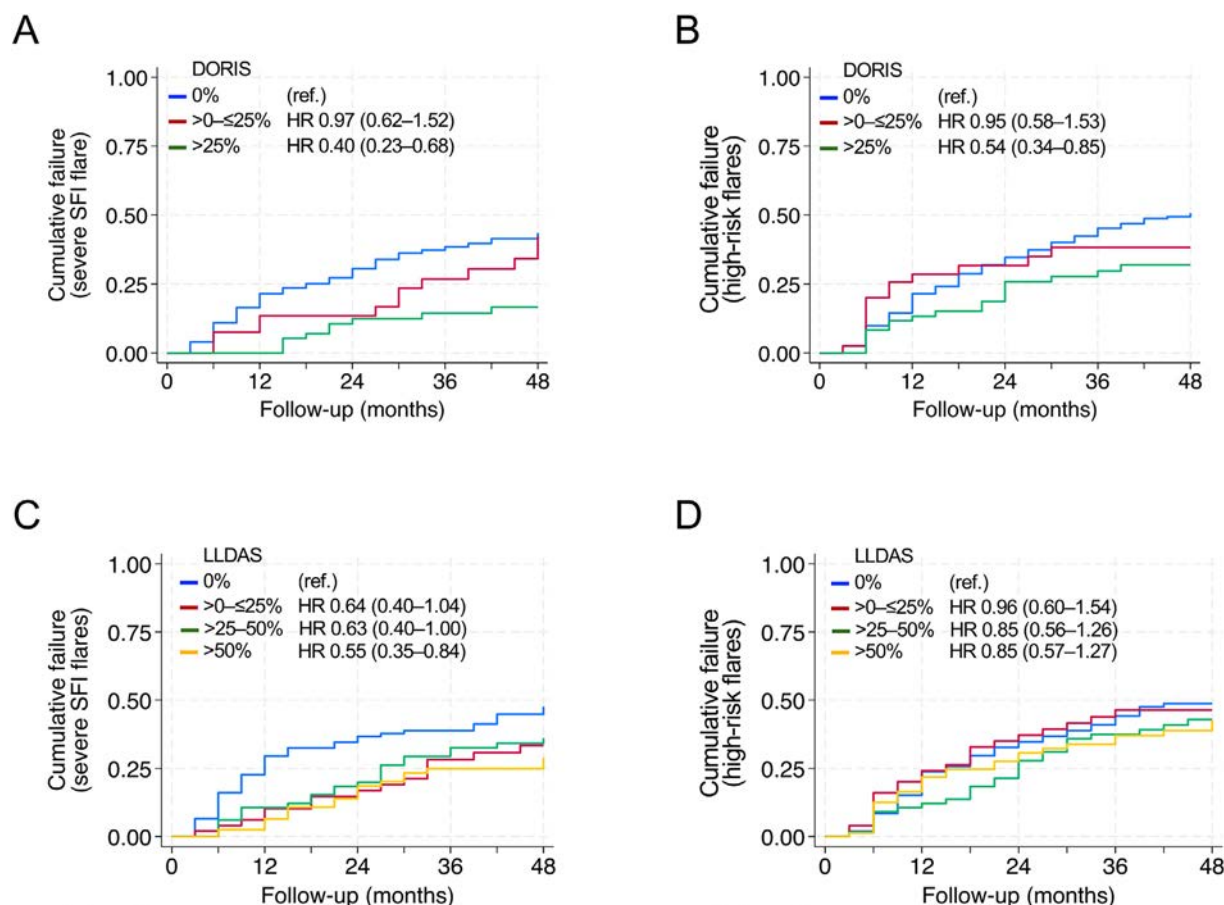


Figure 4. Treatment target attainment and risk of subsequent flares. Landmark-based analysis showing the cumulative incidence of flares occurring after the first 12 months of follow-up, stratified by the cumulative proportion of time spent in DORIS remission during the first 12-month period divided into 3 groups (0% [reference], > 0 to ≤ 5%, and > 25%). Analysis was performed in the derivation cohort (n = 354 patients with SLE). Curves show time to first severe SFI flare (A) and time to first high-risk flare (B). Corresponding analyses stratified by the cumulative proportion of time spent in LLDAS (divided into 4 groups; 0% [reference], > 0 to ≤ 25%, > 25% to 50%, and > 50%) during the first 12 months are shown for severe SFI flares (C) and high-risk flares (D). Hazard ratios (HR) were estimated using Cox models with predefined exposure groups and are shown on the graphs (A–D). DORIS, Definition of Remission in systemic lupus erythematosus; LLDAS, Lupus Low Disease Activity State; SFI, Safety of Estrogens in Lupus Erythematosus National Assessment-systemic lupus erythematosus Disease Activity Index Flare Index; SLE, systemic lupus erythematosus.

Our SLE flare score is driven primarily by treatment-related features, with GC escalation and intensification of ongoing therapy emerging as most influential. These elements reflect clinically meaningful changes in disease status that may not be explicitly captured by standard activity indices. The lower data-driven weight of standalone higher-dose GC escalation relative to mild/moderate oral escalation may reflect differences in GC tapering dynamics and disease control between the 2 dosing strategies, although this requires further investigation.

The SFI was introduced nearly 2 decades ago, when escalation to immunosuppressive agents (and later biologics) was reserved for more severe disease, such that treatment intensification itself partly encoded flare severity. Current recommendations advocate earlier introduction of these therapies both for flare prevention and as GC-sparing strategies, including in patients who might historically have fulfilled only the mild/moderate SFI definition. Consequently, an episode classified as mild/moderate SFI flare may reflect either limited disease activity or a clinically meaningful relapse managed without therapeutic escalation. Importantly, the weights in our score were data-derived, without any *a priori* assumption regarding the appropriateness of specific treatment strategies. Accordingly, within the score, intensification of immunosuppressive/biologic therapy should be interpreted primarily as a clinician-derived

marker of perceived flare severity rather than as an independent driver of damage accrual; therefore, recommendations-concordant treatment escalation should not be viewed as being ‘penalised’ by the scoring system.

The SFI, British Isles Lupus Assessment Group (BILAG)-2004, and more recently the SLE-DAS flare tool each capture different dimensions of flare activity. However, the relative complexity of instruments such as SFI and BILAG-2004 may hinder widespread implementation in routine practice [26]. Previous deconstruction of the SFI suggested that medication-only criteria may misclassify flare severity when considered in isolation [5]. Consistently, we demonstrated that treatment intensification acquires prognostic value especially when it occurs in conjunction with clinical worsening. Accordingly, combinations of clinical manifestations and treatment escalation frequently exceeded the predefined threshold of ≥ 3.5 , thereby designating flares linked to subsequent damage.

This study has several limitations. Recruitment from tertiary centres likely enriched the cohort with more severe and treatment-refractory cases. The cohort was almost exclusively of white patients with a modest representation of severe renal involvement (15.8% at inclusion), consistent with its Southern European composition; both features may limit generalizability. Our study spanned a prolonged calendar period, during which

treatment strategies evolved, particularly with respect to GC dosing and the increasing, albeit still limited, use of biologics with potential disease-modifying effects [27]. BILAG-2004 was not systematically scored, precluding direct comparison with this instrument. For SLE-DAS, head-to-head analyses were performed using a modified version constructed from the available variables. This modification may have reduced sensitivity to longitudinal changes in disease activity, and the possibility that the original SLE-DAS captures damage-relevant signal beyond that detected by the SFI cannot be entirely excluded. The SFI architecture also inherently couples clinical features with treatment decisions, which may vary and lack standardisation across clinicians and centres. As with all observational cohorts relying on scheduled visits, self-limiting mild/moderate flares may be incompletely captured; such underascertainment would, however, bias the corresponding association towards the null, whereas a significant association with damage was nonetheless observed. Laboratory parameters obtained within 30 days of clinical assessment may have introduced temporal discordance between serological and clinical activity. Finally, the retrospective design may be subject to residual confounding.

In summary, we developed a clinician-friendly flare score that provides an outcome-based refinement of SLE flares—beyond the classic mild/moderate vs severe dichotomy—and improves prognostic stratification. High-risk flares (score ≥ 3.5) may arise either from major organ involvement requiring high-dose GCs or from combinations of milder organ disease with treatment escalation, including GC dose increases or immunosuppressive/biologic initiation. These high-score episodes were robustly linked to subsequent damage accrual, and the prognostic relevance of even a single high-risk flare underscores the importance of achieving durable disease control. These findings support further evaluation of this scoring approach and suggest potential utility for clinical monitoring and trial design.

Competing interests

ES received consulting fees from AstraZeneca out of the present work. MG has received fees for sponsored lectures from GSK and AstraZeneca. AB reports consulting fees from GSK. GB reports grants from AstraZeneca, MSD, honoraria and/or consulting fees from GSK, Lilly, Novartis, AstraZeneca, and Otsuka. The remaining authors declare no conflict of interest.

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Contributors

DN and GB conceived and designed the study, interpreted the results, and drafted the manuscript. GB performed statistical analysis. PG and SK performed statistical analysis and drafted parts of the manuscript. SP, DNM, and MN collected data and performed data entry and data curation. ES, AR, AM, CB, AR, and AB evaluated patients and completed data collection forms. MG, PS, AF, DB, and GS contributed to results interpretation and evaluated patients. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. GB supervised the study and acts as guarantor for the overall content of the manuscript.

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Patient consent for publication

Patients' informed consent was obtained for data collection, analysis, and publication of results for both datasets used in this study.

Ethics approval

This study involves human participants, and data collection and analysis were approved by the ethics committees of the participating centres (protocol no. 13960/10-10-2018 and 516/2019/Oss/AOUFe). All patients gave informed consent upon inclusion in the respective registries.

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.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

All data collected for the study 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.2026.06.013](https://doi.org/10.1016/j.ard.2026.06.013).

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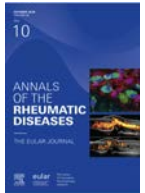
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Systemic sclerosis

Efficacy and safety of nerandomilast in patients with autoimmune disease-related progressive pulmonary fibrosis in the FIBRONEER-ILD trial

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ABSTRACT

Objectives: This study aimed to investigate the effects of nerandomilast in patients with autoimmune disease-related interstitial lung diseases (autoimmune ILDs) and progressive pulmonary fibrosis (PPF) in the FIBRONEER-ILD trial.

Methods: Patients with PPF were randomised to receive nerandomilast 9 mg twice a day, nerandomilast 18 mg twice a day, or placebo. Among patients with autoimmune ILDs, we evaluated the change in forced vital capacity (FVC) at week 52, and time-to-event endpoints and adverse events over the whole trial.

Results: Among 325 patients with autoimmune ILDs, adjusted mean (SE) changes in FVC (mL) at week 52 were −107.1 (25.0) in the placebo group, −61.2 (23.3) in the nerandomilast 9 mg twice a day group (difference vs placebo: 45.9 [95% CI: −20.8 to 112.6]), and −64.9 (23.5) in the nerandomilast 18 mg twice a day group (difference vs placebo: 42.2 [−24.9, 109.3]). Over the whole trial (mean exposure to trial medication: 15.8 months), the hazard ratio (HR) vs placebo for time to first acute exacerbation of ILD, hospitalisation for respiratory cause, or death was 0.66 (95% CI: 0.40–1.10) for nerandomilast 9 mg twice a day and 0.56 (0.33–0.94) for nerandomilast 18 mg twice a day, and the HR for time to death was 0.40 (0.17–0.94) for nerandomilast

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9 mg twice a day and 0.28 (0.11–0.69) for nerandomilast 18 mg twice a day. Adverse events led to treatment discontinuation in 13.0%, 8.0%, and 10.6% of the placebo, nerandomilast 9 mg twice a day, and nerandomilast 18 mg twice a day groups, respectively.

Conclusions: Consistent with the overall trial population, among patients with autoimmune ILDs in FIBRONEER-ILD, nerandomilast slowed progression of pulmonary fibrosis and was well tolerated.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- In the FIBRONEER-ILD trial in patients with progressive pulmonary fibrosis (PPF), nerandomilast reduced decline in forced vital capacity (FVC) vs placebo, supported by benefits on clinical outcomes, including death. Nerandomilast had a favourable safety and tolerability profile.

WHAT THIS STUDY ADDS

- This study shows that among patients with autoimmune disease-related interstitial lung diseases (autoimmune ILDs) in the FIBRONEER-ILD trial, the efficacy of nerandomilast on slowing the decline in FVC and on reducing the risk of clinical outcomes, including death, was consistent with that observed in the overall trial population. Nerandomilast had a favourable safety and tolerability profile irrespective of the use of immunosuppressants.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- These findings support the use of nerandomilast on detection of PPF in patients with autoimmune ILDs.

INTRODUCTION

Interstitial lung disease (ILD) is a common complication of autoimmune diseases [1,2]. Progressive pulmonary fibrosis (PPF) is a term used to describe a progressive course of fibrosing ILD in individuals with a diagnosis other than idiopathic pulmonary fibrosis (IPF) [3]. Patients with autoimmune ILDs and PPF have poor outcomes, including high mortality [4–8]. Most patients with autoimmune ILDs are taking immunomodulatory medications for 1 or more manifestations of their autoimmune disease [6,9–11]. There is some evidence that these medications slow the progression of ILD [12–14], particularly in patients with systemic sclerosis-associated ILD (SSc-ILD) [15–18], but there is limited evidence that these medications improve long-term outcomes, including survival. Nintedanib, a tyrosine kinase inhibitor, slows the decline in lung function in patients with SSc-ILD and PPF, but is associated with gastrointestinal adverse events that often lead to treatment discontinuation [19–22]. New, well-tolerated therapies, which may be used as monotherapy or in combination with other therapies, are needed to slow the progression of PPF in patients with autoimmune ILDs.

Nerandomilast is a preferential inhibitor of phosphodiesterase 4B that has immunomodulatory, vascular, and antifibrotic effects [23–25]. In the phase III FIBRONEER-ILD trial in patients with PPF, nerandomilast 9 mg twice a day and 18 mg twice a day reduced the decline in forced vital capacity (FVC) over 52 weeks vs placebo (the primary endpoint) [26]. In addition, over the whole trial, nerandomilast reduced the risk of clinically significant outcomes, including death [27]. Nerandomilast had a favourable safety and tolerability profile, with similar proportions of patients in the nerandomilast and placebo groups discontinuing due to adverse events [27]. In these analyses, we investigated the efficacy and safety of nerandomilast in patients with autoimmune ILDs and PPF in the FIBRONEER-ILD trial.

METHODS

Study design and participants

The design of the FIBRONEER-ILD trial has been published, together with the protocol [26]. In summary, eligible patients had an ILD other than IPF, an extent of pulmonary fibrosis (reticular abnormality with traction bronchiectasis, with or without honeycombing) on high-resolution computed tomography (HRCT) >10%, FVC ≥45% predicted, and diffusion capacity of the lungs for carbon monoxide (DLco) ≥25% predicted. Patients had met ≥1 of the following criteria for ILD progression at any time within the prior 24 months: relative decline in FVC % predicted ≥10%; relative decline in FVC % predicted ≥5% to <10% with worsened respiratory symptoms; relative decline in FVC % predicted ≥5% to <10% with increased extent of fibrotic changes on imaging; worsened respiratory symptoms and increased extent of fibrotic changes on imaging. Patients had taken nintedanib at a stable dose for ≥12 weeks or had not taken nintedanib for ≥8 weeks. Pirfenidone was not permitted. Prednisone >15 mg/d or equivalent (in the prior 4 weeks), cyclophosphamide, tocilizumab, mycophenolate (in the prior 8 weeks), or rituximab (in the prior 6 months) were not permitted at screening but could be initiated after 6 months of the trial to manage worsening of systemic disease.

Patients were randomised 1:1:1 to receive nerandomilast 9 mg twice a day, nerandomilast 18 mg twice a day, or placebo, stratified by the use of nintedanib and by pattern on HRCT (usual interstitial pneumonia [UIP]/UIP-like fibrotic pattern vs other fibrotic patterns) (centrally reviewed). The primary endpoint was the change from baseline in FVC (mL) at week 52. The first database lock took place once the last patient had completed the week 52 visit. Patients continued to take blinded randomised treatment until the final database lock, which took place once all patients had completed an end-of-treatment visit.

Investigators reported the patient's clinical diagnosis in the case report form. For patients with autoimmune ILDs, investigators could choose categories of rheumatoid arthritis-ILD (RA-ILD), SSc-ILD, or mixed connective tissue disease-ILD (MCTD-ILD). Patients with other autoimmune ILD diagnoses were reported in the category 'other fibrosing ILDs' with a description. The number of patients with ILD associated with idiopathic inflammatory myopathies (IIM)-ILD or Sjögren disease (SjD) was determined based on review of the descriptions provided for patients with 'other fibrosing ILDs'.

The FIBRONEER-ILD trial was carried out in accordance with the principles of the Declaration of Helsinki, the International Council for Harmonisation Harmonised Tripartite Guideline for Good Clinical Practice and applicable regulatory requirements. All patients provided written informed consent before entering the trial. Patients were involved in the design of the trial [28] but were not involved in the reporting or dissemination of the data from the subgroup of patients with autoimmune ILDs.

Outcomes

In these analyses, we report data from the subgroup of patients with autoimmune ILDs. We assessed the following: change from

baseline in FVC (mL) at week 52; categorical changes in FVC (mL) at week 52; time to first acute exacerbation of ILD, hospitalisation for respiratory cause, or death up to final database lock; time to first acute exacerbation of ILD or death up to final database lock; time to first hospitalisation for respiratory cause or death up to final database lock; time to death up to final database lock. We also evaluated the change from baseline in FVC (mL) at week 52 and time to first acute exacerbation of ILD, hospitalisation for respiratory cause, or death up to final database lock in patients with autoimmune ILDs in subsets by HRCT pattern (UIP/UIP-like fibrotic pattern vs other fibrotic patterns), by use of nintedanib (yes/no), and by use of immunomodulatory medications except prednisone (yes/no), at baseline. In the overall subgroup of patients with autoimmune ILDs, we assessed change in DLco % predicted at week 52 and changes in the Living with Pulmonary Fibrosis (L-PF) questionnaire [29] dyspnoea, cough, and fatigue scores at week 52.

Adverse events were coded using preferred terms in the Medical Dictionary for Regulatory Activities v27.1. We evaluated adverse events up to final database lock in subsets by use of nintedanib (yes/no) and by use of immunomodulatory medications except prednisone (yes/no) at baseline.

Analyses

Analyses were conducted in patients with autoimmune ILDs who received ≥ 1 dose of the trial drug. Change from baseline in FVC (mL) at week 52 was analysed using a mixed model for repeated measures (MMRM) with fixed categorical effects of treatment at each visit, baseline nintedanib use at each visit, HRCT pattern (UIP/UIP-like fibrotic pattern vs other fibrotic patterns) at each visit, and treatment by ILD diagnostic subgroup at each visit, and fixed continuous effects of baseline FVC (mL) at each visit. Missing FVC data from patients who died before week 52 were replaced based on the 10th percentile of observed values across treatment arms. The cumulative distribution of change in FVC

(mL) at week 52 was based on observed data. Changes from baseline in DLco and in L-PF questionnaire dyspnoea, cough, and fatigue scores at week 52 were analysed using an MMRM with fixed categorical effects of treatment at each visit, baseline nintedanib use at each visit, baseline HRCT pattern at each visit, and treatment by ILD diagnostic subgroup at each visit (for DLco), and fixed continuous effects of the baseline value of the endpoint in question at each visit. Time-to-event endpoints were analysed using a Cox proportional hazards model that included baseline nintedanib, HRCT pattern, age, FVC % predicted, DLco % predicted (corrected for haemoglobin), diagnosis, treatment, and ILD diagnostic subgroup-by-treatment interaction as covariates. Details of the statistical analyses in subsets of patients with autoimmune ILDs are provided in the [Supplementary material](#).

The analyses of change from baseline in FVC (mL) at week 52; time to first acute exacerbation of ILD, hospitalisation for respiratory cause, or death; and adverse events in the patients with autoimmune ILDs overall were prespecified. The other analyses reported in this manuscript were conducted *post hoc*.

RESULTS

Patients

A total of 325 patients with autoimmune ILDs received ≥ 1 dose of trial drug (100 placebo, 112 nerandomilast 9 mg twice a day, 113 nerandomilast 18 mg twice a day). Of these, 118 (36.3%) had RA-ILD, 75 (23.1%) had SSc-ILD, 47 (14.5%) had MCTD-ILD, 33 (10.2%) had ILD associated with IIM, 27 (8.3%) had SjD-ILD, and 25 (7.7%) had other autoimmune ILDs. At baseline, 212 (65.2%) patients were female, the mean (SD) age was 63.4 (11.2) years, the mean FVC % predicted was 71.5 (15.0), the mean DLco % predicted was 51.5 (16.8), and 256 (78.8%) had a UIP/UIP-like fibrotic pattern on HRCT. Baseline characteristics were similar across treatment groups ([Table 1](#)).

Table 1
Characteristics of patients with autoimmune ILDs in the FIBRONEER-ILD trial

	Placebo (n = 100)	Nerandomilast 9 mg twice a day (n = 112)	Nerandomilast 18 mg twice a day (n = 113)
Female	69 (69.0)	72 (64.3)	71 (62.8)
Age (y)	62.5 (11.3)	63.7 (11.4)	64.0 (10.9)
Weight (kg)	63.3 (14.2)	66.7 (16.0)	66.4 (14.3)
Body mass index (kg/m ²)	24.5 (4.4)	25.4 (4.7)	25.1 (4.6)
Race ^a			
Asian	60 (60.0)	58 (51.8)	54 (47.8)
White	35 (35.0)	46 (41.1)	52 (46.0)
American Indian or Alaska Native	4 (4.0)	3 (2.7)	2 (1.8)
Black/African-American	0 (0)	1 (0.9)	2 (1.8)
Smoking status			
Never	67 (67.0)	67 (59.8)	65 (57.5)
Former	31 (31.0)	43 (38.4)	44 (38.9)
Current	2 (2.0)	2 (1.8)	4 (3.5)
Years since diagnosis of ILD	4.5 (3.7)	4.6 (4.7)	5.4 (5.4)
FVC (mL)	2162 (643)	2257 (720)	2260 (660)
FVC % predicted	70.8 (14.5)	72.5 (16.2)	71.2 (14.3)
DLco % predicted ^b	53.0 (16.4)	51.4 (15.9)	50.2 (18.2)
Taking nintedanib	29 (29.0)	39 (34.8)	43 (38.1)
Taking immunomodulatory medication(s) except prednisone	57 (57.0)	63 (56.3)	59 (52.2)
UIP/UIP-like fibrotic pattern on HRCT	78 (78.0)	89 (79.5)	89 (78.8)
Supplemental oxygen use ^c	18 (18.2)	20 (18.0)	33 (29.5)

DLco, diffusion capacity of the lungs for carbon monoxide; FVC, forced vital capacity; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; UIP, usual interstitial pneumonia.

Data are mean (SD) or n (%) of patients.

^a Data from patients who selected 1 race; 8 patients selected >1 race.

^b Corrected for haemoglobin; n = 110 in nerandomilast 18 mg twice a day group.

^c n = 99 in placebo group, n = 111 in nerandomilast 9 mg twice a day group, n = 112 in nerandomilast 18 mg twice a day group.

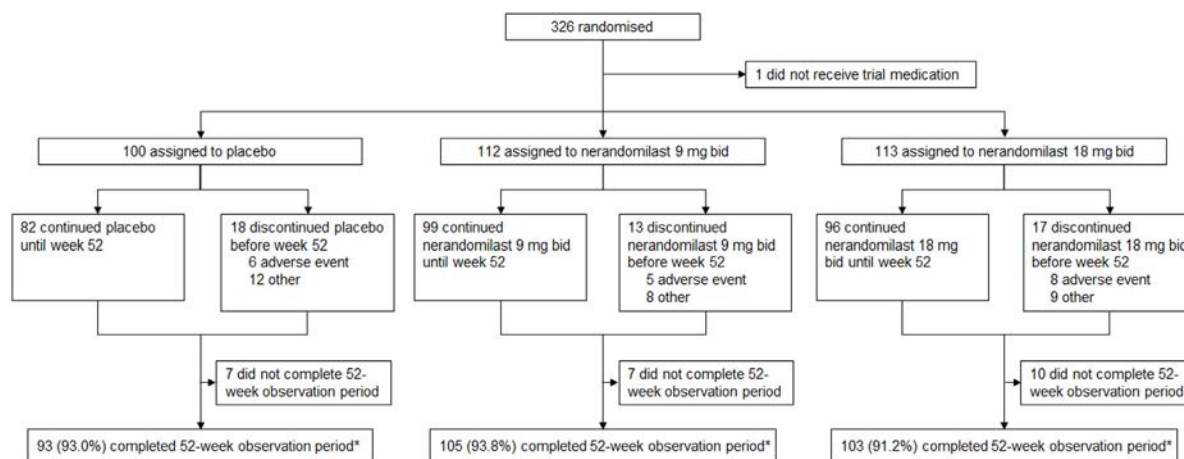


Figure 1. Disposition of patients with autoimmune ILDs up to week 52 in the FIBRONEER-ILD trial. *Forced vital capacity assessment available at week 52 visit or lung transplant or death before week 52. ILD, interstitial lung disease.

At baseline, 111 (34.2%) patients with autoimmune ILDs were taking nintedanib, and 179 (55.1%) were taking immunomodulatory medications except prednisone; 58 patients (17.8%) were taking nintedanib with immunomodulatory medications except prednisone. Methotrexate, azathioprine, and hydroxychloroquine were the most frequently used immunomodulatory medications (Supplementary Tables S1 and S2). Among all patients with autoimmune ILDs, immunomodulatory medications were added during the trial in 14 (14.0%), 22 (19.6%), and 11 (9.7%) patients in the placebo, nerandomilast 9 mg twice a day, and nerandomilast 18 mg twice a day groups, respectively (Supplementary Table S3).

Most patients in each treatment group completed the 52-week observation period (91.2%–93.8%) (Fig 1) and the observation period up to the final database lock (94.7%–98.0%) (Supplementary Fig S1). Up to the final database lock, the mean (SD) exposure to trial medication was 15.8 (5.1) months, and the mean (SD) observation period was 17.3 (3.7) months.

Changes in FVC

In all patients with autoimmune ILDs, adjusted mean (SE) changes in FVC (mL) at week 52 were −107.1 (25.0) in the placebo group, −61.2 (23.3) in the nerandomilast 9 mg twice a day

group (difference vs placebo: 45.9 mL [95% CI: −20.8 to 112.6]), and −64.9 (23.5) in the nerandomilast 18 mg twice a day group (difference vs placebo: 42.2 mL [95% CI: −24.9 to 109.3]) (Fig 2). The FVC curves for nerandomilast and placebo separated early (Supplementary Fig S2). The proportions of patients with stabilisation/improvement (change >0) in FVC (mL) at week 52 were 32%, 43%, and 40% in the placebo, nerandomilast 9 mg twice a day, and nerandomilast 18 mg twice a day groups, respectively (Supplementary Fig S3).

Changes in DLco

Adjusted mean (SE) changes in DLco % predicted at week 52 in patients with autoimmune ILDs were −0.7 (1.4) in the placebo group, −2.2 (1.3) in the nerandomilast 9 mg twice a day group (difference vs placebo: −1.5 [95% CI: −5.2 to 2.3]), and −2.7 (1.4) in the nerandomilast 18 mg twice a day group (difference vs placebo: −2.0 [95% CI: −5.9 to 1.8]).

Patient-reported outcomes

Changes in the L-PF questionnaire dyspnoea, cough, and fatigue scores at week 52 were similar across the placebo and nerandomilast groups (Supplementary Table S4).

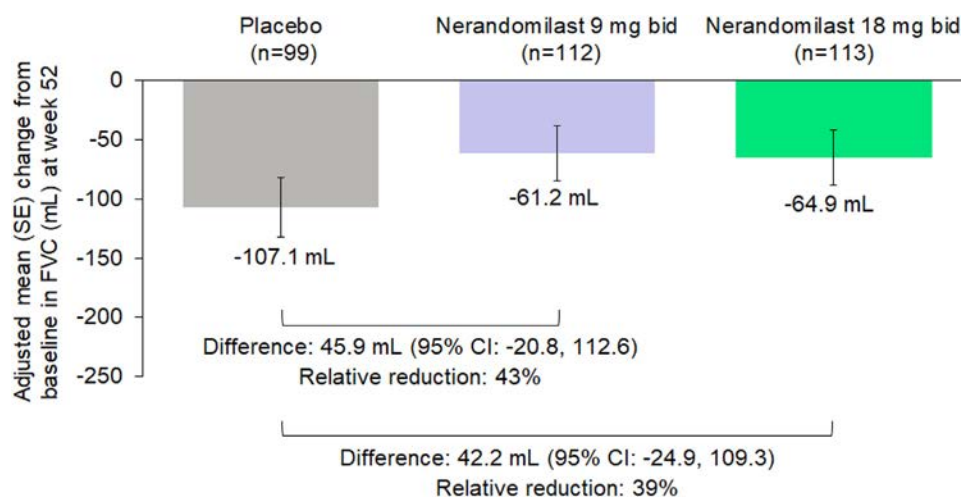


Figure 2. Change in FVC (mL) at week 52 in patients with autoimmune ILDs in the FIBRONEER-ILD trial. FVC, forced vital capacity; ILD, interstitial lung disease.

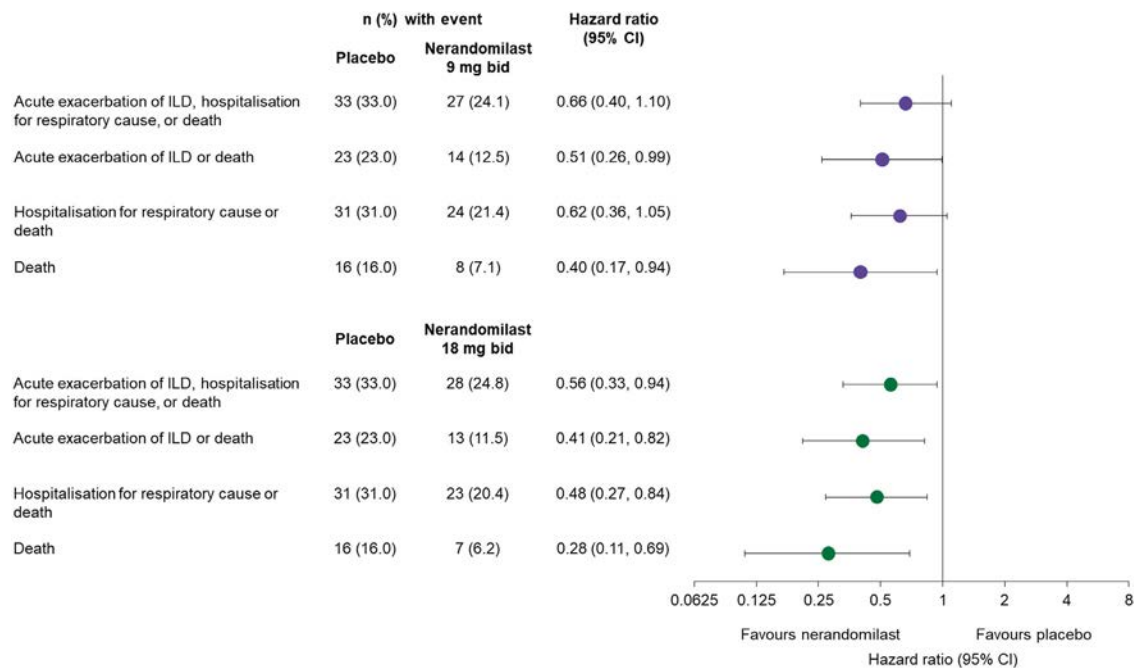


Figure 3. Time-to-event endpoints at final database lock in patients with autoimmune ILDs in the FIBRONEER-ILD trial. ILD, interstitial lung disease.

Time-to-event endpoints

Among all patients with autoimmune ILDs, the composite endpoint of time to first acute exacerbation of ILD, hospitalisation for respiratory cause, or death was experienced by 33 (33.0%) patients in the placebo group, 27 (24.1%) in the nerandomilast 9 mg twice a day group, and 28 (24.8%) in the nerandomilast 18 mg twice a day group. Compared with placebo, there was a 44% reduction in risk with nerandomilast 18 mg twice a day (hazard ratio [HR]: 0.56 [95% CI: 0.33-0.94]), which was nominally significant, and a 34% reduction in risk with nerandomilast 9 mg twice a day (HR: 0.66 [95% CI: 0.40-1.10]) (Figs 3 and 4A).

Both nerandomilast 9 mg twice a day and 18 mg twice a day had a nominally significant benefit on the risk of acute exacerbation of ILD or death. Compared with placebo, there was a 49% reduction in risk with nerandomilast 9 mg twice a day (HR: 0.51 [95% CI: 0.26-0.99]) and a 59% reduction in risk with nerandomilast 18 mg twice a day (HR: 0.41 [95% CI: 0.21-0.82]). For the risk of hospitalisation for respiratory cause or death, compared with placebo, there was a 52% reduction in risk with nerandomilast 18 mg twice a day (HR: 0.48 [95% CI: 0.27-0.84]), which was nominally significant, and a 38% reduction in risk with nerandomilast 9 mg twice a day (HR: 0.62 [95% CI: 0.36-1.05]) (Fig 3).

Death occurred in 16 (16.0%) patients in the placebo group, 8 (7.1%) in the nerandomilast 9 mg twice a day group, and 7 (6.2%) in the nerandomilast 18 mg twice a day group. Compared with placebo, there was a 72% reduction in risk with nerandomilast 18 mg twice a day (HR: 0.28 [95% CI: 0.11-0.69]) and a 60% reduction in risk with nerandomilast 9 mg twice a day (HR: 0.40 [95% CI: 0.17-0.94]) (Figs 3 and 4B). Most deaths were adjudicated to have a respiratory-related cause (Supplementary Table S5).

Subsets by use of immunomodulatory medication

The decline in FVC was smaller in the nerandomilast groups than in the placebo group, both in patients taking

and not taking immunomodulatory medication at baseline. Among patients not taking immunomodulatory medication at baseline, adjusted mean (SE) changes in FVC (mL) at week 52 were –92.8 (35.9) in the placebo group, –81.0 (33.6) in the nerandomilast 9 mg twice a day group, and –69.0 (32.9) in the nerandomilast 18 mg twice a day group, whereas among patients taking immunomodulatory medication, adjusted mean (SE) changes were –113.6 (31.6) in the placebo group, –44.7 (29.7) in the nerandomilast 9 mg twice a day group, and –58.9 (30.8) in the nerandomilast 18 mg twice a day group (Fig 5A,B). The effect of nerandomilast vs placebo on reducing decline in FVC was consistent across the subsets (nominal treatment-by-subset interaction $P = .39$ for nerandomilast 9 mg twice a day and $P = .64$ for nerandomilast 18 mg twice a day). The effect of nerandomilast on the composite endpoint of time to first acute exacerbation of ILD, hospitalisation for respiratory cause, or death was also consistent across the subsets (nominal treatment-by-subset interaction $P = .73$ for nerandomilast 9 mg twice a day and $P = .34$ for nerandomilast 18 mg twice a day) (Fig 5C).

Subsets by HRCT pattern and nintedanib use

The effects of nerandomilast vs placebo on reducing decline in FVC and on the composite endpoint of time to first acute exacerbation of ILD, hospitalisation for respiratory cause, or death were consistent across subsets by HRCT pattern and by use of nintedanib at baseline (Supplementary Figs S4 and S5).

Adverse events

The proportions of patients with any adverse event(s) and with adverse event(s) leading to treatment discontinuation were similar across the nerandomilast and placebo groups (Supplementary Table S6). The most frequent adverse events are shown in Supplementary Table S7.

The proportions of patients with any adverse event(s) and with adverse event(s) leading to treatment discontinuation were similar across the nerandomilast and placebo groups in

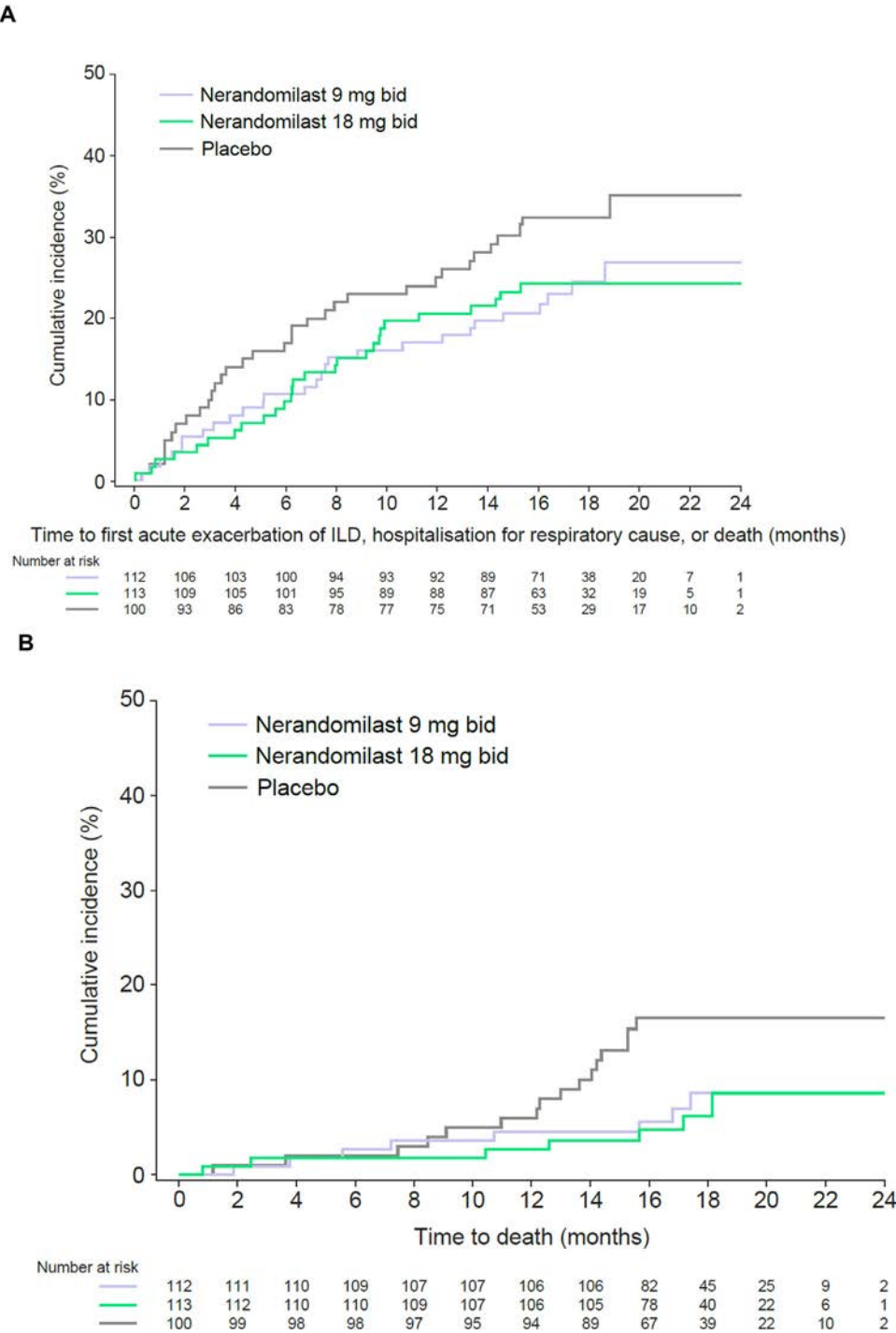


Figure 4. (A) Time to first acute exacerbation of ILD, hospitalisation for respiratory cause, or death and (B) time to death up to final database lock in patients with autoimmune ILDs in the FIBRONEER-ILD trial. ILD, interstitial lung disease.

patients taking and not taking nintedanib at baseline (Supplementary Table S8). Diarrhoea was the most common adverse event and was more frequent in patients taking nintedanib as background therapy than in those not taking it. The proportions of patients with diarrhoea adverse events in the placebo, nerandomilast 9 mg twice a day, and nerandomilast 18 mg twice a day groups were 19.7%, 19.2%, and 22.9%, respectively, in patients not taking nintedanib and 34.5%, 46.2%, and 44.2% in patients taking nintedanib (Supplementary Table S9).

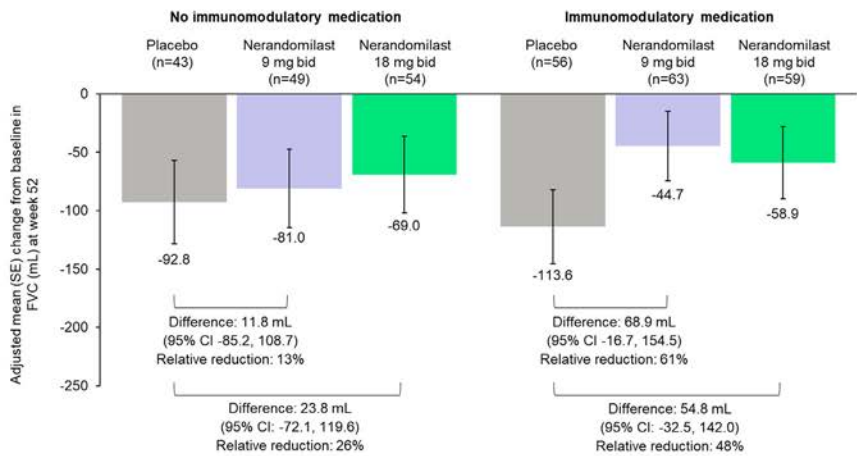
The proportions of patients with any adverse event(s) and with adverse event(s) leading to treatment discontinuation were similar across the nerandomilast and placebo groups in patients taking and not taking immunomodulatory medication at

baseline (Table 2). The proportions of patients with diarrhoea adverse events in the placebo, nerandomilast 9 mg twice a day, and nerandomilast 18 mg twice a day groups were 20.9%, 24.5%, and 25.9%, respectively, in patients not taking immunomodulatory medication and 26.3%, 31.7%, and 35.6% in patients taking immunomodulatory medication (Supplementary Table S10). No increase in upper respiratory tract infections, pneumonia, or sepsis was seen in patients taking nerandomilast and immunomodulatory medication.

DISCUSSION

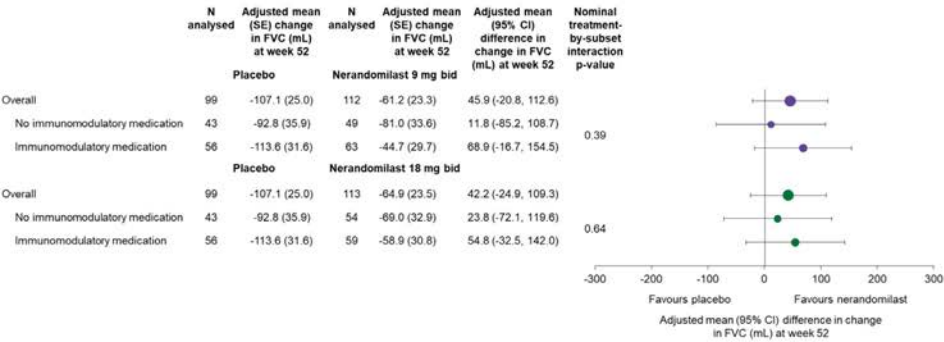
These analyses of data from the FIBRONEER-ILD trial in patients with PPF show that nerandomilast slowed the

A



Nominal treatment-by-subset interaction $p=0.39$ for nerandomilast 9 mg bid and $p=0.64$ for nerandomilast 18 mg bid.

B



C

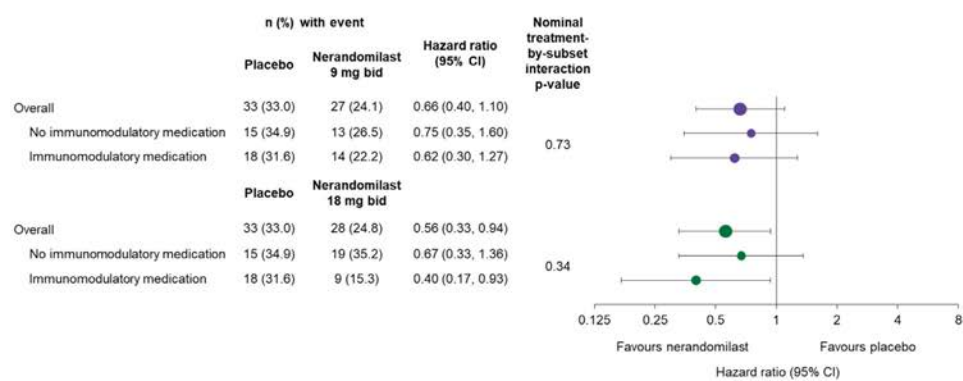


Figure 5. (A) Change in FVC (mL) at week 52 with placebo, nerandomilast 9 mg twice a day and nerandomilast 18 mg twice a day, (B) change in FVC (mL) at week 52 with nerandomilast 9 mg twice a day vs placebo and nerandomilast 18 mg twice a day vs placebo, (C) risk of acute exacerbation of ILD, hospitalisation for respiratory cause, or death with nerandomilast 9 mg twice a day vs placebo and nerandomilast 18 mg twice a day vs placebo at final database lock in subsets by use of immunomodulatory medication (except prednisone) at baseline in patients with autoimmune ILDs in the FIBRONEER-ILD trial. FVC, forced vital capacity; ILD, interstitial lung disease.

progression of pulmonary fibrosis in patients with progressive fibrosing autoimmune ILDs. Compared with placebo, the relative reductions in change in FVC at week 52 with the 2 doses of nerandomilast were 43% and 39% in the patients with autoimmune ILDs, consistent with the reductions of 49% and 41% observed in the overall trial population [26]. In addition, consistent with observations in the overall trial population, in the

patients with autoimmune ILDs, nerandomilast was associated with reductions in the risk of clinically relevant outcomes. Nerandomilast 18 mg twice a day had a nominally significant benefit on the risk of acute exacerbation of ILD, hospitalisation for respiratory cause, or death vs placebo, with a 44% reduction in risk, whereas the 9 mg twice a day dose had a numerical benefit on this outcome, with a 34% reduction in risk. Although the

Table 2

Adverse events in patients with autoimmune ILDs by use of immunomodulatory medications at baseline in the FIBRONEER-ILD trial

	No immunomodulatory medication			Immunomodulatory medication (except prednisone)		
	Placebo (n = 43)	Nerandomilast 9 mg twice a day (n = 49)	Nerandomilast 18 mg twice a day (n = 54)	Placebo (n = 57)	Nerandomilast 9 mg twice a day (n = 63)	Nerandomilast 18 mg twice a day (n = 59)
Any adverse event(s)	41 (95.3)	48 (98.0)	49 (90.7)	52 (91.2)	58 (92.1)	56 (94.9)
Adverse event(s) leading to treatment discontinuation	6 (14.0)	3 (6.1)	6 (11.1)	7 (12.3)	6 (9.5)	6 (10.2)
Diarrhoea adverse event(s) leading to treatment discontinuation	0 (0)	0 (0)	1 (1.9)	0 (0)	1 (1.6)	1 (1.6)
Adverse event(s) leading to treatment interruption	10 (23.3)	9 (18.4)	13 (24.1)	9 (15.8)	11 (17.5)	13 (22.0)
Serious adverse event(s) ^a	22 (51.2)	19 (38.8)	28 (51.9)	19 (33.3)	21 (33.3)	18 (30.5)
Fatal adverse event	4 (9.3)	1 (2.0)	2 (3.7)	5 (8.8)	5 (7.9)	2 (3.4)

ILD, interstitial lung disease.

Data are n (%) of patients with ≥1 such adverse event reported up to final database lock.

^a Event that resulted in death, in hospitalisation or prolongation of hospitalisation, or in persistent or clinically significant disability or incapacity; or was life-threatening, a congenital anomaly or birth defect, or deemed to be serious for any other reason.

number of deaths during the trial was small, both doses of nerandomilast were associated with a nominally significant reduction in the risk of death vs placebo. The reduction in deaths was driven by a reduction in deaths with a respiratory cause. Nerandomilast slowed the decline in FVC and had numerical benefits on clinical outcomes, both in patients taking and not taking nintedanib and in those taking and not taking immunomodulatory medications.

The data from the FIBRONEER-ILD trial provide further evidence that patients with autoimmune ILDs and PPF continue to experience progression and have poor outcomes. In the placebo group, the decline in FVC over a year in patients with autoimmune ILDs was 107 mL, and 19% had a decline in FVC (mL) of >10%. Nearly a third of the patients in the placebo group had an acute exacerbation of ILD, were hospitalised for respiratory cause, or died over a mean observation period of about 17 months, despite one-third of these patients being treated with nintedanib and more than half taking immunomodulatory medications. Also noteworthy is that 16% of patients in the placebo group died during the trial.

Nearly 80% of patients with autoimmune ILDs and PPF in the FIBRONEER-ILD trial had a UIP/UIP-like fibrotic pattern on HRCT. A UIP pattern on HRCT has been associated with a greater risk of ILD progression [30–33]. This may explain why the proportion of patients with a UIP-like pattern in the FIBRONEER-ILD cohort was higher than that observed in real-world cohorts of patients with autoimmune ILDs that did not select for patients with progressive disease [31,33]. The effect of nerandomilast on slowing the decline in FVC and on the risk of acute exacerbation of ILD, hospitalisation for respiratory cause, or death was consistent between patients with a UIP/UIP-like fibrotic pattern and other fibrotic patterns on HRCT.

The safety and tolerability of new medications are a particular concern for patients with autoimmune ILDs, who often have a high medication burden. Adverse events leading to discontinuation and serious adverse events were balanced across the nerandomilast and placebo groups. As expected based on the adverse event profile of nintedanib [20–22], across the treatment groups, diarrhoea was more common in patients taking nintedanib. Diarrhoea was more frequent in patients taking nerandomilast than in those taking placebo, but it infrequently led to treatment discontinuation. Weight loss was also identified as an adverse event associated with nerandomilast. Importantly, there was no increase in upper respiratory tract infections or pneumonia in patients treated with nerandomilast compared with placebo.

Our analyses have some limitations. The FIBRONEER-ILD trial was not powered to assess the efficacy of nerandomilast in patients with autoimmune ILDs, and the number of patients with individual autoimmune disease diagnoses was too small to enable robust analyses of data from these specific diagnostic subsets. The analyses of subsets based on HRCT pattern, use of nintedanib, and use of immunosuppressants should be regarded as exploratory. The number of patients in some subsets was small. Only 3 Black patients with autoimmune ILDs participated in the trial. Although over half of the patients with autoimmune ILDs were taking immunomodulatory medications at baseline, some commonly used immunomodulatory medications, including mycophenolate and other medications recommended as treatment options for autoimmune ILD in guidelines issued by the American College of Rheumatology/American College of Chest Physicians [34] and the European Respiratory Society/European Alliance of Associations for Rheumatology [2], were excluded. The efficacy and tolerability of nerandomilast in patients taking medications excluded from the trial are unknown. Our findings are only applicable to the population studied, ie, to patients with progressive fibrosing autoimmune ILDs. A benefit of nerandomilast on patient-reported outcomes was not demonstrated.

In conclusion, among patients with autoimmune ILDs and PPF in the FIBRONEER-ILD trial, the efficacy of nerandomilast on slowing the decline in FVC was consistent with that observed in the overall trial population. Nerandomilast reduced the risk of clinically relevant outcomes, including death. Nerandomilast had a favourable safety and tolerability profile. These findings support the use of nerandomilast as a treatment for PPF in patients with autoimmune ILDs.

CRediT authorship contribution statement

Anna-Maria Hoffmann-Vold: Writing – review & editing, Investigation, Conceptualization. **Shervin Assassi:** Writing – review & editing, Investigation, Conceptualization. **Vincent Cottin:** Writing – review & editing, Investigation, Conceptualization. **Michael Kreuter:** Writing – review & editing, Investigation, Conceptualization. **Claudia Valenzuela:** Writing – review & editing, Investigation, Conceptualization. **Marlies S Wijsenbeek:** Writing – review & editing, Investigation, Conceptualization. **Hui Gu:** Writing – review & editing, Formal analysis, Conceptualization. **Madhu Kanakapura:** Writing – review & editing, Conceptualization. **Ivana Ritter:** Writing – review & editing, Conceptualization. **Susanne Stowasser:** Writing –

review & editing, Conceptualization. **Gerrit Weimann:** Writing – review & editing, Conceptualization. **Toby M Maher:** Writing – review & editing, Investigation, Conceptualization.

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Competing interests

A-MH-V reports grants from Boehringer Ingelheim and Janssen; consultancy fees from AbbVie, Avalyn Pharma, Boehringer Ingelheim, Bristol Myers Squibb, Calluna Pharma, Genentech, Janssen, Merck Sharp & Dohme, Pliant Therapeutics, Roche, and Werfen; speaker fees from Boehringer Ingelheim, Janssen, Medscape, Merck Sharp & Dohme, Novartis, and Roche; travel support from Boehringer Ingelheim; was the convenor of the European Respiratory Society/European Alliance of Associations for Rheumatology (ERS/EULAR) guideline for connective tissue disease-associated ILD; and is the EULAR study group leader on the lung in rheumatic and musculoskeletal diseases. SA reports grants from AbbVie, aTyr, Boehringer Ingelheim, and the Scleroderma Research Foundation; consultancy fees from AbbVie, AstraZeneca, aTyr, Boehringer Ingelheim, Candid, CSL Behring, Merck, Mitsubishi Tanabe, Regeneron, the Scleroderma Research Foundation, Takeda, TeneoFour, and UCB; and speaker fees from Boehringer Ingelheim. VC reports consultancy fees from AbbVie, AstraZeneca, Avalyn Pharma, Boehringer Ingelheim, Bristol Myers Squibb, CSL (Behring, Vifor), Ferrer/United Therapeutics, GlaxoSmithKline, Gossamer Bio, Liquidia, Pliant Therapeutics, PureTech, Roche, Roivant, and Sanofi; fees for lectures from Boehringer Ingelheim, Ferrer/United Therapeutics, and Sanofi; support for attending meetings from Boehringer Ingelheim and Sanofi; and has served on Data and Safety Monitoring Boards for Calluna Pharma, GlaxoSmithKline and Molecure, and on an Adjudication Committee for FibroGen. MK reports consultancy fees from Boehringer Ingelheim, AstraZeneca, Bristol Myers Squibb, Chiesi, and GlaxoSmithKline. CV reports consultancy fees from Boehringer Ingelheim. MSW reports grants from AstraZeneca/Daiichi, Boehringer Ingelheim, Erasmus MC, Hoffmann-La Roche, Sarcoidosis.nl, The Dutch Lung Foundation, The Dutch Pulmonary Fibrosis Patients Association, The Netherlands Organization for Health Research and Development, and The Thorax Foundation; consultancy fees (with payments made to her institution) from Agomab, Amgen, AstraZeneca, Avalyn Pharma, Boehringer Ingelheim, Bristol Myers Squibb, Calluna Pharma, Chiesi, CSL Behring, Galapagos, Galecto, GlaxoSmithKline, Hoffmann-La Roche, Horizon Therapeutics, Kinevant Sciences, Merck Sharp & Dohme, Molecure, NeRRé Therapeutics, Novartis, Pulmongene, PureTech Health, Trevi, and Vicore; speaker fees (with payments made to her

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Patient consent for publication

Not applicable.

Ethics approval

The FIBRONEER-ILD trial was carried out in accordance with the principles of the Declaration of Helsinki, the International Council for Harmonisation Harmonised Tripartite Guideline for Good Clinical Practice and applicable regulatory requirements. All patients provided written informed consent prior to entering the trial.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

To ensure independent interpretation of clinical study results and enable authors to fulfil their role and obligations under the ICMJE criteria, Boehringer Ingelheim grants all authors access to relevant clinical study data. In adherence with the Boehringer Ingelheim Policy on Transparency and Publication of Clinical Study Data, scientific and medical researchers can request access to clinical study data, typically 1 year after approval has been granted by major regulatory authorities or after termination of the development programme. Researchers should use <https://vivli.org/> to request access to study data and visit <https://www.mystudywindow.com/msw/datasharing> for further information.

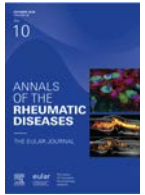
Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ard.2026.03.027](https://doi.org/10.1016/j.ard.2026.03.027).

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Systemic sclerosis

Pulmonary arterial hypertension susceptibility and mortality among patients with systemic sclerosis with the DNASE1L3 rs35677470 (Arg206Cys) variant

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ABSTRACT

Objectives: A loss-of-function variant in deoxyribonuclease 1-like 3 (*DNASE1L3*), encoding Arg to Cys substitution at amino acid 206 (R206C), was previously identified as a heritable susceptibility factor for systemic sclerosis (SSc). We analysed mortality and pulmonary outcomes in patients with SSc with or without the *DNASE1L3* R206C variant.

Methods: Mortality, pulmonary hypertension (PH), and interstitial lung disease (ILD) were ascertained in the UTHealth Houston, Royal Free Hospital, and Johns Hopkins University cohorts. Mortality risk and precapillary PH time-to-event from SSc onset were estimated using Cox proportional hazards models. Meta-analyses were performed to generate overall risk estimates.

Results: In the mortality analysis (n = 2366 patients, 46.6% of whom were deceased), after adjustment for age of SSc onset and sex, *DNASE1L3* R206C was associated with significantly greater mortality (hazard ratio [HR]: 1.16, 95% CI: 1.02–1.32). In the precapillary PH analysis (n = 1170 patients, 20.3% of whom had precapillary PH), after adjustment for age of SSc onset, sex, and the presence of ILD, *DNASE1L3* R206C was associated with significantly greater precapillary PH risk (HR: 1.69, 95% CI: 1.31–2.19). The majority of patients with precapillary PH did not have ILD, indicating that they had WHO Group I, ie pulmonary arterial hypertension (PAH).

Conclusions: We report a previously unknown impact of *DNASE1L3* polymorphism on PAH susceptibility in SSc. The association between loss-of-function of an immunoregulatory gene (*DNASE1L3*) and PAH susceptibility supports further exploration of downstream sequelae of *DNASE1L3* dysfunction in SSc pathogenesis and the mechanisms underlying the well-recognised but poorly understood susceptibility of patients with SSc to PAH.

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WHAT IS ALREADY KNOWN ON THIS TOPIC

- Roughly 10% of patients with systemic sclerosis (SSc) develop pulmonary arterial hypertension (PAH), but the genetic risk factors and mechanisms underlying this risk are unclear.

WHAT THIS STUDY ADDS

- A loss-of-function polymorphism in the immunoregulatory gene deoxyribonuclease 1-like 3 (*DNASE1L3*), previously identified as a susceptibility locus for SSc per se, was found to be associated with greater mortality and PAH susceptibility among patients with SSc, but was not positively associated with interstitial lung disease.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- The results of this study can provide a framework for research on the downstream sequelae of *DNASE1L3* dysfunction in SSc pathogenesis and on the mechanisms underlying PAH susceptibility among patients with SSc.

INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune disease characterised by vasculopathy and fibrosis affecting the skin and internal organs [1,2]. SSc has the highest standardised mortality ratio among the major autoimmune rheumatic diseases. Interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH) are the first and second leading causes of SSc-related mortality, respectively [3–7], with ILD being especially prominent among those with diffuse cutaneous SSc (dcSSc) and PAH proportionately more prominent as a cause of death among those with limited cutaneous SSc (lcSSc) [7]. Treatments that can slow progression of ILD and PAH have emerged [8,9], but the development of targeted therapies remains hampered by an incomplete understanding of SSc pathogenesis.

Genetic association studies have helped to elucidate the underlying pathogenic mechanisms of SSc by identifying several SSc susceptibility loci within and outside of the human leukocyte antigen (HLA) region [10]. Although most of the identified non-HLA single-nucleotide polymorphisms (SNP) lie within non-coding regions, a notable exception is the rs35677470 SNP in deoxyribonuclease 1-like 3 (*DNASE1L3*). The minor allele, encoding Arg to Cys substitution at amino acid 206 (R206C), was found by 2 independent groups of investigators to be associated with SSc [11,12]. The association was strongest with lcSSc and, concordantly, with anticentromere antibodies (ACA).

DNASE1L3 is an endonuclease expressed predominantly by immune cells within the spleen, liver, and gastrointestinal tract [13,14]. Importantly, *DNASE1L3* is secreted into the circulation and thereby exerts cell-extrinsic activity, digesting circulating self-DNA fragments in vesicles derived from apoptotic cells and in neutrophil extracellular traps [13,15]. Genetic evidence has demonstrated a critical role of *DNASE1L3* in protection against autoimmunity, as follows: (i) Rare *DNASE1L3* loss-of-function mutations were identified in cases of familial systemic lupus erythematosus (SLE) and hypocomplementemic urticarial vasculitis syndrome [16,17]. (ii) Mice with genetic deficiency of *Dnase1l3* spontaneously develop antinuclear antibodies (ANA) [13,14]. (iii) The variant encoding *DNASE1L3* R206C, in addition to increasing SSc susceptibility [11,12], has also been identified as a susceptibility locus for rheumatoid arthritis and SLE [18,19]. We and others have demonstrated that the R206C substitution causes loss-of-function, resulting in incomplete

fragmentation of circulating cell-free DNA and therefore a greater proportion of long DNA fragments within the circulation [19–23]. Aberrant activation of the immune system and/or direct organ damage induced by incompletely digested DNA fragments could plausibly underlie the SSc risk associated with *DNASE1L3* loss-of-function, but the causal chain in SSc pathogenesis is unknown.

To help elucidate the downstream sequelae of *DNASE1L3* loss-of-function in the context of SSc, we examined the relationship between the *DNASE1L3* R206C variant and clinical outcomes.

METHODS

Patients and healthy controls

Patients with SSc had been recruited from 4 cohorts: (i) The Scleroderma Family Registry and DNA Repository: a multicentre US and Canadian cross-sectional Registry with DNA samples stored at the UTHealth Houston Division of Rheumatology [24]. Patients with SSc met the 1980 preliminary American College of Rheumatology (ACR) classification criteria [25] or had at least 3 of the 5 features of CREST syndrome, with sclerodactyly being mandatory. Because the Registry is cross-sectional and lacks chest computed tomography and right heart catheter data (RHC) in most cases, Registry patients were not included in the analyses of ILD and PH. Registry patients were included in the mortality analysis since we obtained vital status data from the US National Death Index (NDI) (see below). In the mortality analysis, UTHealth Houston Registry and patients in GENISOS (Genetics vs Environment in Scleroderma Outcomes Study) were analysed together. (ii) GENISOS: a prospective, longitudinal cohort among UTHealth Houston, UTMB Health (Galveston), and UTHealth San Antonio, enrolling from 1998 to present [26]. Inclusion criteria are (i) age >18 years, (ii) satisfaction of the ACR 1980 preliminary SSc classification criteria (for those enrolled from 1998 to 2013) or the 2013 ACR/EULAR (European Alliance of Associations for Rheumatology) SSc classification criteria [27], and (iii) disease onset (defined as the first non-Raynaud's phenomenon symptom) within the previous 5 years. (iii) The Royal Free Hospital cohort: Inclusion criteria are age >18 years, with an expert confirmed diagnosis of SSc. Patients fulfil the 2013 ACR/EULAR SSc classification criteria. (iv) The Johns Hopkins University Scleroderma Center Research Registry: a prospective, longitudinal cohort that has enrolled patients since 1990 who meet 1980 or 2013 ACR/EULAR SSc classification criteria, or have at least 3 of 5 CREST syndrome criteria, or have definite Raynaud's phenomenon with abnormal nailfold capillaries and an SSc-specific autoantibody. Disease duration at cohort enrollment was recorded for the UTHealth Houston and Johns Hopkins cohorts. For the Royal Free cohort, disease duration at the time of blood withdrawal for genetic studies was recorded for most patients and is reported in the Results, but does not necessarily reflect the disease duration at the time that the patients began clinical follow-up at the Royal Free Hospital for SSc.

All eligible white patients with available genotype data for the *DNASE1L3* allele were included in this study. Additionally, white healthy controls (HCs) in the Registry with available genotype data were analysed for mortality. HCs were not blood relatives of patients with SSc and had no personal history of any autoimmune rheumatic disease. Race and sex were self-reported.

All participants provided written informed consent. See [Supplementary methods](#) for details on institutional approvals of research.

Ascertainment of vital status

Deaths among UThHealth Houston Registry and GENISOS subjects prior to 2010 had been identified from the US NDI and Social Security Death Index, as previously described [28]. Vital status for the remaining subjects (alive at the time of the 2010 query or newly enrolled after the 2010 query) was determined via an NDI query performed in December of 2021 and through the institution’s electronic medical records. Vital status in the Royal Free Hospital cohort was confirmed by case notes review that is updated automatically from National Health Service records. At Johns Hopkins, mortality is assessed using the Social Security Death Index, obituary searches, Lexis Nexis data, and death dates captured in the institution’s electronic medical record system and Precision Medicine Analytics Platform.

Ascertainment of precapillary pulmonary hypertension and ILD

The prevalence of pulmonary hypertension (PH) was assessed in the GENISOS and Johns Hopkins cohorts as follows: patients with at least 1 available echocardiogram and/or RHC were assessed. Patients who did not have an elevated estimated right ventricular systolic pressure (RVSP), or did not have an RVSP estimate due to insufficient tricuspid regurgitation and also did not have evidence of right ventricular dilation on echo, and had not undergone RHC were adjudicated as negative for PH. Patients who had undergone RHC were adjudicated according to the current hemodynamic definitions, with mean pulmonary artery pressure (PAP) >20 mm Hg considered positive for PH [29]. Patients were excluded from analyses in the following scenarios, due to inability to adjudicate confidently as negative or positive for precapillary PH: (i) Patients with an elevated estimated RVSP based on echocardiogram, but without an RHC. (ii) Patients with PH by RHC who met the criteria for postcapillary PH based on pulmonary capillary wedge pressure (PCWP) and/or pulmonary vascular resistance values. Patients in the Royal Free cohort were adjudicated similarly, with PH confirmed by RHC in all cases and defined by contemporary diagnostic thresholds for mean PAP. Those with PCWP >15 mm Hg were defined as WHO Group II. Patients with more than 1 RHC available were adjudicated as positive for precapillary PH if any of their RHC results met the hemodynamic definition of precapillary PH. In 5 cases in GENISOS, patients were counted as positive for precapillary PH without an RHC after an extensive chart review, based on the following criteria: echocardiogram with estimated RVSP >60 mm Hg and evidence of right ventricular dysfunction without evidence of left ventricular dysfunction.

Table 1
Demographics and clinical characteristics of patients with SSc in the mortality analysis

	UThHealth Houston Registry and GENISOS (n = 1234)	Royal Free College London (n = 506)	Johns Hopkins (n = 626)
^a Minor allele frequency (%)	9.7	9.1	8.3
Female, n (%)	1089 (88.2)	431 (85.2)	537 (85.8)
Age at SSc onset (y), median (IQR)	46.3 (36.8, 54.8)	49.0 (39.0, 58.0)	47.7 (37.2, 55.8)
Disease duration at study enrolment ^b in years, median (IQR)	6.0 (2.5, 13.7)	15.0 (10.0, 21.0)	2.5 (1.0, 8.2)
Diffuse skin involvement, n (%)	457 (37.0)	143 (28.3)	228 (36.4)
^c Censoring time in years, median (IQR)	20.1 (14.1, 27.2)	21.0 (14.0, 27.0)	18.2 (10.7, 24.9)
Deceased, n (%)	535 (43.4)	227 (44.9)	341 (54.4)
Anticentromere antibody, n (%)	366 (29.7)	185 (36.6)	201 (32.1)

DNASE1L3, deoxyribonuclease 1-like 3; GENISOS, Genetics vs Environment in Scleroderma Outcomes Study; SSc, systemic sclerosis.
^a Allele encoding DNASE1L3 R206C.
^b For Royal Free, the disease duration at blood draw for genetic research.
^c From time of SSc onset to the time of death or censoring.

ILD was defined as follows: GENISOS—bilateral interstitial pulmonary infiltrates seen on chest radiography or CT in the absence of heart failure or infection. Royal Free—Radiologist’s visual read of chest CT and absence of alternative lung pathology. Johns Hopkins—evidence of ILD on high-resolution chest CT, or evidence of restriction on pulmonary function tests in the presence of rales or radiographic evidence of ILD.

Genotype and autoantibody determination

See [Supplementary methods](#).

Statistical analyses

Mortality was assessed using an additive Cox proportional hazards model in which 1 copy of the minor allele encoding DNASE1L3 R206C (heterozygous) counted as 1 and 2 copies (homozygous) counted as 2. In a preliminary analysis of the UThHealth Houston Registry and GENISOS (shown as [Supplementary data](#)), age at study enrollment was used as the starting point for censoring. For the multicohort analyses of time from SSc onset to mortality or precapillary PH diagnosis, 1-stage individual participant data (IPD) meta-analyses were performed, in which individual-level data from the 3 cohorts were analysed simultaneously using additive Cox proportional hazard models. The models were stratified by cohort, allowing baseline hazards to vary across the cohorts. Mortality analyses included the UThHealth Houston Registry and GENISOS, Royal Free Hospital, and Johns Hopkins. Precapillary PH analyses included GENISOS, Royal Free Hospital, and Johns Hopkins, without the UThHealth Houston Registry, since it is cross-sectional and lacks chest CT and RHC data in most cases. See [Supplementary methods](#) for additional description of statistical analyses.

RESULTS

Preliminary evidence of mortality and PAH risk in the UThHealth Houston cohorts

This study originated with a mortality analysis of the UThHealth Houston Scleroderma Registry and GENISOS cohort. Demographics and clinical characteristics of the patients with SSc (n = 1234) are shown in [Table 1](#). Mortality was significantly greater among patients with SSc with the minor allele (encoding the DNASE1L3 R206C variant) after adjustment for age at study enrollment and sex ([Supplementary Table S1](#)).

Mortality was greatest among patients homozygous for the minor allele (Supplementary Fig S1A), suggesting a dose effect.

HCs who underwent genotyping in the Immunochip genetic association study [11] were also included in the NDI query (n = 682). Demographics are shown in Supplementary Table S2. Remarkably, the DNASE1L3 R206C variant was associated with significantly lower mortality after adjustment for age at enrollment and sex—the opposite of what was observed among patients with SSc (Supplementary Table S1 and Supplementary Fig S1B). This discordance suggests that the DNASE1L3 R206C variant may not be pathologic per se, but can become so in the presence of 1 or more effect modifiers involved in SSc pathogenesis.

Considering that the DNASE1L3 R206C variant is associated mostly with ACA-positive SSc [11,12], it was counterintuitive that this variant was associated with increased mortality among patients with SSc, since ACA portends a more favourable prognosis compared with other SSc subgroups [30]. However, when the analysis was restricted to ACA-positive patients with SSc (n = 366), the mortality effect of the DNASE1L3 R206C variant persisted (Supplementary Table S1). Since some historical literature suggests that ACA-positive patients have a relatively low risk of progressive ILD compared with other SSc subgroups, but are susceptible to PAH [30], these results suggested to us the hypothesis that the DNASE1L3 R206C variant predisposes to SSc-PAH.

Within the UTHealth Houston Scleroderma Registry, ACA-positive patients with the DNASE1L3 R206C variant had significantly higher echocardiogram-based RVSP estimates on average than ACA-negative or ACA-positive patients without this variant (Supplementary Fig S2A). Forced vital capacity % predicted was significantly higher among ACA-positive patients with the DNASE1L3 R206C variant than among ACA-negative patients without the variant, and was not significantly different from that of ACA-positive patients without the variant (Supplementary Fig S2B). Although the Registry is cross-sectional and lacks RHC and high-resolution chest CT data for most patients, this preliminary analysis suggested increased susceptibility to PAH but not severe ILD among patients with SSc with the DNASE1L3 R206C variant, in particular among those with ACA. We next sought to determine mortality and PAH risk associated with the DNASE1L3 R206C variant in 3 cohorts with many years of longitudinal follow-up, regular screening for PH, RHC data for most patients suspected to have PH, and vital status ascertainment.

Mortality association with DNASE1L3 R206C variant in patients with SSc

A total of 2366 patients with SSc across all cohorts (UTHealth Houston Registry and GENISOS, Royal Free Hospital, Johns

Table 2
Cox proportional hazards meta-analysis of mortality across all cohorts, with SSc onset as the starting point for censoring

Variable	Hazard ratio (95% CI)	P value
Copy number of minor allele ^a	1.16 (1.02, 1.32)	.024
Age at SSc onset (y)	1.07 (1.06, 1.08)	<.001
Female	0.53 (0.45, 0.62)	<.001

DNASE1L3, deoxyribonuclease 1-like 3; SSc, systemic sclerosis.
^a Allele encoding DNASE1L3 R206C.

Hopkins University) were analysed for mortality in a meta-analysis, 46.7% of whom were deceased. Median follow-up time from SSc onset to death or censoring ranged from 18 to 21 years across the 3 cohorts. Demographics and clinical characteristics of the patients are shown in Table 1.

After adjustment for age at SSc onset and sex, the DNASE1L3 R206C variant was associated with significantly greater mortality, whether analysed as an IPD meta-analysis (hazard ratio [HR]: 1.16, 95% CI: 1.02-1.32) (Table 2) or a group-level meta-analysis (Supplementary Fig S3). The HR estimate was accentuated by inclusion of limited/diffuse cutaneous status in the model (Supplementary Table S3). The overall HR estimate was similar when the analysis was restricted to ACA-positive patients, but was not statistically significant (HR: 1.21, 95% CI: 0.99-1.47) (Supplementary Table S4).

PAH susceptibility in patients with SSc with the DNASE1L3 R206C variant

A total of 1219 patients with SSc across 3 longitudinal cohorts (GENISOS, Royal Free Hospital, Johns Hopkins University) were examined for precapillary PH prevalence, after exclusion of those with uncertain cases as detailed in the Methods section. Of these patients, 1170 with available dates of SSc onset and PH diagnoses were included in a time-to-event analysis. Patient demographics and clinical features are summarised in Table 3.

In each cohort, precapillary PH was more prevalent among patients with the DNASE1L3 R206C variant. Based on group-level meta-analysis, the estimated relative risk of precapillary PH associated with the DNASE1L3 R206C variant was 1.70 (95% CI: 1.28, 2.25) (Fig 1). The association was numerically stronger among ACA-positive compared with ACA-negative patients (Supplementary Fig S4).

The risk of precapillary PH associated with the DNASE1L3 R206C variant was assessed further in a time-to-event analysis, with SSc onset as the starting time and precapillary PH or last

Table 3
Demographics and clinical characteristics of patients with SSc in the precapillary PH analysis

	GENISOS (n = 193)	Royal Free College London (n = 555)	Johns Hopkins (n = 471)
^a Minor allele frequency (%)	8.0	9.2	7.7
Female, n (%)	162 (83.9)	476 (85.8)	408 (86.6)
Age at SSc onset (y), median (IQR)	46.8 (38.3, 55.0)	49.0 (39.0, 58.0)	46.9 (36.4, 55.1)
Disease duration at study enrolment ^b in years, median (IQR)	2.2 (1.1, 3.3)	14.0 (7.0, 20.0)	2.2 (0.9, 7.0)
Diffuse skin involvement, n (%)	110 (57.0)	151 (27.2)	171 (36.3)
Time from disease onset to last follow-up assessment in years, median (IQR)	8.3 (4.3, 11.4)	20.0 (12.3, 26.0)	16.2 (7.6, 23.4)
Precapillary PH, n (%)	15 (7.8)	110 (19.8)	123 (26.1)
Anticentromere antibody, n (%)	32 (16.6)	203 (36.6)	158 (33.5)

DNASE1L3, deoxyribonuclease 1-like 3; GENISOS, Genetics vs Environment in Scleroderma Outcomes Study; ILD, interstitial lung disease; PH, pulmonary hypertension; SSc, systemic sclerosis.
^a Allele encoding DNASE1L3 R206C.
^b For Royal Free, the disease duration at blood draw for genetic research.

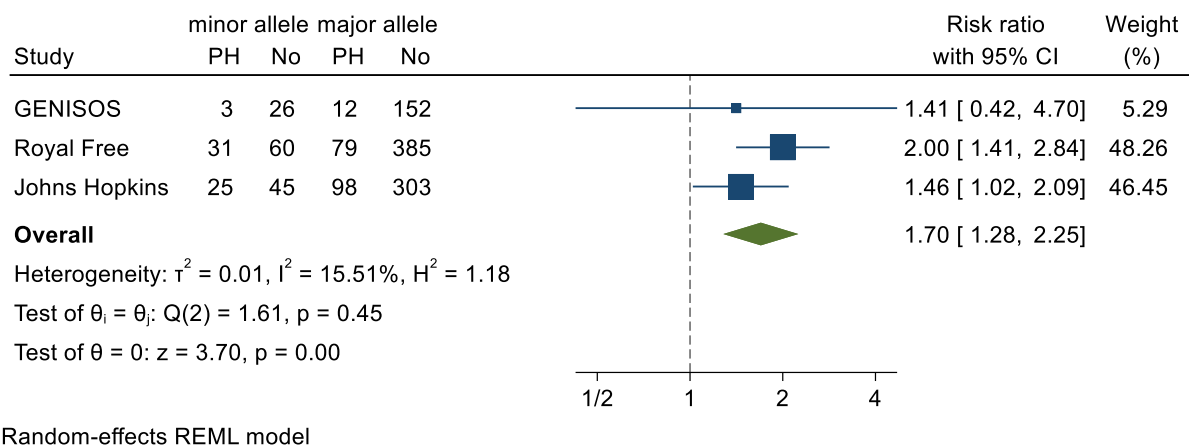


Figure 1. Forest plot of the relative risk of precapillary PH (prevalence) associated with the DNASE1L3 R206C variant in the 3 cohorts, based on the Chi-squared test. Minor allele: heterozygous or homozygous for the minor allele encoding DNASE1L3 R206C; major allele: homozygous for the major allele; DNASE1L3: deoxyribonuclease 1-like 3; PH, precapillary pulmonary hypertension; REML: restricted maximum likelihood.

clinical assessment as the censoring time. As shown by the Kaplan-Meier curve, the DNASE1L3 R206C variant was associated with a greater risk of precapillary PH incidence over time (Supplementary Fig S5). In the Cox proportional hazards model, age at SSc onset and sex were included as potential confounders, since both were previously shown to affect PH risk in the setting of SSc [31–33]. The presence of ILD was also included in the model, since ILD can lead to WHO Group III PH with a different pathophysiology than WHO Group I (PAH). After adjustment for age of SSc onset, sex, and presence of ILD, the DNASE1L3 R206C variant was associated with a greater risk of precapillary PH incidence (HR: 1.69, 95% CI: 1.31–2.19) (Table 4). Inclusion of the limited/diffuse cutaneous subset in the model did not impact the result (Supplementary Table S5). Among ACA-positive patients, the risk was similar and was also statistically significant (HR: 1.56, 95% CI: 1.10–2.23) (Supplementary Table S6).

In each cohort, the prevalence of ILD was equal or numerically lower in patients with vs without the DNASE1L3 R206C variant overall (Supplementary Fig S6A). Among patients with precapillary PH, the prevalence of ILD was lower in patients with vs without the DNASE1L3 R206C variant in each cohort (Supplementary Fig S6B). The overall prevalence of ILD among patients with the DNASE1L3 R206C variant and precapillary PH was 39.0% (range 33.3%–44.0%), indicating that the majority of cases were WHO Group I, ie PAH. It is likely that many of the patients with mild ILD also had WHO Group I/PAH, but this possibility was not explored further due to the lack of a standardised threshold of ILD severity by which to adjudicate between WHO Groups I and III.

Prevalence of ischaemic digital ulcers and digital gangrene in patients with SSc with the DNASE1L3 R206C variant

As a preliminary analysis to explore the possibility that the DNASE1L3 R206C variant might increase susceptibility to other vascular manifestations of SSc, we examined the prevalence of digital tip ulcers and digital gangrene in the GENISOS and Johns Hopkins cohorts. There was a modest positive association between the DNASE1L3 R206C variant and the prevalence of digital tip ulcers or gangrene, but the effect size in GENISOS was minimal and not statistically significant (Supplementary Table S7). Additional research will be needed to determine the extent to which DNASE1L3 polymorphism influences vasculopathy outcomes other than SSc-PAH.

DISCUSSION

Examining a large group of patients with SSc in 3 independent cohorts, we found evidence of increased PAH susceptibility and mortality in patients with a loss-of-function variant in the immunoregulatory gene DNASE1L3. These results build on the previous discovery of this variant as a susceptibility factor for SSc per se [11,12], by showing a more severe disease trajectory and increased susceptibility to a specific vascular end-organ outcome.

Elucidation of genetic associations with PAH among patients with SSc can suggest novel targeted treatment strategies for this lethal complication. Previous research suggests that alleles in the transforming growth factor beta gene family that increase susceptibility to idiopathic and familial PAH do not have a significant association with PAH among patients with SSc [34]. These important negative results suggest the possibility of incomplete overlap of PAH pathogenic mechanisms in the setting of SSc compared with idiopathic and familial cases. In this context, a genetic variant in the promoter region of UPAR, a component of the plasminogen activation system involved in vascular remodelling and inflammation, was found to be positively associated with digital ulcers and PAH in patients with SSc [35]. Two studies also suggest a link between dysfunction of immunoregulatory genes and precapillary PH risk among patients with SSc. A SNP in the immunoregulatory gene TNFAIP3 was found to be associated with SSc susceptibility, particularly dcSSc, antitopoisomerase I antibody (ATA), ILD, and precapillary PH [36]. A coding variant in the Toll-like receptor 2 gene was found to be associated specifically with ATA-positive SSc, and this variant was associated with increased susceptibility to precapillary PH in a cohort of patients with SSc [37]. The potential impact of WHO Group III PH on the PH association in those 2 studies is unclear.

Genetic evidence has demonstrated a critical role of DNASE1L3 in maintaining self-tolerance, as evidenced by ANA positivity in Dnase1l3-null mice, and susceptibility to several autoimmune diseases associated with DNASE1L3 loss-of-function [11–14,16–19]. We and others have demonstrated that the R206C substitution in DNASE1L3 can be considered a loss-of-function variant, in that it leads to diminished digestion of circulating genomic DNA fragments from apoptotic cells [19–23]. Interaction between improperly digested circulating self-DNA and the immune system could underlie the susceptibility to

Table 4
Cox proportional hazards meta-analysis of precapillary PH incidence across 3 cohorts, with SSc onset as the starting point for censoring

Variable	Hazard ratio (95%CI)	P value
Copy number of minor allele ^a	1.69 (1.31, 2.19)	<.001
Age at SSc onset (y)	1.05 (1.04, 1.06)	<.001
Female	0.60 (0.43, 0.83)	.002
Presence of ILD	2.23 (1.71, 2.91)	<.001

DNASE1L3, deoxyribonuclease 1-like 3; ILD, interstitial lung disease; PH, pulmonary hypertension; SSc, systemic sclerosis.

^a Allele encoding DNASE1L3 R206C.

autoimmunity [13], although the causal chain is unclear. Our study is the first to identify a specific end-organ outcome associated with *DNASE1L3* polymorphism, and 1 of only a few to identify a genetic susceptibility locus for SSc-associated PAH. Since the DNASE1L3 R206C variant is also a susceptibility locus for SLE [19], our results also raise the question of the extent to which *DNASE1L3* loss-of-function impacts susceptibility to PAH among patients with SLE.

This study has several strengths. Three independent SSc cohorts with long-term follow-up were examined, enabling ascertainment of outcomes that often take several years to transpire. Patients in the PAH analysis underwent regular clinical assessments and screening echocardiograms, and the majority with suspicion of PH underwent RHC, allowing us to rely on the gold standard diagnostic test for PH ascertainment. Each cohort used national databases for ascertainment of vital status. Taken together, the rigour of the study design allows for a robust conclusion that can serve as an anchor for future exploration of the downstream physiologic sequelae of *DNASE1L3* loss-of-function and mechanisms underlying SSc-associated PAH.

The study also has limitations. Since only white subjects from the United States and United Kingdom were included in the analyses, the generalisability of our findings to other populations cannot be determined. We examined an SSc cohort from Barcelona, Spain, comprising over 200 patients, but the minor allele frequency was only 0.016 (7 total patients with the DNASE1L3 R206C variant), precluding a statistically powered analysis (data not shown). Similarly, among Black/African American patients in the GENISOS cohort, only 4 had the DNASE1L3 R206C variant (data not shown). Since the allele frequency of the variant encoding DNASE1L3 R206C is highest among populations of northern European descent [38], investigating its potential impact in other populations would require larger sample sizes. For patients with ILD, precapillary PH cases could not be distinguished definitively between WHO Group I (PAH) and WHO Group III due to lung fibrosis. However, the prevalence of ILD was lower among precapillary patients with PH with vs without the DNASE1L3 R206C variant. Therefore, any impact of Group III PH on PAH prevalence estimates would be expected to bias towards the null by inflating the PAH estimates among patients without DNASE1L3 R206C. We could not systematically assess for chronic thromboembolic (WHO Group IV) PH due to lack of V/Q scan data on most patients. Although there is no published evidence to our knowledge of increased thromboembolism risk in humans associated with DNASE1L3 dysfunction, there is evidence from a mouse model that impaired degradation of neutrophilic extracellular trap DNA, in the setting of double deficiency of *Dnase1* and *Dnase1l3*, can lead to vascular

occlusion distinct from the classical coagulation cascade [15]. It is reasonable to hypothesise that this phenomenon could contribute to PH susceptibility in the setting of DNASE1L3 dysfunction. This possibility is worthy of future research. However, chronic thromboembolic PH is exceptionally rare among patients with SSc based on the authors' experience. Moreover, among the 47 patients in this study with PH for whom V/Q scans were available, >90% were reported as low or very low probability of pulmonary embolism, and none as high probability (data not shown). One patient with the *DNASE1L3* risk allele (and 3 patients without this allele) was reported as intermediate probability. Based on the combination of clinical experience and empiric V/Q scan data, we believe that chronic thromboembolic PH had minimal or no influence on the PH susceptibility comparison in our study. Although PAH is a plausible explanation for the increased mortality observed in patients with SSc with the DNASE1L3 R206C variant, we cannot determine the causes of death in most cases and cannot exclude other variables that were not measured. HCs in the mortality analysis were not demographically matched to patients with SSc, with a greater mean age at study enrollment (52.4 vs 46.3 years) and proportionately fewer females (52.8% vs 88.2%). These differences might influence the results of the mortality analyses, although our adjustments for age at study enrollment and sex in the analyses are expected to have a mitigating effect on this issue. Although our results provide a novel biological insight into the pathogenesis of SSc-PAH, they do not provide sufficient justification at this stage to screen for *DNASE1L3* genotype as a prognostic aid in clinical practice. Any potential utility of the *DNASE1L3* genotype as a clinical risk stratification tool would require further research to determine the prognostic value added to currently available demographic, clinical, and serologic variables.

We also acknowledge that a lack of functional mechanistic data at this point leaves critical unanswered questions on the pathways by which *DNASE1L3* loss-of-function increases PAH susceptibility. It is plausible that pulmonary vascular damage could be a sequela of aberrant immune system activation, for example, through the action of vasoactive cytokines, autoantibodies, or infiltration of immune cells autoreactive to the pulmonary vasculature. Other possibilities, not mutually exclusive, are a direct pathologic effect of improperly processed circulating self-DNA fragments on pulmonary vascular endothelial cells, or a cell-intrinsic effect of *DNASE1L3* expressed within the lungs. The latter possibility is suggested by the detection of *DNASE1L3* expression in a small percentage of pulmonary endothelial cells (<https://gtexportal.org/home/>, accessed on 04/01/2026), although the functional impact of endothelial cell *DNASE1L3* expression is unknown. The discordant mortality impact of the DNASE1L3 R206C variant between patients with SSc (increased mortality) and HCs (reduced mortality) suggests that this variant may not be intrinsically pathologic, but can become pathologic in the presence of 1 or more effect modifiers involved in SSc pathogenesis. Identification of these effect modifiers, and how they interact with DNASE1L3 R206C or its downstream sequelae (eg, incompletely digested self-DNA fragments) to predispose to SSc-PAH, also warrants further research.

In summary, we report a previously unknown impact of loss-of-function of the immunoregulatory gene *DNASE1L3* on PAH susceptibility among patients with SSc. This discovery can provide a framework for research on the pathologic sequelae of *DNASE1L3* loss-of-function and on unique mechanisms that cause susceptibility to PAH in the setting of SSc.

Competing interests

BS reports that financial support was provided by National Institutes of Health and Rheumatology Research Foundation. SA reports that financial support was provided by US Department of Defense and National Institutes of Health. MDM reports financial support was provided by National Institutes of Health and US Department of Defense. CPD reports that financial support was provided by Royal Free Charity. AAS reports financial support was provided by the National Institutes of Health. 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

Brian Skaug: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Stefano Rodolfi:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Christopher P. Denton:** Writing – review & editing, Investigation, Formal analysis, Data curation, Conceptualization. **Ami A. Shah:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Bich Na Choi:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Nelson Gonzalez:** Writing – review & editing, Investigation, Data curation. **Marialbert Acosta Herrera:** Writing – review & editing, Investigation, Data curation. **Javier Martin:** Writing – review & editing, Investigation, Data curation. **Julio Charles:** Writing – review & editing, Investigation, Data curation. **Vijaya L. Murthy:** Writing – review & editing, Investigation, Data curation. **Meng Zhang:** Writing – review & editing, Data curation. **Maureen D. Mayes:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Shervin Assassi:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Patient consent for publication

Not applicable.

Ethics approval

This research was approved by the UTHealth Houston Committee for the Protection of Human Subjects (protocol numbers HSC-MS-02-004, HSC-MS-02-046, and HSC-MS-02-161), the London-Hampstead (IRAS Ref. 270295) and London-Fulham (IRAS Ref. 279682) NHS National Research Ethics Committees, and the Johns Hopkins Medicine IRB (IRB00226995, IRB00430231).

Provenance and peer review

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Supplementary materials

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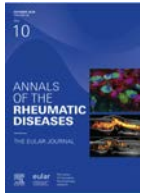
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Systemic sclerosis

Phenotype-specific associations of mosaic chromosomal alterations in systemic sclerosis

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ABSTRACT

Objectives: Mosaic chromosomal alterations (mCAs) increase with age and are associated with many diseases, including autoimmune diseases. The associations between mCAs and systemic sclerosis (SSc) and its clinical subtypes have not been explored.

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Methods: We recruited study subjects from 2 independent datasets (set 1: 635 SSc, 4401 controls; set 2: 347 SSc, 2170 controls) and detected mCAs (loss, loss of heterozygosity [LOH], gain, and mosaic loss of the X chromosome [mLOX]) from their peripheral blood samples. Logistic regression analyses were conducted with covariates in each cohort, and the results were meta-analysed. We also conducted stratified analyses by age groups, the age at disease onset, clinical phenotypes based on the skin lesions, autoantibody profiles, and the presence of complications.

Results: We observed a trend of increased loss in SSc, especially in old age ($P = .0063$). The association of loss was strengthened in certain subtypes of SSc, including lcSSc (odds ratio [OR] = 2.22, $P = .019$) and SSc with vascular complications (digital ulcers, pulmonary hypertension, or renal crisis; OR = 3.30, $P = .0054$). The effect sizes of Loss increased in patients with high cell fractions (CFs). We also observed that mLOX was significantly associated with SSc, limited cutaneous systemic sclerosis (lcSSc), and anticentromere antibody-positive systemic sclerosis (ACA-SSc) only for subjects with high CFs. mLOX was significantly associated with lcSSc and ACA-SSc even compared with dcSSc and ATA-SSc, respectively. These associations were consistently observed in each of the 2 datasets. Finally, we identified majority of the associations of Loss were mainly driven by SSc with late age at onset.

Conclusions: Loss and mLOX were significantly and differentially associated with SSc and its subtypes, underscoring potential phenotype-specific contributions of mCAs.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Systemic sclerosis (SSc) is a heterogeneous disease, with its phenotypes and disease outcomes varying among patients.
- Age-related mosaic chromosomal alterations (mCAs) in blood and subsequent clonal haematopoiesis are associated with various adverse health outcomes.
- mCAs have also been linked to several immune-mediated diseases, such as late-onset rheumatoid arthritis (LORA), and hence may influence immune cells and their functions.

WHAT THIS STUDY ADDS

- Autosomal loss is increased in SSc in aged subjects.
- Autosomal loss was associated with limited cutaneous systemic sclerosis (lcSSc), anticentromere antibody-positive systemic sclerosis (ACA-SSc), interstitial lung disease-associated systemic sclerosis (ILD-SSc), and vascular complication-associated systemic sclerosis (VC-SSc) in a dose-dependent manner of cell fraction.
- Mosaic loss of the X chromosome (mLOX) was associated with SSc and its subtypes only in patients with high cell fraction.
- Late-onset SSc and its subtypes show stronger associations with autosomal loss with higher effect sizes compared with nonlate-onset SSc.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Our study facilitates further research to recapitulate the current findings in independent cohorts as well as in different ancestries.
- Incorporating profiles of autosomal loss and mLOX in blood into conventional clinical information may enable a better stratification of SSc patients and the development of a better management strategy.
- Further experimental approaches that investigate the underlying molecular mechanisms of phenotypic heterogeneity of SSc driven by autosomal loss and mLOX, such as whole-genome sequencing and single-cell RNA sequencing, are also warranted.

INTRODUCTION

Systemic sclerosis (SSc) is a complex disease characterised by progressive fibrosis of the skin and internal organs and vasculopathy, which is thought to be caused by abnormal immune

activation [1,2]. SSc affects predominantly females and presents heterogeneous phenotypes based on the organ involvement (limited cutaneous systemic sclerosis [lcSSc] and diffuse cutaneous systemic sclerosis [dcSSc]) and serological profiles (anticentromere antibody [ACA], antitopoisomerase I antibody [ATA]), which are closely related to disease outcomes and hence prognosis of affected individuals [1,2]. Clinical manifestations that significantly impact the prognosis include interstitial lung disease (ILD), pulmonary arterial hypertension (PAH), and other vascular manifestations (digital ulcers (DU), scleroderma renal crisis (SRC)) [1–3]. For patients with SSc, ILD and vascular complications can be fatal [3], and the current international guideline emphasises early assessment of organ involvement and phenotype-based management [4]. However, in clinical settings, we still often encounter treatment-resistant cases or cases with adverse effects due to intensive immunosuppressive treatments [1,2,5,6].

Genetic and environmental factors interact in the development of SSc [7]. Twin studies estimated the disease heritability to be ~4.7% [8], and the recent genome-wide association studies (GWAS) for Europeans (EUR) and East Asians (EAS) suggested that heritability explained by common single-nucleotide polymorphisms (SNPs) was 0.5% and 1.3% in EUR [9] and EAS [10], respectively, indicating the relatively low heritability compared with other autoimmune diseases, such as rheumatoid arthritis (RA). Well-known environmental factors, including silica exposure [11] and chemotherapeutic agents [12] cannot fully explain the susceptibility to SSc, implicating the presence of additional acquired contributors.

Mosaic chromosomal alterations (mCAs) are acquired structural changes in autosomal and sex chromosomes and include autosomal copy loss (loss), autosomal copy gain (gain), autosomal copy-neutral loss of heterozygosity (CN-LOH), mosaic loss of the X chromosome (mLOX), and mosaic loss of the Y chromosome (mLOY) [13–21]. It is known that mCAs increase with age, and several risk factors have also been well documented, such as cigarette smoking [13–23]. Large-scale GWASs have also identified associations between genetic variants and specific mCAs (13,19–21). Beyond their role as a signature of ageing, mCAs have been linked to multiple diseases, especially haematologic malignancies [13,15–18]. Considering the impacts on clone sizes of B and T lymphocytes, or immune cell functions, it is expected that mCAs can also affect susceptibility to and severity of certain immune-related diseases [18].

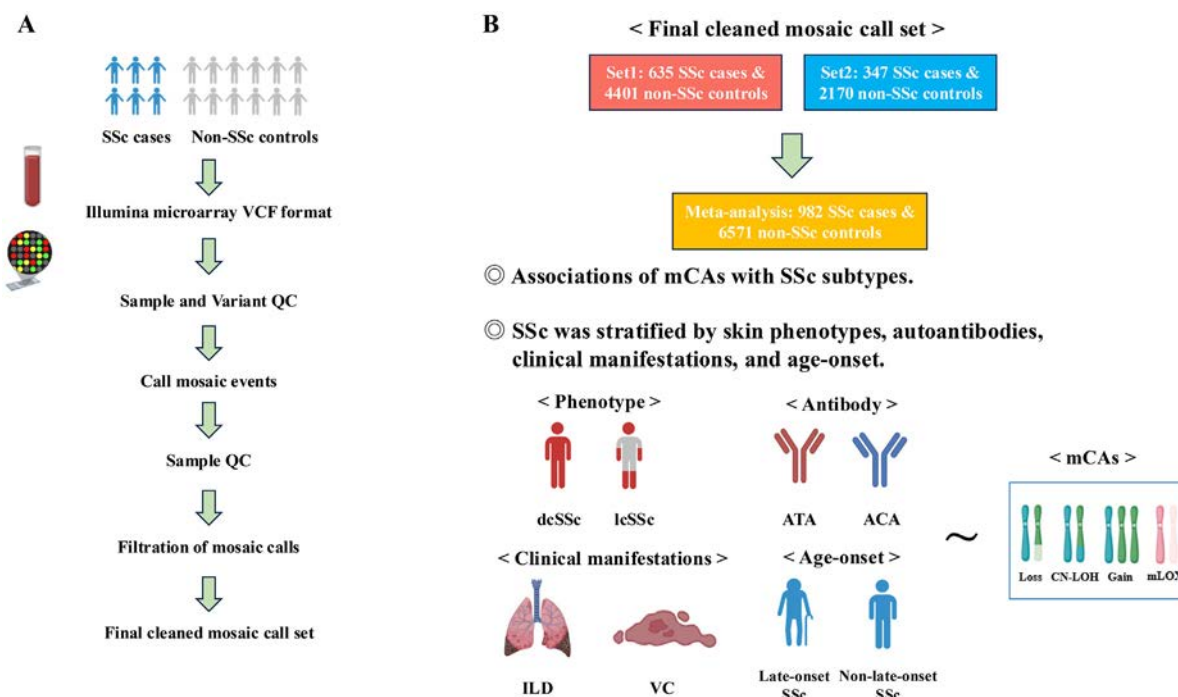


Figure 1. Overview of the present study. A, A workflow of the mosaic call. B, The design of the present study.

ACA, anticentromere antibody; ATA, anti-topoisomerase I antibody; CN-LOH, copy-neutral loss of heterozygosity; dcSSc, diffuse cutaneous SSc; Gain, autosomal gain; ILD, interstitial lung disease; lcSSc, limited cutaneous SSc; Loss, autosomal loss; mCA, mosaic chromosomal alteration; mLOX, mosaic loss of chromosome X; QC, quality control; SSc, systemic sclerosis; VC, vascular complications; VCF, variant call format.

Indeed, we have recently identified a significant correlation between mLOY and late-onset RA (LORA), RA characterised by the development at age ≥ 60 years old, a higher proportion of male patients, and clinical features distinct from young-onset RA (YORA) [24]. In contrast, YORA showed a negative association with mLOY, implicating that mLOY can play distinct roles between LORA and YORA in their pathology [24]. Regarding SSc, SSc typically affects individuals aged between 20 and 50 years [25]. However, 10% to 20% of SSc cases develop in patients aged 60 and 65 years or even older, and the clinical features of such late-onset SSc differ from those of non-late-onset SSc [25–27]. Thus, an age-at-onset-dependent association of mCA in SSc is worth investigating. Not only the age of onset, but also heterogeneity of clinical phenotypes in SSc have not been well explained by genetics as well as environmental factors thus far [9,10].

To fill the gaps, here we quantify mCA in peripheral blood obtained from subjects with or without SSc. We investigate associations of mCA with SSc and its clinical subtypes, autoantibody profiles, and major clinical manifestations relevant to prognosis. We also examine the dose-dependent effect of mCA burdens based on cell fractions (CFs) (estimated proportion of peripheral blood cells carrying given mCAs). Additionally, we investigate the age-at-onset-dependent differences of the effect sizes among SSc subjects, focusing on late-onset or nonlate-onset SSc.

METHODS

Study design and participants

A total of 1256 cases were enrolled from multiple centres. A total of 6736 controls were enrolled from Hospital-based Epidemiological Research Program at Aichi Cancer Center (HERPACC) [28]. All the study subjects were Japanese. The diagnosis of SSc was made by physicians according to the ACR/EULAR classification criteria (2013) [29]. The presence of ILD was based on chest

roentgenograms and/or computed tomography (CT) scan images and determined in individual institutions. Patients with either PAH, digital ulcers (DU), or scleroderma renal crisis (SRC) were classified as those with vascular complications (VCs). The breakdown of VC is shown in [Supplementary Table S1](#). Late-onset SSc was defined as SSc with onset at age 60 years or older.

Genotyping

The details of genotyping are shown in [Supplementary Methods](#).

Variant- and sample-level quality control

We performed variant- and sample-level quality control (QC) before phasing for calling of mosaic events ([Fig 1A](#), [Supplementary Fig S1](#)) as described in our previous work [24]. Following the Mosaic Chromosomal Alterations (MoChA) Workflow Description Language (WDL) protocols [13,30], we excluded variants that met any of the following criteria: genotyping call rate $< 97\%$, variants falling in segmental duplications with low divergence ($< 2\%$), variants with excess heterozygosity ($P < 1e-6$, Hardy–Weinberg equilibrium test). In sample QC, we removed genetically identical samples showing PIHAT > 0.9 estimated by PLINK 1.9 [31] and outliers from East Asian clusters after we mapped the samples with the 1000 Genomes Project [32] using principal component analysis. We also removed subjects with a sample call rate < 0.97 and samples with phased B-allele frequency (BAF) autocorrelation > 0.03 , which indicates poor quality or contamination of DNA. The call-rate failures were not evenly distributed across recruiting centres but were concentrated in a small subset of sites. The final cleaned mosaic call set included set 1, consisting of 635 SSc cases and 4401 non-SSc controls, and set 2, consisting of 347 SSc cases and 2170 non-SSc controls ([Fig 1B](#)).

Mosaic event detection

Mosaic call detection was performed as described in our previous work [24] and summarised in Figure 1A. After sample- and variant-level QC, the long-range haplotype phasing was performed using SHAPEIT5 [33]. mCAs were detected using MoChA WDL pipelines (2024 09-27 version) [13,30]. After running MoChA, we excluded subjects that met one of the following criteria: subjects aged less than 16 years old at the time of sampling, those with sex discordance between genotypes and phenotypes, or subjects with missing data on their sex and/or age. Mosaic calls were filtered by excluding CNP-class events and retaining only calls supported by BAF- and coverage/length-based criteria (thresholds on bdev, n50_hets, lod_BAF metrics, rel_cov, and event length). Regarding autosomal events, we excluded constitutional mosaic events (lod_baf_phase <10), and germline duplications (length < 500 lt; 500 kbp and relative coverage > 2.5). To reduce false-positive chromosome X loss calls due to sex-chromosome aneuploidy (XXX or XXY), mLOX events were restricted to those with relative coverage (rel_cov) < 2.5.

Autosomal mCA events were detected by copy number state (autosomal loss, CN-LOH, or autosomal gain) and by p versus q arm for autosomal loss and CN-LOH events. mCAs for which we could not determine the copy number state were categorised as undetermined and included in mCAs. mLOX was detected only in females. For each autosomal loss and mLOX that passed QCs, CF was calculated as $4 \times \text{bdev} / (1 + 2 \times \text{bdev})$, where bdev represents the BAF deviation estimated from 0.5.

Statistical analysis

We performed logistic regression analyses to evaluate the association between SSc and mCAs as follows:

$$SSc \sim mCAs + Sex + age$$

where mCAs are any autosomal mCA, autosomal loss, LOH, autosomal gain, or mLOX. mLOX was evaluated only in female participants; therefore, analyses were restricted to females and did not include sex as a covariate. Given the female predominance of SSc and the limited number of male patients in our cohorts, mLOY was not evaluated because of insufficient statistical power.

As a sensitivity analysis, we additionally performed female-only analyses for overall autosomal mCAs and autosomal loss to assess the potential impact of the sex imbalance between SSc cases and controls.

We tested the association of autosomal loss or mLOX with SSc subtypes as follows:

$$SSc \text{ subtype} \sim \text{autosomal loss} + Sex + age$$

$$SSc \text{ subtype} \sim mLOX + age$$

where subjects were stratified by skin phenotypes (lcSSc and dcSSc), autoantibody profiles (ACA and ATA), the presence of ILD or VC (only autosomal loss), or ages (≥ 60 , <60, only autosomal loss). Intracase analyses of associations between mLOX and skin phenotypes or autoantibody profiles were also conducted using this model.

For conditional analyses, the associations of SSc subtypes and autosomal loss were conditioned on the skin phenotypes or autoantibody profiles as follows:

$$SSc \text{ subtype} \sim \text{autosomal loss} + Sex + age \\ + \text{Autoantibody or Skin phenotype}$$

where SSc subtypes were ILD or VC.

To test the dose-dependent effect of CF, we compared subjects who carried autosomal loss or mLOX with CF > 5% and subjects without any mCAs as follows:

$$SSc \text{ or } SSc \text{ subtype} \sim \text{autosomal loss (CF > 5\%)} + Sex + age$$

$$SSc \text{ or } SSc \text{ subtype} \sim mLOX(\text{CF > 5\%}) + age$$

where subjects were stratified by skin phenotypes (lcSSc), autoantibody profiles (ACA), or the presence of ILD or VC (only autosomal loss).

We used a CF threshold of 5% for autosomal loss. This threshold was selected based on prior evidence [17].

For association analyses of the age of disease onset, we tested the association of autosomal loss or mLOX with late-onset or non-late-onset SSc or its subtypes (lcSSc, ACA(+), ILD(+), or VC(+)) in the following model:

$$SSc \text{ or } SSc \text{ Subtype} \sim \text{autosomal loss} + Sex + age$$

To assess potential nonlinear effects of age, we performed sensitivity analyses additionally including an age-squared term in the logistic regression models.

All the analyses were performed separately in the 2 datasets (sets 1 and 2), and then the results were meta-analysed (Fig 1B). Meta-analyses were performed using an inverse variance fixed-effects model. In the case of complete separation, associations were estimated using Firth regression, and inference was performed using profile likelihood.

Statistical analyses were conducted using R (version 4.5.0) and Python (version 3.11.8). The estimated risk is expressed as an odds ratio (OR) with its 95% confidence interval (CI). Statistical significance was defined based on the meta-analysis results, with supportive evidence from a consistent direction of association across the 2 datasets. For primary analyses, Bonferroni correction was applied within each predefined analysis set. Specifically, the significance threshold was set at 0.05/5 for the overall case–control analyses across 5 mCA categories and at 0.05/2 for age-stratified autosomal loss analyses, skin subtype analyses, autoantibody subtype analyses, and complication-stratified analyses. The overall late-onset SSc analysis was evaluated as a single predefined analysis at $P < .05$.

Conditional analyses were evaluated at nominal $P < .05$ to assess whether the observed associations were independent of autoantibody status. CF-stratified analyses were also evaluated using nominal P values, because these analyses were intended to examine whether effect sizes were strengthened in high-CF subsets rather than to define primary associations based on P value thresholds. These conditional and CF-stratified analyses, as well as mLOX and late-onset subtype analyses, were considered supporting exploratory analyses.

Ethical and public involvement statements

All patients provided informed consent. This study was approved by the local ethics committee of each institute as previously described [10]. Patients were not involved in any part of this study, including the study design, formal analyses, result interpretation, writing, or editing of the manuscript.

Table
Demographic features of the subjects in each dataset

Datasets	Set 1		Set 2	
	SSc	Controls	SSc	Controls
Number of subjects,	635	4401	347	2170
age in median (IQR)	56 (42–65)	57 (47–64)	65 (54–72)	57 (47–64)
Sex, n (%)				
Male	69 (10.9%)	2552 (58.0%)	51 (14.7%)	1259 (58.0%)
Female	566 (89.1%)	1849 (42.0%)	296 (85.3%)	911 (42.0%)
Skin phenotype, n (%)				
lcSSc	381 (60.0%)	—	231 (66.6%)	—
dcSSc	254 (40.0%)	—	115 (33.1%)	—
Autoantibody, n (%)				
ACA	225 (35.4%)	—	169 (48.7%)	—
ATA	161 (25.4%)	—	76 (21.9%)	—
Clinical manifestations, n (%)				
ILD	289 (45.5%)	—	143 (41.2%)	—
VC	176 (27.7%)	—	88 (25.4%)	—
Age at onset, n (%)				
Late-onset	56 (8.8%)	—	121 (34.9%)	—
Nonlate-onset	132 (20.8%)	—	222 (64.0%)	—

Late-onset of the disease is defined as the disease onset at the age ≥ 60 years old.
ACA, anticentromere antibody; ATA, anti-topoisomerase I antibody; dcSSc, diffuse cutaneous systemic sclerosis; ILD, interstitial lung disease; IQR, interquartile range; lcSSc, limited cutaneous systemic sclerosis; SSc, systemic sclerosis; VC, vascular complications.

RESULTS

Demographic and clinical features of study participants

The demographic features of the participants in sets 1 and 2 are listed in the Table. As expected, patients with SSc included more females than controls in both datasets. Consistent with previous reports [13–21], the prevalence of mCAs increases with age in both SSc and control cohorts, confirming the validity of the current results (Supplementary Fig S2, Supplementary Table S2).

An age-dependent positive association of autosomal loss with SSc

To investigate if the presence of specific types of mCAs is associated with SSc, we applied the logistic regression models with covariates (see the Methods section). We identified a trend of increased mCAs in SSc and this was driven by consistent positive associations between autosomal loss and SSc across the datasets (OR, 1.72; 95% CI, 0.94–3.14; $P = .079$, meta-analysis) (Fig 2A, Supplementary Table S3). Thus, we primarily focused on autosomal loss in the following analyses. Though it is well-known that mCAs increased with age

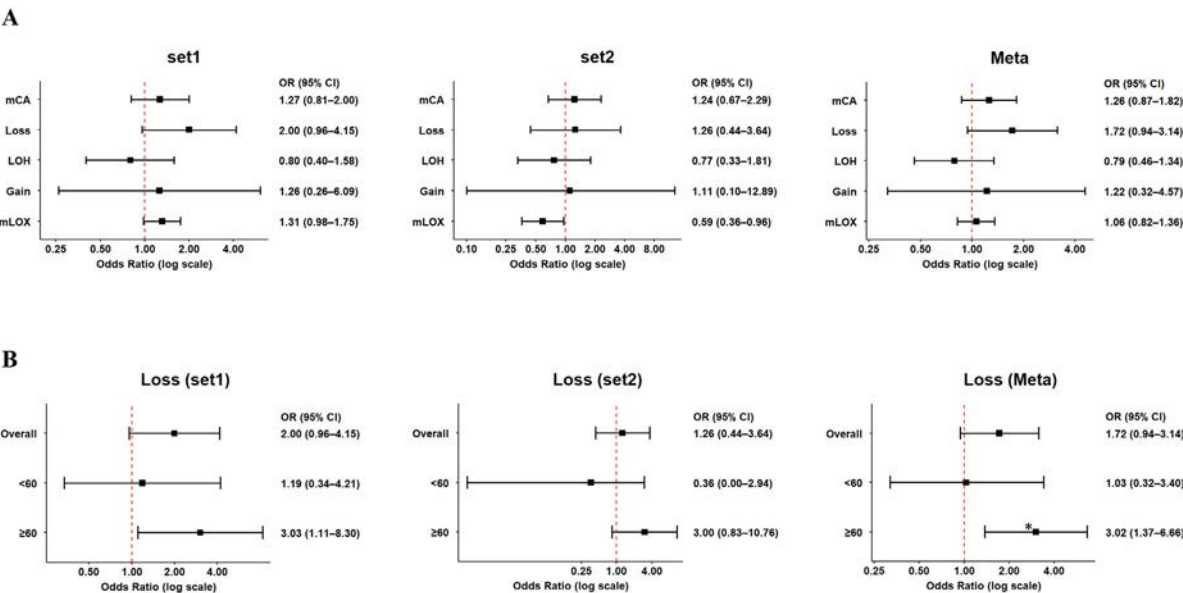


Figure 2. Association between SSc and mCAs. A, Forest plots showing associations between SSc and mCAs or its subtypes (autosomal loss, LOH, autosomal gain, and mLOX) in 2 independent datasets (set 1, set 2) and those of meta-analysis are presented. B, Forest plots showing associations between SSc and autosomal loss across different age groups (age < 60 and age ≥ 60 years) in 2 independent datasets (set 1, set 2) and those of meta-analysis are presented. Odds ratios (ORs) are presented in a log scale, and error bars indicate the 95% confidence intervals (CIs). Statistical significance is denoted based on Bonferroni-adjusted 2-sided P values < 0.025 (*).

Gain, autosomal gain; Loss, autosomal loss; LOH, loss of heterozygosity; mCA, mosaic chromosomal alteration; mLOX, mosaic loss of chromosome X.

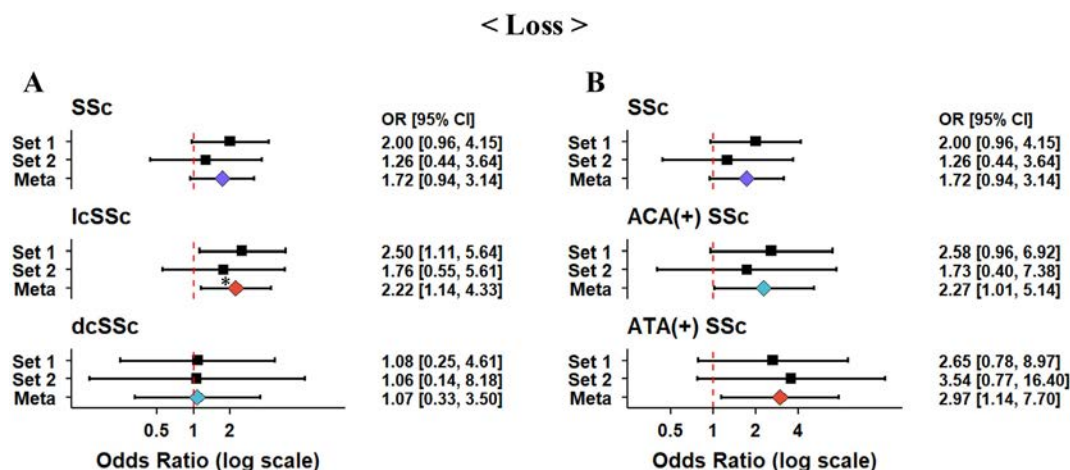


Figure 3. Associations of autosomal loss with major clinical subtypes of SSc.

A-B, Associations between skin subtypes (lcSSc and dcSSc) and autosomal loss (A) or between autoantibody profiles (ACA and ATA) and autosomal loss (B) are presented. The associations between SSc and autosomal loss are presented for comparison. Presented are the results of a case–control study. Odds ratios (ORs) are presented in a log scale, and error bars indicate the 95% confidence intervals (CIs). Statistical significance is denoted based on Bonferroni-adjusted 2-sided P values < 0.025 (*).

ACA, anticentromere antibody; ATA, antitopoisomerase I antibody; dcSSc, diffuse cutaneous SSc; lcSSc, limited cutaneous SSc; Loss, autosomal loss; SSc, systemic sclerosis.

even in healthy individuals [13–21] (Supplementary Fig S2, Supplementary Table S2), we observed that the effect sizes of autosomal loss on SSc were significantly higher in patients aged 60 years or more (OR, 3.02; 95% CI, 1.37–6.66; $P = .0063$), which was consistent across the datasets (Fig 2B, Supplementary Table S4).

These findings suggest that autosomal loss is significantly associated with SSc especially for patients with advanced age.

In female-only analyses, differences in mCA and autosomal loss frequencies between SSc and controls widened (Supplementary Table S5), with stronger associations; autosomal loss was significantly associated with overall SSc and more strongly with SSc in females aged ≥ 60 years (Supplementary Table S6). Because autosomal mosaic events are generally more frequent in males [14], the higher male proportion among controls may have slightly biased the comparison toward the null, despite adjustment for sex in the statistical model.

Phenotype-specific associations of autosomal loss

We investigated if the heterogeneity of SSc phenotypes, such as skin manifestations (lcSSc or dcSSc) or autoantibody profiles (ACA or ATA), is associated with a specific type of mCAs. We identified a significant positive association of autosomal loss with lcSSc (OR, 2.22; 95% CI, 1.14–4.33; $P = .019$; meta-analysis), which is consistent across the datasets (Fig 3A, Supplementary Table S7). We also observed consistent positive associations of autosomal loss with ACA-SSc (OR, 2.27; 95% CI, 1.01–5.14; $P = .049$; meta-analysis) (Fig 3B, Supplementary Table S8). On the other hand, although dcSSc has no association with autosomal loss, ATA-SSc shows positive association with autosomal loss (OR, 2.97; 95% CI, 1.14–7.70; $P = .026$; meta-analysis; Fig 3A,B; Supplementary Tables S7 and S8).

Then, we sought potential associations of clinical phenotypes other than skin manifestations and autoantibody profiles, namely ILD and VC, both of which are intractable to treatment and can even be fatal [3]. We identified that autosomal loss had a significant positive association with ILD (OR, 2.44; 95% CI, 1.16–5.14; $P = .019$; meta-analysis) and VC (OR, 3.30; 95% CI, 1.42–7.66; $P = .0054$; meta-analysis; Fig 4A,B; Supplementary

Table S9). Of note, the effect sizes tended to be larger than that of SSc and autosomal loss. Furthermore, the associations between autosomal loss and ILD or VC remained significant even after conditioning on the presence of ACA or ATA (Fig 4C, D; Supplementary Table S10). Conditioning on the skin phenotypes (lcSSc or dcSSc), the associations were attenuated; but a trend of positive association still remained (Supplementary Fig 3; Supplementary Table S10).

Together, these results suggest the phenotype-specific association of autosomal loss.

Dose-dependent associations of autosomal loss with SSc subsets

Considering the survival advantage, and clonal expansion of the haematopoietic stem and progenitor cells (HSPCs) with given mCAs (13,34), the higher burden of mCAs can confer larger effects on phenotypes.

To test such a dose-dependent effect, we extracted the subjects with autosomal loss with CF $> 5\%$ and compared the risk of each phenotype in a case–control setting. As we expected, we observed increased effect sizes in associations of autosomal loss with SSc, lcSSc, ACA-SSc, ILD-SSc, and VC-SSc in subjects with high CF (Fig 5, Supplementary Table S11).

Associations of mLOX were mainly observed in subjects with high CF

The increased effect sizes in subjects with high CF for autosomal loss motivated us to analyse other types of mCAs in detail for associations only observed in subjects with high CF. As a result, we identified consistent positive associations between mLOX with high CF and SSc (OR = 2.17; 95% CI, 1.00–4.70; $P = 0.050$), lcSSc (OR = 2.84; 95% CI, 1.27–6.34; $P = 0.011$), and ACA-SSc (OR = 3.72; 95% CI, 1.60–8.65; $P = 0.0023$), that were not apparent without considering CF (Fig 6A–C, Supplementary Table S12).

We also found that mLOX showed significant associations in intracase analyses of SSc subsets, namely, positive associations of lcSSc in comparison with dcSSc (OR, 2.49; 95% CI, 1.37–4.54; $P = .0027$) and positive associations of ACA-SSc in

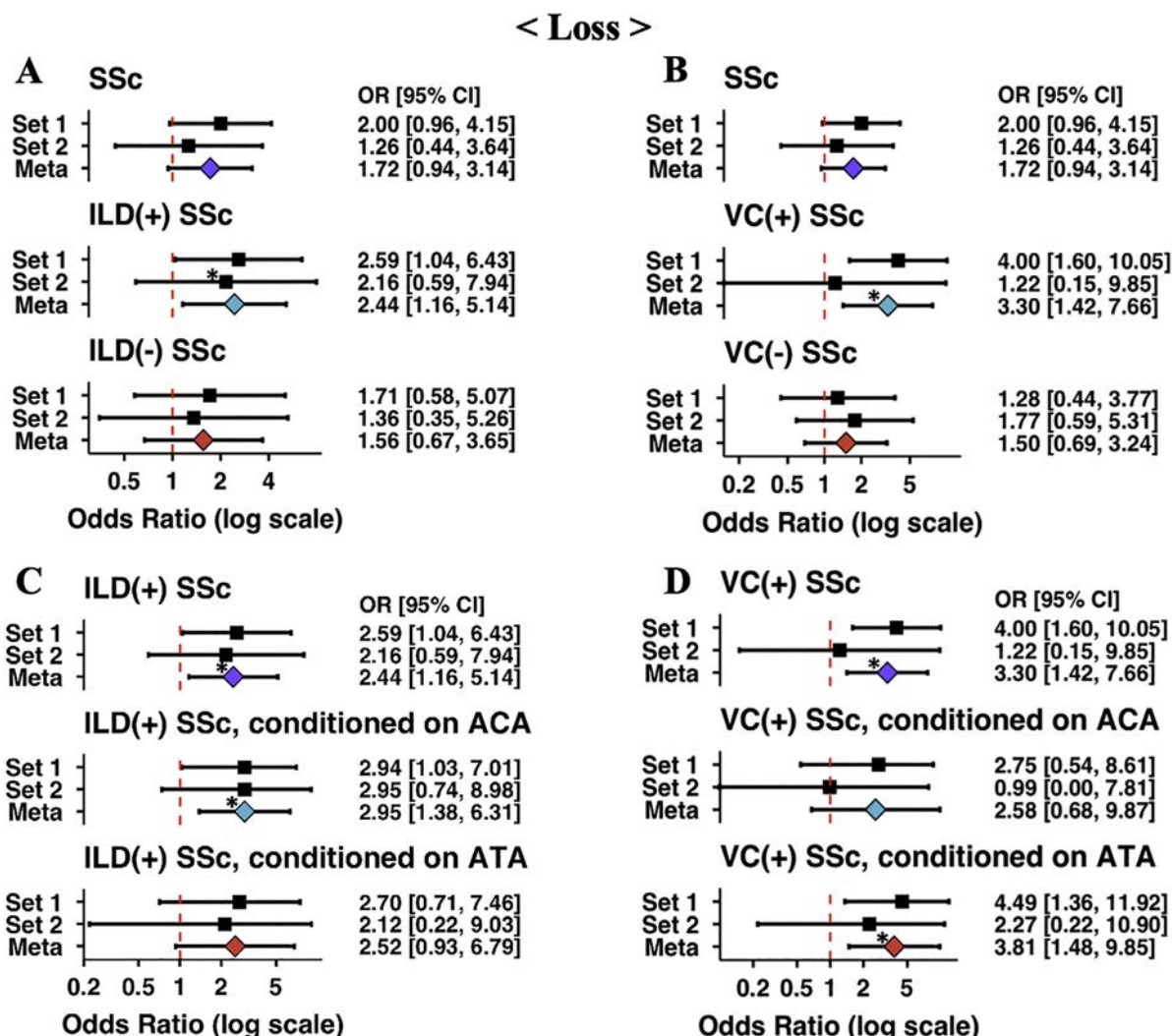


Figure 4. Associations between autosomal loss and SSc with interstitial lung disease or vascular complications. A, B, Associations between SSc with or without interstitial lung disease (ILD) and autosomal loss (A) and SSc with or without vascular complications (VC) and autosomal loss (B) are presented. The associations between SSc and autosomal loss are presented for comparison. C, D, Associations between ILD-SSc and autosomal loss (C) or VC-SSc and autosomal loss conditioning on autoantibody profiles (ACA, ATA) are presented. The associations between ILD-SSc or VC-SSc and autosomal loss are presented for comparison. The results of a case-control study are shown. Odds ratios (ORs) are presented in a log scale, and error bars indicate the 95% confidence intervals (CIs). Statistical significance is denoted based on Bonferroni-adjusted 2-sided P values < 0.025 (*) in primary analyses and based on 2-sided nominal P values < 0.05 (*) in conditioning analyses.

ACA, anticentromere antibody; ATA, antitopoisomerase I antibody; ILD, interstitial lung disease; Loss, autosomal loss; SSc, systemic sclerosis; VC, vascular complications.

comparison with ATA-SSc (OR, 3.16; 95% CI, 1.49–6.68; $P = .0026$) (Fig 6D,E; Supplementary Table S13). These effect sizes in intracase analyses were further increased in the analyses restricting to subjects with high CF although the associations were no longer significant due to the limited sample sizes (Supplementary Fig S4, Supplementary Table S14). Interestingly, we did not observe significant associations of autosomal loss in the intracase analyses (Supplementary Fig S5, Supplementary Table S15).

These results suggest the dose-dependent involvement of mLOX with SSc and its subtypes. Additionally, exploratory analysis showed that autosomal loss–mLOX overlap tended to be more frequent in SSc than controls (4/862 [0.46%] versus 3/2760 [0.11%]; Fisher exact test, OR, 4.28, 95% CI 0.96–19.18, $P = .060$), although cases were few (Supplementary Table S16). In overlap cases, autosomal loss consistently had a higher CF than mLOX (Supplementary Fig S6, Supplementary Table S16), and their CFs were positively correlated (Pearson's $r = 0.91$,

$P = .0042$), suggesting related clonal populations. The higher autosomal loss fraction may indicate earlier occurrence, with subsequent mLOX in a subclone; however, these findings require caution given the small sample size and lack of single-cell data.

The stronger association of autosomal loss with late-onset SSc and its subtypes

Based on our previous findings of LORA-specific associations, we hypothesised that there might be differential associations between subjects with different ages of disease onset. Referring to the previous studies [24,25,27], we stratified the patients with SSc or its clinical subtypes according to the age of disease onset (≥ 60 and < 60 years) (see the Methods section).

We observed stronger associations of autosomal loss and late-onset subsets of SSc (OR, 3.94; 95% CI, 1.43–10.86; $P = .0080$), lcSSc (OR, 4.77; 95% CI, 1.48–15.35; $P = .0088$), ACA-SSc (OR,

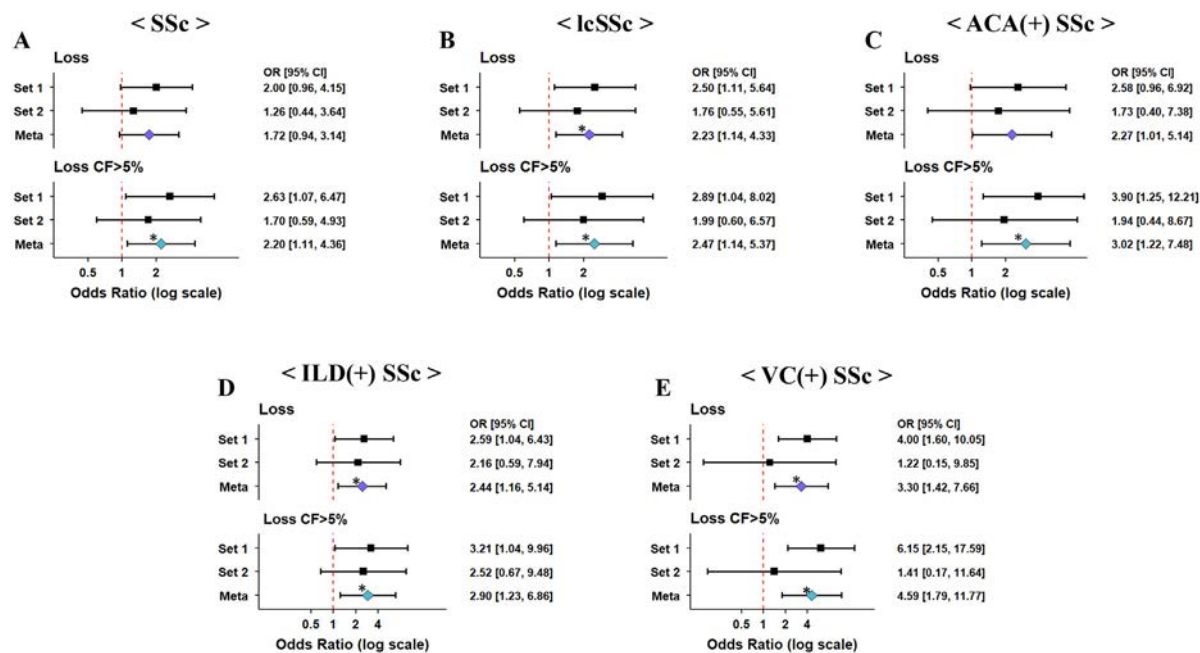


Figure 5. Dose-dependent effect of the cell fraction of autosomal loss on SSc and its subtypes. A–E, Associations between autosomal loss and SSc (A), lcSSc (B), ACA-SSc (C), ILD-SSc (D), or VC-SSc (E) with (bottom) or without (top) cell fraction (CF) threshold of 5% (autosomal loss CF > 5%) are presented. The results of a case–control study are shown. Odds ratios (ORs) are presented on a log scale, and error bars indicate the 95% confidence intervals (CIs). Statistical significance is denoted based on 2-sided nominal P values < 0.05 (*).

ACA, anticentromere antibody; ATA, antitopoisomerase I antibody; ILD, interstitial lung disease; Loss, autosomal loss; SSc, systemic sclerosis; VC, vascular complications.

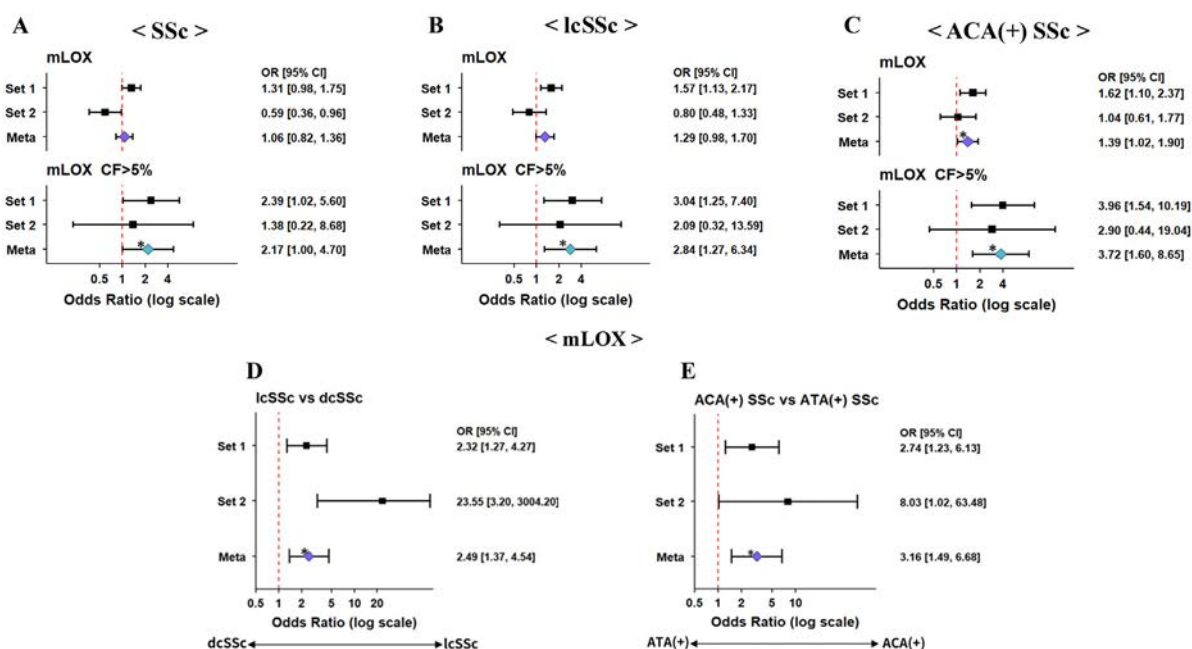


Figure 6. Intracase association of mLOX with major clinical subtypes of SSc and dose-dependent effect of the cell fraction of mLOX on SSc and its subtypes. A–C, Associations of mLOX with SSc (A), lcSSc (B), and ACA-SSc (C), with (bottom) or without (top) cell fraction (CF) threshold of 5% (mLOX CF > 5%) are presented. The results of a case–control study are shown. D–E, Intracase associations of mLOX comparing skin subtypes (lcSSc and dcSSc) (D) and autoantibody profiles (ACA and ATA) (E) are presented.

Odds ratios (ORs) are presented in a log scale, and error bars indicate the 95% confidence intervals (CIs). Statistical significance is denoted based on 2-sided nominal P values < 0.05 (*).

ACA, anticentromere antibody; ATA, antitopoisomerase I antibody; dcSSc, diffuse cutaneous SSc; lcSSc, limited cutaneous SSc; Loss, autosomal loss; SSc, systemic sclerosis.

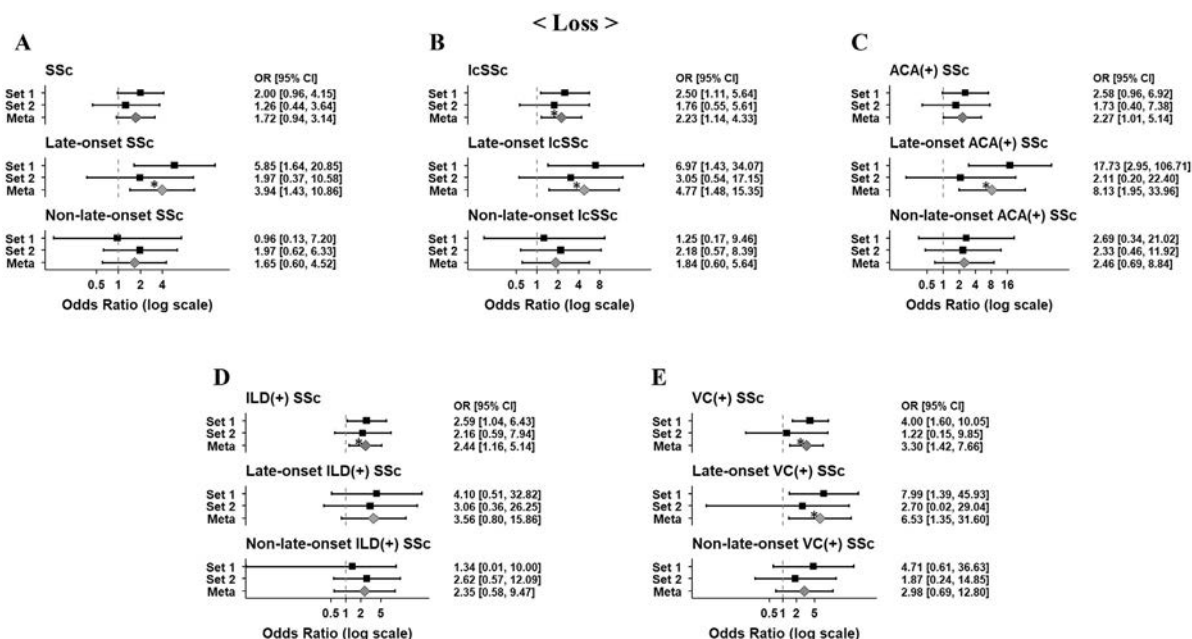


Figure 7. Stronger associations of autosomal loss with late-onset SSc and its subtypes.

A–E, Associations between autosomal loss and SSc (A), lcSSc (B), ACA-SSc (C), ILD-SSc (D), and VC-SSc (E) for the whole subset (top), the subset with late-onset (middle), and those with non-late-onset (bottom) are presented. The results of a case–control study are shown. Odds ratios (ORs) are presented on a log scale, and error bars indicate the 95% confidence intervals (CIs). Statistical significance is denoted based on 2-sided nominal P values < 0.05 (*).

SSc, systemic sclerosis; lcSSc, limited cutaneous SSc; ACA, anticentromere antibody; ILD, interstitial lung disease; VC, vascular complications; Loss, autosomal loss.

8.13; 95% CI, 1.95–33.96; $P = .0040$), ILD-SSc (OR, 3.56; 95% CI, 0.80–15.86; $P = .096$), and VC-SSc (OR, 6.53; 95% CI, 1.35–31.60; $P = .020$) with higher effect sizes than those of overall and nonlate-onset subsets (Fig 7, Supplementary Tables S17 and S18). On the other hand, we did not observe consistent patterns in mLOX (Supplementary Tables S19 and S20).

These results support the notion that phenotypes of late-onset SSc are different from those of nonlate-onset SSc, and autosomal loss can partially explain such phenotypic differences.

The autosomal loss events in SSc tended to be skewed in chr5q

Since we observed that associations of autosomal loss with SSc and its subtypes, we analysed whether autosomal loss events in SSc recurrently involved a specific genomic region. Supplementary Table S21 shows the frequency of loss by chromosomal arm in SSc and controls. We observed autosomal loss events in the long arm of chromosome 5 (5q) were the most frequent in SSc (20.0%). The 5q autosomal loss were the second most frequent in the controls (9.20%). Although this study with low statistical power did not reveal a significant result, the 5q region may be associated with SSc.

DISCUSSION

In the present study, we identified associations of autosomal loss and mLOX with SSc and its subtypes. The consistent association patterns across datasets support the validity of the results. A part of associations were pronounced by stratifications based on biological age, clinical phenotypes and age at disease onset, highlighting the potential roles of mCAs in the pathologic basis of SSc and its heterogeneous phenotypes. We also confirmed that inclusion of an age-squared term as an additional covariate

did not substantially change the results (Supplementary Table S22).

Although there is no established evidence how mCAs impact on immune cells so far, the present study together with our previous finding of mLOY in LORA suggest that mCAs might contribute to the expansion of aberrant immune cell clones and subsequent dysregulation of immune responses [24]. Of note, previous works have suggested that somatic genome alterations play some roles in SSc biology. A study with target sequence of genes frequently mutated in myeloid malignancies in peripheral blood mononuclear cells from SSc and healthy donors identified enrichment of clonal haematopoiesis of intermediate potential (CHIP) in younger (age < 50 lt; 50 years) SSc patients [35]. Another study with whole-genome sequence (WGS) of cultured lung fibroblasts from SSc and healthy subjects demonstrated increased somatic mutational burden and chromosomal instability in SSc lung fibroblasts [36]. In addition, recent work has further highlighted chromosomal instability and telomere attrition in SSc [37]. The present study is complementary to these observations.

The association between autosomal loss and VC suggests that such chromosomal alterations may lead to endothelial dysfunction and injury with impaired vascular repair process, leading to vascular lesions observed in SSc, including DU, PAH, and SRC. We observed the dose-dependent associations of autosomal loss as well as associations of mLOX only in subjects with high CF, suggesting that the expansion of autosomal loss-bearing clones (in either autosome or X chromosome) is likely to exert measurable effects (e.g., vascular impairment) leading to specific phenotype manifestations (e.g., VC). Importantly, the effect sizes of autosomal loss were pronounced in late-onset SSc, especially in lcSSc and ACA phenotypes, well concordant with the previous finding that late-onset SSc exhibits a higher prevalence of

lcSSc and ACA phenotypes [25–27]. These data may suggest that age-related chromosomal instability and clonal haematopoiesis of autosomal loss underlie the phenotypic shift toward the limited form (rather than the generalised form) of skin phenotype or the vascular form (rather than the immune-fibrotic form) in late-onset SSc. Together, these data suggest that mCAs, particularly autosomal loss and mLOX, might serve as biomarkers, partly in a dose-dependent manner, and hence contribute to the development of phenotype-based clinical management of SSc.

The predominance of chromosomal loss in late-onset SSc may reflect the intersection between age-related clonal haematopoiesis and the distinctive biology of late-onset SSc [13,14,17,24–27]. Chromosomal losses are a common manifestation of age-related mosaic chromosomal alterations in blood-derived DNA and may arise from accumulated DNA damage, telomere dysfunction, or impaired DNA repair, followed by clonal selection of haematopoietic cells [13,14,17,30,34]. Given accumulating evidence that SSc tissues show features of DNA damage, our findings raise the possibility that chromosomal loss marks a subset of individuals with heightened genomic instability, in whom somatic loss events in haematopoietic cells may either contribute to immune dysregulation or serve as a surrogate marker of a broader DNA damage-prone state [18,35–39].

The effect sizes of the mLOX associations showed some variability between the 2 datasets, particularly in the intracase analysis of lcSSc versus dcSSc (Fig 6D,E; Supplementary Table S13). This likely reflects the instability of mLOX-related estimates when carrier numbers are small. A similar between-cohort variability of mLOX analyses was also noted in our previous RA mosaic study [24].

Comparison with our previous RA study [24] is informative. Both SSc and RA showed stronger mCA associations in late-onset disease, suggesting relevance of age-related clonal mosaicism to late-onset autoimmune phenotypes [24]. However, the associated mCA classes differed. Specifically, SSc was mainly positively associated with autosomal loss, whereas RA showed a negative association with autosomal mCAs [24], indicating disease-specific contributions of acquired chromosomal alterations.

Several limitations should be addressed in future work. First, the cross-sectional design precludes causal inference between mCAs and SSc phenotypes; therefore, mechanistic interpretations will require longitudinal and functional studies. Second, this study remains underpowered for some subgroup analyses, and phenotypic information, including smoking status and detailed treatment history, should be further expanded. In particular, ILD defined by high-resolution CT would be an important phenotype to evaluate in relation to mCA associations. Finally, DNA damage and genomic instability, both of which are closely related to mCAs and CHIP, have been implicated in SSc [36,38,39]. Future studies integrating expanded mCA analyses with whole-exome sequence (WES)/WGS data may help clarify the potential links among these phenomena.

Nevertheless, our data provide novel biological insights that illuminate unrecognised immune-haematopoietic pathways underlying clinical heterogeneity in SSc. Further expansion of data, including samples from other ancestries and sequencing data (WES/WGS and single-cell RNA sequencing), would help us to consolidate the current findings and elucidate the cellular and molecular contributions of mCAs to SSc pathophysiology.

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Contributors

YN and CT conceived the project. YY and CT supervised the project. YN and CT analysed the data. YI, SU, ST, and TK shared the statistical codes. CT, HY, MK, MA, HN, SM, MH, YA, SN, YT, YK, and MK generated the SSc data. YS, YN, KH, RN, and YE collected additional clinical information of SSc. YNK, KM, and II generated the control data. CT, YI, SU, and XL participated in technical discussions of mCAs. YN, YI, and CT wrote the manuscript. All authors have critically reviewed and approved the final version of the manuscript. CT is the guarantor.

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Competing interests

All other authors declare they have no competing interests. SN has received speaking fees and/or honoraria from GlaxoSmithKline, Eli Lilly, Asahi Kasei, Bristol Myers Squibb, Otsuka Pharmaceutical, Eisai, AstraZeneca, AbbVie, Astellas Pharma, Tanabe Pharma, Janssen, Chugai Pharmaceutical, Taisho Pharmaceutical, Ayumi, Gilead Sciences, UCB, Pfizer and has received consulting fees, lecture fees from Asahi Kasei, Otsuka Pharmaceutical, Boehringer Ingelheim, and Tanabe Pharma. MK has received consulting fees from AbbVie, argenx, AstraZeneca, Boehringer Ingelheim, GSK, Kissei, MBL, Mochida, Novartis, and Mitsubishi Pharma; research grants from Boehringer Ingelheim and MBL; and speaking fee from Asahi Kasei Pharma, Boehringer Ingelheim, Bristol Myers Squibb, Chugai, Eli Lilly, MSD, and Ono Pharmaceuticals.

Patient consent for publication

Not applicable.

Ethics approval

All patients provided informed consent, and this study was approved by the local ethics committee of each institute.

Data availability

Statistical codes are available from Chikashi Terao (ORCID 0000-0002-6452-4095) at any time, only on reasonable request. The Mosaic Chromosomal Alterations pipelines used for mosaic calling (mocha.wdl) are available at (<https://github.com/free seek/mochawdl>).

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used ChatGPT (OpenAI) to improve the clarity and readability of the English text. After using this tool, the authors reviewed and edited the manuscript and take full responsibility for the content of the published article.

Supplementary materials

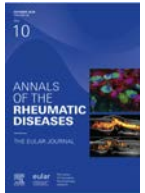
Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2026.06.006.

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Autoinflammatory disorders

Peripheral blood *UBA1* variant burden predicts poor outcomes in VEXAS syndrome: a nationwide prospective study

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ABSTRACT

Objectives: Vacuole, E1 enzyme, X-linked, autoinflammatory, somatic (VEXAS) syndrome (VS) is a severe autoinflammatory disease caused by acquired pathogenic *UBA1* variants and is associated with high mortality. This study aimed to identify predictors of poor outcomes in VEXAS syndrome.

Methods: We conducted a nationwide prospective registry in Japan, in which all suspected cases underwent next-generation sequencing to detect pathogenic *UBA1* variants and estimate the variant allele frequency (VAF) at baseline. Disease activity (VEXAS Current Activity Form [VEXASCAF]), serum C-reactive protein (CRP) level, prednisolone (PSL) dose, adverse events (AEs), mortality, and transfusion dependency were evaluated every 3 months for 1 year.

Results: Among the screened patients, we included 60 patients with pathogenic *UBA1* variants (all males; median age, 74 years) and 45 male patients with VS-like syndrome of similar age but without *UBA1* variants served as controls. During a median follow-up of 343 days, the VEXASCAF and CRP levels decreased, whereas the PSL dose did not significantly change. Grade ≥ 3 AEs occurred in 50% of patients within 1 year. In the multivariable Cox regression analysis, a higher baseline VAF and higher PSL dose independently predicted poor prognosis, defined as death or transfusion dependence. The association between a higher VAF and poor outcomes was confirmed in 34 independent patients with VS. After adjusting for disease onset, patients carrying the *UBA1* p.Met41Leu variant showed better survival. A similarly poor prognosis was observed in VEXAS-like patients.

Conclusions: Baseline pathogenic *UBA1* VAF serves as a potent prognostic biomarker for poor outcomes in VS and highlights its potential role in risk stratification.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Vacuole, E1 enzyme, X-linked, autoinflammatory, somatic (VEXAS) syndrome is a severe autoinflammatory disease with high mortality, for which reliable prognostic factors are urgently needed.
- Although retrospective studies have suggested that *UBA1* variant allele frequency (VAF) may correlate with disease severity and prognosis, definitive prognostic factors have not been identified in prospective studies.

WHAT THIS STUDY ADDS

- This is the first nationwide prospective study demonstrating that baseline peripheral blood *UBA1* VAF independently predicts poor outcomes (death or transfusion dependence) in VEXAS syndrome.
- Higher baseline prednisolone dose also predicted adverse outcomes, suggesting the limitations of current glucocorticoid-based management.
- VEXAS-like conditions similarly exhibited poor outcomes, underscoring the need for further investigation.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Monitoring the VAF of pathogenic *UBA1* variant may be important for risk stratification development in VEXAS syndrome.

INTRODUCTION

Vacuole, E1 enzyme, X-linked, autoinflammatory, somatic (VEXAS) syndrome (VS) is a life-threatening inflammatory disorder with haematological overlap caused by acquired pathogenic variants in the ubiquitin-like modifier activating enzyme (*UBA1*) gene arising in haematopoietic progenitors [1]. Retrospective studies have shown that most patients are men in their 50s or older and present with a broad inflammatory phenotype, including cutaneous lesions, pulmonary involvement, and chondritis [2,3]. Approximately 95% of patients with VS harbour a substitution at *UBA1* Met41, which impairs ubiquitination, promotes cell death, and amplifies inflammation via damage-associated molecular pattern release, raising the possibility that a higher mutation burden could drive more severe disease [4,5]. The remaining 5% of patients with VS carry *UBA1* variants outside Met41, such as variants in exon 14. These are often referred to as noncanonical *UBA1* variants and have been associated with fewer inflammatory manifestations and a greater tendency towards haematological diseases [6].

Outcomes are poor, with a 5-year survival reported of approximately 18–50% [7], partially due to a high rate of complications associated with prednisolone (PSL) dependence. International guidance from the American College of Rheumatology recommends interleukin (IL)-6 inhibitors (IL-6i), Janus kinase inhibitors (JAKi), or IL-1 inhibitors for PSL-dependent VS [8]. However, tocilizumab (TCZ) is not sufficiently effective to allow discontinuation of PSL [9,10]. Analyses of data from the French VEXAS study group, (FRENEX), have shown limited treatment efficacy for ruxolitinib and TCZ [11,12]. Moreover, the disease trajectory of VS was not affected by TCZ or JAKi [13]. No PSL-sparing treatment has been developed, and most patients with VS are maintained on high-to-moderate doses of PSL, which naturally leads to a high incidence of adverse events (AEs) and mortality, especially in older individuals. Short-term results from the world's first prospective VS registry from our group

indicated a persistently high mean PSL dose of 13 mg/d accompanied by low remission rates and a substantial AE burden [14].

PSL dependence may reflect the persistence of pathogenic *UBA1* clones that have not been eradicated by anticytokine therapy. In contrast, azacitidine is the only agent reported to reduce the *UBA1* variant allele frequency (VAF): in a retrospective series, a $\geq 25\%$ VAF reduction ('molecular response') occurred in 65% of treated patients and was accompanied by inflammatory and haematologic responses; VAF fell to $< 2\%$ in 43% of the cases [15]. These data suggest that quantifying the mutational clone burden using VAF may be clinically informative for VS.

Indeed, *UBA1* VAF has been considered to correlate with disease severity and treatment response [4,16], and a low VAF variant is found in asymptomatic individuals [17]. Some patients with low VAF ($\leq 10\%$) may initially be asymptomatic and subsequently develop overt disease [18]. However, multiple studies have failed to confirm the association between VAF and mortality. Instead, *UBA1* genotypes associated with cytosolic *UBA1b* translation have been proposed as useful predictors of VS prognosis in multiple studies. For instance, the *UBA1* p.Met41Val, with the lowest *UBA1b* level, has been linked to reduced survival, whereas p.Met41Leu, with the highest *UBA1b* level, has been associated with improved survival rates [4,16].

Overall, the prognostic factors proposed to date are primarily based on retrospective studies and may be affected by selection bias favouring surviving cases as well as by ambiguities in the follow-up period. Identifying reliable prognostic factors for survival is essential for risk stratification, optimisation of treatment, enabling early intervention, guiding clinical decisions, informing patients, and supporting drug development to improve overall outcomes. Such objectives can be achieved only through prospective studies that can identify causal relationships between variables and outcomes. To identify the predictive factors for poor prognosis, we analysed data from a prospective VS registry that has not been previously reported. In this study, we report that VAF is a predictor of poor prognosis in VS.

METHODS

Patients and disease activity measure

Thirty-nine facilities in Japan participated in this study (Supplementary Fig S1). Patients with suspected VS-like symptoms were enrolled in this study. Inclusion criteria were a clinical score ≥ 3 [3]; age ≥ 50 years (1 point), cutaneous lesions (1 point), lung involvement (1 point), chondritis (1 point), and macrocytic anaemia (2 points). *UBA1* variant screening was performed for all patients. For *UBA1* variant-positive VS cases, only those with at least 3 months of follow-up data were included in the analysis. Clinical data were collected by the attending physicians at registration, at 12, 24, 36, and 48 weeks, and at the end-of-study follow-up (Fig 1). In the *UBA1*-variant-negative VS-like group, patients without postregistration data and female patients were excluded to match the VS group according to sex. Clinical data were collected during registration and at the end of the observation period. Short-term clinical data from the initial 30 VS cases in this prospective registry have been published together with case reports [14,19–21]. All patients provided written informed consent, and the study was approved by the Yokohama City University (YCU) Certified Institutional Review Board (F240500053). The study protocol was registered in the

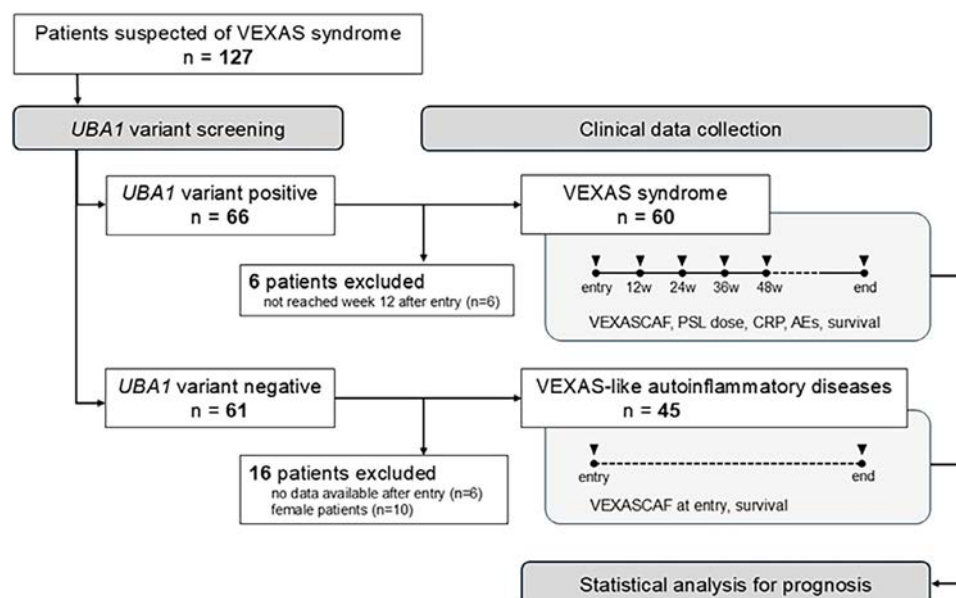


Figure 1. Study flowchart. Flowchart showing the number of patients screened for the entire *UBA1* gene and their estimated variant allele frequency (VAF). Patients positive for pathogenic *UBA1* variants were followed every 3 months after enrolment and assessed for VEXASCAF scores, prednisolone (PSL) dose, survival, transfusion dependency status, and adverse events (AEs). CRP, C-reactive protein; VEXAS, Vacuole, E1 enzyme, X-linked, autoinflammatory, somatic; VEXASCAF, VEXAS Current Activity Form.

University Hospital Medical Information Network database (UMIN000053426).

We previously developed a disease activity index (DAI) called the VEXAS Current Activity Form (VEXASCAF), which evaluates 11 typical clinical symptoms of VS over the past 28 days, as determined by an attending physician [14]. The VEXASCAF score, treatment, and AEs were monitored at entry and every 12–48 weeks by the attending physician. Among the laboratory data assessed, we evaluated C-reactive protein (CRP) levels, which are considered a biomarker for VS [12]. Complete remission (CR) was also evaluated using the definition proposed by FRENEX as follows: no symptoms caused by VEXAS, CRP ≤ 10 mg/L, and PSL-equivalent therapy ≤ 10 mg/d [12]. The grades of AEs during the follow-up period were determined based on the Common Terminology Criteria for Adverse Events v5.0 (<https://ctep.cancer.gov/>): grade 1, mild; grade 2, moderate; grade 3, severe but not immediately life-threatening, hospitalisation, or prolongation of existing hospitalisation indicated; grade 4, severe, life-threatening requiring immediate intervention; and grade 5, death. We report AEs greater than grade 3 in this article. The AEs in our study included both drug-induced and non–drug-induced events.

To validate the association between poor outcomes and the VAF of the pathogenic *UBA1* variant, we referred to our recently published retrospective dataset [22]. Therefore, we examined poor outcomes encompassing both death and transfusion dependency in a prospective analysis. We obtained information on 34 patients not included in the prospective study regarding their VAF, observation period from the time of VAF evaluation, and poor outcome events.

Symptom onset was defined as the first day of the month when VS-related symptoms initially appeared. Event-free survival was defined as the period from symptom onset to the development of transfusion dependency or death. ‘Poor outcome’ was defined as death; transfusion dependency (receiving ≥ 2 units of red blood cells per month for ≥ 6 months); or haematopoietic stem cell transplantation, at the last observation time

point, as specified by the U.S. National Institutes of Health (NIH) group [13].

Patient involvement

Patients and/or the public were not involved in the design, conduction, reporting, or dissemination of this research.

Genetic testing

As previously reported [14], whole-blood DNA was extracted from all patients at the start of the study. Next-generation sequencing (NGS) was performed at the Kazusa DNA Research Institute (Kisarazu, Chiba, Japan) at a depth of over 500 $\times$, covering all exons of the *UBA1* gene to identify pathogenic variants. We did not perform whole-exome sequencing in this study, and genetic analyses were limited to *UBA1*. Genomic DNA sequences were analysed using targeted NGS using a hybrid capture method [23]. The obtained nucleotide sequences were compared with a publicly available human genome reference sequence (GRCh38/hg38) to detect variants. Library preparation was performed using the NEXTFLEX Rapid XP V2 DNA-Seq kit (Revvity), and NGS was conducted using NextSeq2000 (Illumina). Data analyses were performed using Fastp, BWA, Mutect2 (GATK) 4.4.0.0, VarDict 1.0, and LoFreq 2.1.5. Variants meeting the following criteria were reported: (i) An allele frequency of less than 1% in both Japanese and global databases, (ii) A VAF of 2% or higher, (iii) A ‘Pass’ variant call by Mutect2. The VAF was also estimated from the Mutect2 data. For retrospective data, droplet digital polymerase chain reaction (PCR) was used to determine the VAF as previously described [3].

Statistical analysis

Associations with overall survival were evaluated using Cox proportional hazards models. Two-group comparisons were

performed using the Mann-Whitney *U* test, multigroup comparisons were performed using one-way analysis of variance (ANOVA), symptom frequencies were compared using the chi-square test, and longitudinal changes were analysed using a mixed-effects model. Survival differences between the groups were assessed by Kaplan–Meier analysis and the log-rank test. Correlation analyses were performed using Spearman's rank correlation coefficients. A *P* value <.05 was considered significant and adjusted using the Bonferroni correction, if necessary. All statistical analyses were performed using GraphPad Prism 10 (GraphPad Software) or SPSS software (IBM), and R (<https://www.r-project.org/>).

RESULTS

Patients and baseline characteristics

From May 2023 to September 2025, 127 consecutive patients with suspected VS were screened, of whom 66 (all male; median age, 74 years) carried pathogenic *UBA1* variants (Fig 1). We included 60 male patients with VS and 45 male patients of a similar age and without *UBA1* variants (patients with VS-like syndrome) for comparative analyses. The median peripheral-blood *UBA1* VAF at enrolment was 56.0 % (IQR: 39.7–66.6; range: 2.8–96.1). VAFs differed by Met41 genotype, with lower VAF in p.Met41Val and p.Met41Thr than in p.Met41Leu (Fig 2A), consistent with our prior retrospective findings [3].

The baseline features were broadly similar between the groups, except for observation duration and macrocytic anaemia prevalence (Table). The median observation period for the 60 patients with VS was 343 days, and 31 of these patients reached

the planned 48-week visit at the time of the analysis (Supplementary Table S1).

Forty-eight-week trajectories of disease activity, PSL, and CRP

Of the 60 patients with VS, 44 (73.0%) were already on PSL at enrolment, with a median dose of 10 mg/d (IQR: 20.3; range: 0–72 mg/d); however, only 2 patients (3.3%) were PSL-free after 48 weeks. During the 48-week period, 25 patients received biologics (TCZ, *n* = 23; infliximab, *n* = 1; etanercept, *n* = 1; golimumab, *n* = 1) and 4 patients received JAKi (ruxolitinib *n* = 2, baricitinib *n* = 2). Five patients required red blood cell transfusions.

Longitudinal modelling showed significant decreases in VEX-ASCAF and CRP levels by week 48, with stabilisation after month 3, whereas the PSL dose did not significantly change (at entry median PSL dose 10 mg/d, and after 1 year 10 mg/d, Fig 2B–D; Supplementary Table S1). Eleven patients (18.6%) met the FRENEX criteria for CR at least once during the 48-week period [12]. These data indicate that while PSL cannot be discontinued, patients with VS experience fewer symptoms after 1 year. We also evaluated the outcomes in patients with VS who received JAKi. Although only 4 patients were treated with JAKi, 2 achieved FRENEX CR (Supplementary Table S2).

Predictors of poor outcome in the prospective cohort

Nine patients with VS and 15 VS-like died during the follow-up period up to 2 years (p.Met41Thr, *n* = 4, p.Met41Leu *n* = 3, p.Met41Val *n* = 2). Early deaths (≤3 months post-registration) in patients with VS included sudden death, mycobacterial infection, and respiratory failure due to lung disease.

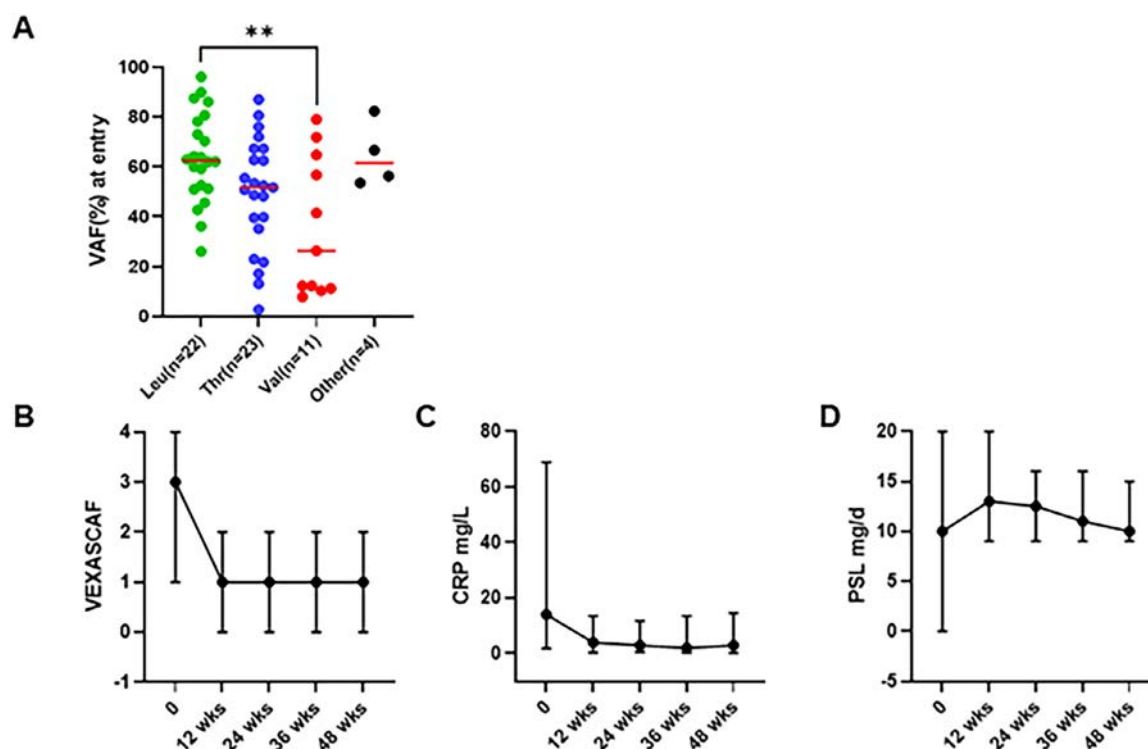


Figure 2. Peripheral blood variant allele frequency (VAF) at patient enrolment and longitudinal changes in various clinical parameters in VEXAS syndrome patients. (A) Differences in pathogenic *UBA1* variant allele frequency (VAF) in peripheral whole blood at the time of prospective study enrolment. Adjusted *P* = .0037 by one-way analysis of variance (ANOVA) with Dunnett's multiple comparisons. (B–D) Longitudinal changes in VEXASCAF scores, C-reactive protein (CRP), and prednisolone (PSL) dose over 48 wk. Data are presented as median (IQR). Mixed-effect analysis with fixed effects showed *P* < .0001 for VEXASCAF, *P* = .0064 for CRP, and *P* = .34 for PSL. VEXAS, Vacuole, E1 enzyme, X-linked, autoinflammatory, somatic; VEXAS-CAF, VEXAS Current Activity Form.

Table

Characteristics of patients with VEXAS syndrome and VEXAS-like autoinflammatory disease at study enrolment included in the Cox regression analysis

variable	VEXAS	VEXAS-like	P
Participants, n	60	45	
Male sex, n (%)	60 (100)	45 (100)	1.0
Median age at time analysis (range)	74.0 (55–89, IQR 71.0–77.0)	73.0 (43–88, IQR 67.0–81.0)	.35
Observation period (d)	343	213	.0033
Pathogenic <i>UBA1</i> variant, n (%)	60 (100)	0 (0)	
p. Met41Thr (c.122T > C), n (%)	22 (36.6)	0	
p. Met41Val (c.121A > G), n (%)	12 (20.0)	0	
p. Met41Leu (c.121A > C), n (%)	22 (36.6)	0	
Splice variant, n (%)	3 (5.0)	0	
p. Ala478Ser, n (%)	1 (1.7)	0	
VEXASCAF (range)	3 (0–8)	3 (0–7)	.93
<i>VEXASCAF components</i>			
Fever, n (%)	28 (48.3)	29 (64.4)	.10
Auricular chondritis, n (%)	13 (22.0)	6 (13.3)	.26
Nose chondritis, n (%)	5 (8.8)	1 (2.2)	.16
Respiratory symptoms, n (%)	15 (25.9)	14 (31.1)	.56
Arthralgia, n (%)	25 (43.1)	20 (44.4)	.89
Arthritis, n (%)	12 (21.1)	7 (15.6)	.48
Thrombosis, n (%)	5 (8.8)	6 (13.3)	.46
Dermatosis, n (%)	36 (61.0)	25 (55.6)	.58
Eye symptoms, n (%)	16 (27.6)	8 (17.8)	.24
Vasculitis, n (%)	7 (13.2)	8 (18.6)	.47
Periorbital oedema, n (%)	4 (7.0)	4 (8.9)	.73
<i>Haematological manifestation</i>			
Myelodysplastic syndrome, n (%)	24 (45.3)	23 (54.8)	.36
Macrocytic anaemia, n (%)	50 (83.3)	22 (48.9)	.0002 ^a

VEXAS, Vacuole, E1 enzyme, X-linked, autoinflammatory, somatic; VEXASCAF, VEXAS Current Activity Form.

P values were calculated using the Mann-Whitney U test for continuous variables and the chi-square test for categorical variables. The listed symptoms were those present at the time of registry enrolment.

^a P values <.0033 after Bonferroni correction (14 variables) were considered statistically significant.

To identify predictors of poor prognosis, we adopted a composite poor-outcome endpoint proposed by the NIH group—death, transfusion dependence, and/or haematopoietic stem-cell transplantation [13], although none of our patients underwent stem cell transplantation. All transfusion-dependent cases in the endpoint had a follow-up period of at least 6 months after enrolment. Covariates recorded at baseline, including age, peripheral whole blood VAF of p.Met41 variants, serum CRP level, PSL dose, and observation period, were analysed using Cox proportional hazards models. The results indicated that poor outcome events were significantly associated with higher VAF and PSL doses at study entry ($P = .023$, hazard ratio [HR] 1.036, 95% CI: 1.005–1.069, and $P = .033$, HR 1.042, 95% CI 1.003–1.082, respectively, [Supplementary Table S3](#)). This means that every 10% increase in VAF was associated with a 1.43-fold higher risk of poor outcomes. Correlation analyses between a VAF and various clinical parameters collected in this study revealed no significant associations ([Supplementary Fig S2](#)).

In our recent work, *RNASE1* mRNA levels were shown to correlate with disease activity and *UBA1* VAF in VS [24]. We therefore evaluated whether a combined baseline model incorporating *RNASE1* and *UBA1* VAF enhances the prediction of poor outcomes in a subset of patients with VS. The results showed that no significant interaction between *RNASE1* and VAF was observed, and their combination did not improve prognostic performance beyond VAF alone. However, we observed a trend linking lower *RNASE1* mRNA expression to poorer prognosis in VS ([Supplementary Table S4](#)).

Genotype and survival after adjusting for disease onset

Although previous retrospective studies have repeatedly shown an association between better outcomes and the *UBA1* p. Met41Leu variant [4,13,16], survival based on *UBA1* genotype was comparable in our prospective data ([Fig 3A](#)). We believe that variability in the observation period from the onset of VS symptoms may have affected the results. Therefore, we retrospectively determined the initial date of symptom onset after adjusting for the observation period. After this adjustment, we found that patients with the p.Met41Leu variant had a better prognosis than those with other genotypes, which is consistent with previous reports ([Fig 3B,C](#)).

AEs in prospective data

Within 1 year, 48 AEs grade ≥ 3 occurred in 30 patients (50%), including 6 malignancies in 4 patients, 2 thrombotic events, and 17 microbiologically confirmed infections (including 4 mycobacterial infections in 3 patients) ([Supplementary Table S5](#)). Lung involvement (pneumonia or interstitial lung disease) was the most common grade 3 AE ($n = 14$). To identify factors associated with AEs in the VS, variables recorded at baseline, including pathogenic *UBA1* variant VAF, age at entry, serum CRP level, and PSL dose, along with the observation period until AE occurrence, were analysed using Cox proportional hazard regression. However, none of these variables was significantly associated with AEs. We assessed whether grade ≥ 3 AEs within 48 weeks were related to cumulative PSL exposure and whether these

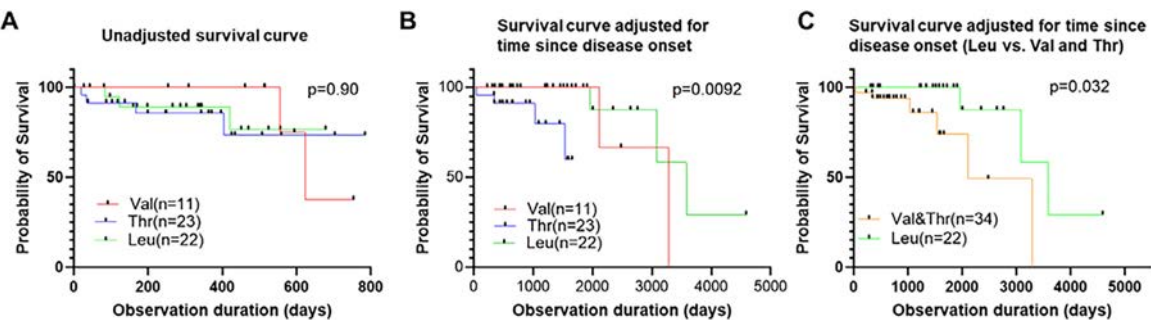


Figure 3. Survival by *UBA1* Met41 genotype with adjustment for symptom onset. Kaplan–Meier curves are shown for 56 patients with VEXAS syndrome (VS) enrolled in this study (3 patients with splice variants and 1 patient with the p.A478S variant were excluded from the analysis). Curves are stratified by *UBA1* p.Met41 variant: p.Met41Val (red), p.Met41Thr (blue), p.Met41Leu (green), and combined p.Met41Val/p.Met41Thr (orange). *P* values were calculated using the log-rank test. (A) Overall survival from registration by *UBA1* p.Met41 genotype without adjustment for symptom onset. (B) Revised version of the survival curve in panel A, after incorporating the time interval from initial VS symptom onset to registration. (C) Panel B was modified to compare p.Met41Leu versus other p.Met41 variants (p.Met41Val and p.Met41Thr combined). VEXAS, Vacuole, E1 enzyme, X-linked, autoinflammatory, somatic.

might confound the association with *UBA1* VAF. In patients who were followed up through week 48 (*n* = 31), the cumulative PSL exposure was approximated as the sum of the prescribed doses across 5 scheduled visits. Pearson’s analysis showed no significant correlation between VAF and cumulative PSL exposure. In addition, VAF did not differ between patients with and without AEs.

Validation of the VAF—outcome association in an independent retrospective cohort

To confirm the association between baseline VAF and poor outcomes, we performed a validation analysis using our previously reported retrospective cohort. In our retrospective study

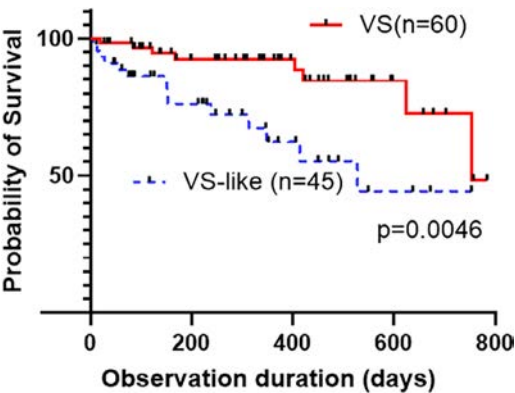


Figure 5. Survival in VEXAS syndrome vs VEXAS-like autoinflammatory disease. The figure shows survival curve differences between patients with VEXAS syndrome (VS) and those with VS-like autoinflammatory diseases (VS-like). The red line represents the survival curve for VS, and the blue dotted line represents that for VS-like autoinflammatory diseases. *P* values were calculated using the log-rank test. VEXAS, Vacuole, E1 enzyme, X-linked, autoinflammatory, somatic.

[22], the median observation period was 384 days, during which 17 patients (48.9%) died, and 2 patients became transfusion dependent. A comparison between patients with and without poor outcomes revealed that those who died had significantly higher VAF of the pathogenic *UBA1* variant at study entry, which is consistent with the findings from the prospective data (Fig 4A,B) [22]. Information on the AEs was not available in the retrospective data. Taken together with prospective data, our findings indicate that a higher pathogenic *UBA1* mutant burden is associated with poorer outcomes in VS.

Survival in VS versus VS-like autoinflammatory disease

As shown in Table and Figure 5, we compared the survival outcomes of patients with VS to a VS-like control group (similar age, sex, and phenotype, and confirmed to be negative for pathogenic *UBA1* variants using Kaplan–Meier analysis). Vital status was confirmed at the last follow-up in all patients in both the VEXAS and VEXAS-like groups. The results showed that patients in the VS-like group had a significantly shorter life expectancy than those of patients with VS. The 1-year survival rates were 92.3% and 67.3% in the VS and VS-like group, respectively. Of note, 7 patients with trisomy-8 associated autoinflammatory

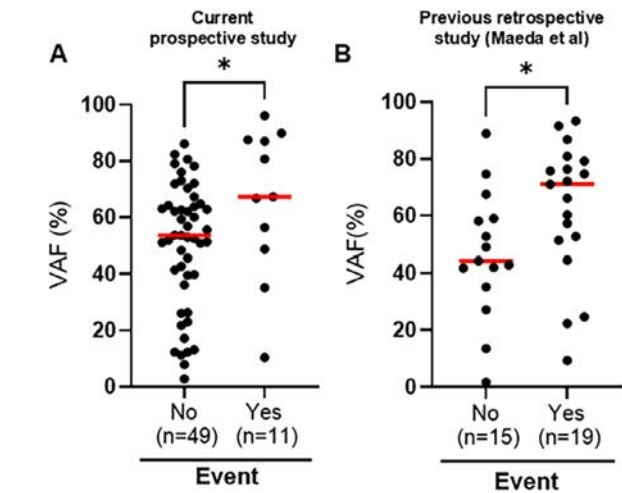


Figure 4. Baseline VAF and poor outcomes in prospective and retrospective cohorts. (A) Among 60 patients with VEXAS syndrome enrolled in the current prospective study, the peripheral blood variant allele frequency (VAF) at enrolment (y-axis) was compared according to the presence or absence of poor prognostic outcomes at last observation time. ‘Yes’ indicates cases with poor prognostic outcomes (death and transfusion dependence). (B) In an independent retrospective cohort of patients with VEXAS syndrome (Maeda et al. [22]), 34 patients who did not participate in the prospective registry were analysed, and VAF at the time of first *UBA1* genetic testing was compared according to poor prognostic outcomes. ‘Yes’ indicates cases with poor prognostic outcomes. Mann–Whitney *U* test: A, *P* = .038; B, *P* = .017. VEXAS, Vacuole, E1 enzyme, X-linked, autoinflammatory, somatic.

disease were found in the VEXAS-like controls. The causes of death in patients with VS-like syndrome varied, as shown in [Supplementary Table S6](#).

DISCUSSION

In this nationwide prospective study, the baseline peripheral blood *UBA1* VAF independently predicted 1-year poor outcomes in VS, defined as death or transfusion dependence. This association was reproduced in an independent retrospective cohort study, supporting the use of VAF as a robust and readily measurable prognostic biomarker. A higher baseline PSL dose was also associated with poor outcomes, underscoring the limitations and risks of PSL dependence in the older population.

Genotype–outcome relationships reported in retrospective series—better survival with p.Met41Leu and worse outcomes with p.Met41Val—were not evident when survival was indexed to registry enrolment but emerged once survival was measured from symptom onset [4,13,16]. This discrepancy likely reflects the heterogeneity in pre-enrolment disease duration and the relatively small number of deaths in our prospective window, which can obscure the genotype effects that accrue over longer intervals.

Stratification of the VAF of pathogenic *UBA1* variants by genotype consistently yielded reproducible results, with p.Met41Val showing the lowest VAF and p.Met41Leu showing the highest ([Fig 2A](#)). This VAF distribution appears to contradict conventional reports indicating that p.Met41Val is associated with a worse prognosis than p.Met41Leu. This phenomenon suggests higher pathogenicity of the p.Met41Val variant at the cellular level. Indeed, our *in vitro* experiments using THP-1 cell lines harbouring the p.Met41Val mutation demonstrated higher levels of cell death, inflammatory responses, interferon signatures, and necroptosis response gene signatures compared with p.Met41Leu-mutated cells [24]. Taking these findings together, p.Met41Val variant cells may exhibit pathogenicity even at low VAF, whereas p.Met41Leu variant cells may have relatively low cellular impact despite high clonal burden. This hypothesis may explain longer survival in patients with p.Met41Leu, potentially due to less inflammation ([Fig 3C](#)). However, at the patient level, the environment in which normal and mutant cells coexist in the circulating blood may play a critical role. In fact, deaths and AEs were frequently observed even in patients carrying the supposedly ‘benign’ p.Met41Leu variant in both our prospective and retrospective cohorts [3,14,22]. High circulating VAF probably reflects a greater systemic burden of mutant cells with cumulative inflammatory signalling and relative immunodeficiency from the depletion of normal myeloid compartments, thereby predisposing patients to adverse outcomes. These considerations support the incorporation of VAF into initial risk assessment and, where feasible, into longitudinal monitoring. Although differences in inflammatory activity and cell death between *UBA1* genotype may be key determinants for patients’ survival, larger prospective studies with longer follow-up are needed to fully clarify why patients with the p.Met41Leu variant have a more favourable prognosis.

This prospective study highlights the limitations of the current therapies. Continuous PSL therapy did not reduce disease activity for more than 1 year. Moreover, reports from the NIH group indicate that even with the use of IL-6i or JAKi, which are recommended under international guidance by the American College of Rheumatology [8,13,25], halting the progression of VS remains challenging [13]. In our cohort, JAKi were used in only 4 patients with VS; therefore, we could not assess the effectiveness. However, notably, 2 of the patients who were

administered JAKi achieved CR (determined based on FRENVEX criteria [12]), suggesting that JAKi could benefit some patients. Prospective comparative real-world studies are needed to determine which second-line therapies or combinations provide the greatest benefit to patients with VS. A randomised trial of the JAKi pacritinib is currently underway to reduce PSL exposure [25]. Such therapeutic developments would provide the first evidence-based option for reducing the PSL dose.

Furthermore, since VAF is associated with the overall prognosis, therapies capable of reducing VAF are promising. Although we have not assessed longitudinal changes in VAF nor VAF in bone marrow, prospective evaluation of VAF-modifying therapies—and of VAF as a surrogate endpoint—should be pursued [15]. Beyond anti-inflammatory approaches, an emerging therapeutic concept in VS is the suppression or eradication of *UBA1*-mutant clones. The hypomethylating agent azacitidine has been reported to reduce *UBA1* VAF in parallel with improvements in inflammation and cytopenia, and large retrospective registry data suggest that molecular responses frequently co-occur with clinical benefits [15]. In selected patients, allogeneic haematopoietic cell transplantation can lead to the eradication of *UBA1* mutations within months and concomitant resolution of the VEXAS phenotype [26]. Although these observations indicate that *UBA1* VAF is a meaningful pharmacodynamic biomarker for clone-directed interventions, it has not yet been established as a surrogate endpoint across treatment classes. Clinical responses to downstream anti-inflammatory therapies may occur without VAF reduction, and discordance between clinical improvement and clonal dynamics has been reported [12]. Therefore, we considered *UBA1* VAF to be best positioned as an exploratory endpoint until prospective studies validated that VAF changes reliably predict long-term outcomes.

In this study, the baseline DAI and inflammatory markers were not associated with mortality or AEs in the VS [27,28]. Our analysis demonstrated that VEXASCAF could be significantly reduced by PSL in combination with other drugs; however, deaths occurred despite this improvement in disease activity, suggesting that the underlying condition driving patient mortality may not be assessed by clinical findings alone. Several factors may account for this lack of association. First, a single baseline VEXASCAF or CRP level may not adequately capture the longitudinal inflammatory burden, which directly influences poor outcomes. Second, outcomes in VS are driven not only by inflammation but also by haematological factors and treatment- or disease-related immunodeficiency, which may not be fully reflected by baseline CRP or VEXASCAF. Finally, our study may have been underpowered to detect modest associations between these baseline markers.

Although a higher clonal burden is expected to be associated with greater inflammation, peripheral blood *UBA1* VAF was not significantly associated with clinical manifestations or CRP levels ([Supplementary Fig S2](#)). This lack of association may, in part, reflect confounding by treatment, and suggests that biomarkers beyond *UBA1* VAF are needed to fully capture disease activity and prognosis in VS. We recently identified RNASE1 as a potential biomarker of disease activity in VS [24,27]. In addition, emerging evidence implicates the type I interferon signature in the VS pathobiology [29]. As we did not evaluate biomarkers beyond CRP in the current study, integrating additional biomarkers and, in the future, *in silico* and/or structural analyses of the *UBA1* gene alongside *UBA1* VAF may improve prognostic assessment and treatment stratification.

This study has several limitations. First, this study did not evaluate quality of life (QOL) indicators or patient-reported

outcomes (PRO), which may be associated with DAI and represent clinically important endpoints in VS. Specifically, PRO domains such as pain, fatigue, physical functioning, and treatment burden may not be fully captured by DAI and should be assessed using validated instruments (eg EuroQol 5-Dimensions (EQ-5D), 36-Item Short Form Health Survey (SF-36)) as well as PRO specific for patients with VS [30]. Future studies incorporating QOL assessments along with DAI are necessary to further clarify the role of DAI in VS. In this context, a new DAI, the VEX-ASDAI, has been developed for clinical trials [28] and is being assessed alongside QOL measures in an ongoing clinical trial. Evaluating the roles of DAI, QOL, and PRO in various aspects of patient care and the advancement of therapeutic strategies remain critical.

Second, this study was limited by its small sample size, short follow-up period, and low number of poor outcomes, which prevented analysis of multiple prognostic factors. Additionally, the presence of clonal haematopoiesis of indeterminate potential (CHIP), which has recently been reported to be associated with poor outcomes [13], was not evaluated. Notably, a subset of patients experienced poor outcomes despite low peripheral blood VAF, which could indicate an underestimation of clonal burden in bone marrow or tissue compartments, temporal changes in VAF not captured by a single baseline measurement, *UBA1*-variant-specific biological effects, and/or the contribution of comorbidities and broader immune dysregulation, such as the presence of CHIP and myelodysplastic syndrome (MDS)-related somatic mutations (eg *TET2*, *DNMT3A*, *ASXL1*, *SRSF2*). Longitudinal and compartment-specific VAF assessments together with multigene sequencing may help refine prognostic models.

Third, as our patients were recruited exclusively from Japan, differences in genetic background, symptom-driven referral patterns in settings where *UBA1* genetic screening is not currently approved, and country-specific clinical practices, including limited access to ruxolitinib and azacitidine for patients with VS, might influence patient outcomes; hence, external validation in prospective, multiethnic international cohorts is warranted.

Finally, the follow-up period was significantly shorter in the VS-like group, which may have biased survival comparisons. Moreover, the cause-of-death information was incomplete for some patients with VS like, further limiting the prognosis interpretation in this group. However, our findings underscore the need for dedicated studies on patients with VS-like with larger cohorts and longer, more uniform follow-up periods to clarify the underlying aetiologies and outcomes.

In conclusion, the baseline peripheral blood *UBA1* VAF was an independent predictor of 1-year death or transfusion dependence in VS, with replication in a separate cohort. VAF is a pragmatic biomarker for risk stratification, may inform patient selection for trials, and may serve as a pharmacodynamic readout for clone-directed therapies.

Competing interests

YK reports speaking fees from Amgen and Novartis, grants from Amgen, and advisory boards and consultation fees from Sobi and Novartis outside the submitted work. KI reports grants from Mitsubishi-Tanabe and Eli Lilly, and speaker honoraria from AbbVie, Eli Lilly, Eisai, Gilead, AstraZeneca, Asahi Kasei, Pfizer, Mitsubishi-Tanabe, Janssen, Novartis, UCB, Daiichi-Sankyo, Chugai, and Bristol-Myers Squibb outside the submitted work. DK has received lecture fees from AbbVie GK, Asahi Kasei Pharma Corp., Astellas Pharma Inc., Bristol-Myers Squibb Company, Chugai Pharmaceutical Co., Ltd., Eisai Co., Ltd., Eli Lilly

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Patient consent for publication

Not applicable.

Ethics approval

All patients provided written informed consent, and the study was approved by the YCU Certified Institutional Review Board (F240500053).

Provenance and peer review

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Data availability statement

Original data are available on request from the corresponding author.

Supplementary materials

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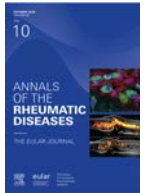
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Autoimmunity

Integrated transcriptomic and epigenomic profiling reveals conserved molecular subtypes across systemic autoimmune diseases

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ABSTRACT

Objectives: Rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and primary Sjögren's syndrome (pSS) frequently share overlapping clinical features, yet exhibit substantial intradisease heterogeneity in organ involvement and severity. This study aimed to define conserved molecular subtypes across these systemic autoimmune diseases (SADs) and delineate their transcriptional and epigenetic underpinnings.

Methods: We profiled 262 treatment-naïve Chinese Han patients (RA: n = 109; SLE: n = 69; pSS: n = 84), predominantly female (83.2%), who were divided into discovery (n = 119) and validation (n = 143) cohorts. Bulk RNA sequencing defined molecular subtypes, which were correlated with clinical phenotypes. Chromatin accessibility and super-enhancer landscapes were mapped by Assay for Transposase-Accessible Chromatin with high-throughput sequencing and cleavage under targets and tagmentation, and single-cell RNA sequencing (scRNA-seq) was performed to delineate subtype-specific cellular compositions.

Results: Integrative analyses uncovered 2 robust and reproducible molecular subtypes, megakaryocyte-enriched and B-cell-enriched, which were consistent across all 3 SADs and both cohorts. The megakaryocyte-enriched subtype was associated with higher platelet counts, greater disease activity, and broader organ involvement. Epigenomic profiling identified distinct chromatin accessibility and regulatory architectures, with megakaryocyte-associated genes (eg, *ZFP36L1* and *RAD51B*) linked to active superenhancers in the megakaryocyte-enriched subtype, and B-

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cell-associated genes (eg, *MAF* and *PRDM1*) in the B-cell-enriched subtype. scRNA-seq confirmed expansion of platelet-producing megakaryocytes and enhanced B-cell-activation signatures in their respective subtypes.

Conclusions: Integrative multiomics profiling defines 2 conserved molecular subtypes across RA, SLE, and pSS with distinct cellular and regulatory programmes. This cross-disease taxonomy may inform precision stratification and the development of lineage-targeted therapies in SADs. Further multicentre validation and investigation of the epigenetic mechanisms underlying molecular subtypes are warranted.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and primary Sjögren's syndrome (pSS) share overlapping clinical phenotypes, autoantibody profiles, and molecular signatures, suggesting a common immunopathogenic basis.
- Substantial intradisease heterogeneity within each disorder contributes to variable organ involvement and disease severity.

WHAT THIS STUDY ADDS

- We identified 2 conserved molecular subtypes, megakaryocyte-enriched and B-cell-enriched, across RA, SLE, and pSS.
- Each subtype is defined by distinct gene expression programmes, transcription factors, epigenetic regulators, and immune cell compositions.
- The megakaryocyte-enriched subtype is associated with multi-organ involvement and greater disease severity.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- This molecular classification provides a framework for immune stratification across systemic autoimmune diseases, with the potential to guide personalised therapy and inform the design of mechanism-based clinical trials.

INTRODUCTION

Rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS) are the most prevalent systemic autoimmune diseases (SADs), with estimated global prevalences of 0.21%, 0.043%, and 0.06%, respectively [1–3]. These disorders predominantly affect women, with female-to-male incidence ratios of 3:1 in RA, 9:1 in SLE, and 12:1 in pSS, and frequently co-occur within individuals and families [4–6]. Clinically, they exhibit overlapping manifestations, autoantibody repertoires, and immune-mediated tissue injury, frequently leading to diagnostic reclassifications during disease evolution [7]. Such similarities have supported the application of convergent therapeutic approaches, exemplified by B-cell-targeted agents such as CD20 inhibitors [8–10].

At the molecular level, accumulating evidence supports a shared immunogenetic foundation among these diseases [11–13]. Our prior genomic and transcriptomic studies revealed shared genetic variants in common immune pathways and highly similar gene expression profiles in peripheral blood mononuclear cells (PBMCs) from patients with RA, SLE, and pSS [14,15], suggesting a convergent immunological architecture underlying distinct clinical phenotypes and organ tropisms. However, how these cross-disease molecular signatures can be leveraged to improve patient stratification and guide precision therapy remains poorly understood.

Despite these molecular commonalities, substantial intradisease heterogeneity persists, reflected in variable clinical

courses, therapeutic responses, and long-term outcomes. Molecular subtyping has been described separately in RA, SLE, and pSS, but these disease-specific frameworks fail to capture the shared immune dysregulation across SADs [16–25]. Although integrative analyses of the transcriptome, methylome, and genome have identified 4 molecular classes (inflammatory, lymphoid, interferon [IFN], and undefined) across 7 SADs including RA, SLE, and pSS [26], the transcriptional–epigenetic regulatory relationships and their cellular determinants remain incompletely understood. Consequently, the clinical translation of these molecular classifications into personalised therapeutic strategies remains limited.

Epigenetic regulation, including transcription factors (TFs) and superenhancers (SEs), provides a critical layer of gene control and cellular identity, shaping the transcriptional landscape that drives immune phenotypes. Integrating epigenomic landscapes with transcriptomic data provides an opportunity to elucidate the regulatory circuitry underlying disease heterogeneity and molecular subtypes.

In this study, we performed integrated transcriptomic and epigenomic profiling of PBMCs from well-characterised cohorts of patients with RA, SLE, and pSS. We identified 2 conserved immune subtypes, each defined by distinct cellular compositions, chromatin accessibility patterns, and TF networks. This cross-disease molecular taxonomy offers a framework for immune stratification and may inform the development of lineage-targeted therapeutic strategies across SADs.

METHODS

Participants and experimental procedures

Peripheral blood samples were collected from treatment-naïve patients with RA (n = 109), SLE (n = 69), and pSS (n = 84), along with healthy controls (HCs; n = 20). Patients were assigned to a discovery cohort (n = 119) and a validation cohort (n = 143). Detailed demographic and clinical information for all groups is summarized in [Supplementary Table S1](#). Bulk RNA sequencing (RNA-seq) was performed on PBMCs from all participants to define molecular subtypes. Representative samples were further profiled using cleavage under targets and tagmentation (CUT&Tag) and Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq) to characterise epigenetic regulation, whereas single-cell RNA sequencing (scRNA-seq) was performed to delineate the cellular origins and transcriptional programmes underlying each subtype. Clinical and serological data were collected in parallel, and key cellular findings were validated by flow cytometry. Detailed experimental procedures and computational pipelines are provided in the [Supplementary Materials](#). All participants provided written informed consent, and the study was approved by the Ethics Committee of Shantou Central Hospital (Approval No. [2022] KY-006). Patients and members of the public were

not involved in the design, conduct, reporting, or dissemination of this research.

Statistical analysis

All statistical analyses were performed using R software (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism (version 10.4.0; GraphPad Software, Boston, MA, USA). Continuous variables with a normal distribution are presented as the mean $\pm$ SD and were compared using unpaired Student's *t*-tests. Non-normally distributed variables are expressed as the median (interquartile range, IQR) and were analysed using the Mann-Whitney U test. Categorical variables were compared using Pearson's χ^2 test. All tests were 2-tailed, and *P* < .05 was considered statistically significant.

RESULTS

Two consensus molecular subtypes across diseases in the discovery cohort

The overall study design is summarized in Figure 1A. To elucidate shared and disease-specific immune transcriptomes across RA, SLE, and pSS, we examined gene expression patterns in the discovery cohort (RA: *n* = 48, SLE: *n* = 33, pSS: *n* = 38, HCs: *n* = 20). Differential expression analysis comparing each disease to HCs revealed a profound overlap of dysregulated genes across SADs (Supplementary Fig S1A,D). Crucially, functional enrichment of the commonly upregulated genes prominently highlighted platelet activation and blood coagulation (Supplementary Fig S1B,C). Apart from the expected enhancement of antibody-related signatures in SLE, cross-disease transcriptomic variation dominated the overall landscape, suggesting that molecular variation was driven by intrinsic immune states rather than by diagnostic category (Supplementary Figs S2–S5).

Across diseases, 2 distinct molecular subtypes emerged (Fig 1B, Supplementary Fig S6). These subtypes, designated C1 (*n* = 59) and C2 (*n* = 60), comprised balanced proportions of RA, SLE, and pSS samples, indicating that molecular identity transcends clinical diagnosis. In the principal component analysis (PCA) plot, C2 samples clustered closer to HCs, whereas C1 represented a more divergent and activated immune state, suggesting a gradient of deviation from health (Fig 1C,D).

The 2 subtypes were characterised by distinct transcriptional programmes. C1 highly expressed platelet-related genes (*PPBP*, *GP9*, *PF4*, *TREML1*, and *CXCL5*), whereas C2 was enriched for immunoglobulin (IgG) genes (*IGHV* and *IGKV*) (Fig 1E). Functional enrichment analysis corroborated these transcriptional contrasts, with C1 enriched for wound healing, haemostasis, coagulation, and platelet activation, whereas C2 was enriched for IgG production and adaptive immune effector processes (Fig 1F,G).

TF activity analysis reinforced this dichotomy. C1 exhibited a megakaryocyte (MK)- and platelet-enriched profile with strong activation of lineage regulators such as *GATA1*, *MEIS1*, *PITX1*, and *NFIB*, alongside *GFI1B*, *KLF1*, *TAL1*, and *ZFPM2*, which are core TFs driving MK differentiation. This programme indicated enhanced platelet lineage engagement and potential platelet-driven immunopathology. In contrast, C2 showed upregulation of *PRDM1*, *REL*, and *STAT1*, consistent with B-cell/plasma cell activation and antibody production (Fig 1H).

Cell-type deconvolution aligned with these transcriptional signatures. C1 showed enrichment for MK/platelet signatures,

whereas C2 was enriched in plasma cells (Fig 1I,J). Independent validation using Web-based Cell-type Specific Enrichment Analysis (WebCSEA) confirmed that C1-upregulated genes were mapped to MKs and platelets, whereas C2-upregulated genes were linked to plasma cells and B cells (Fig 1K,L).

Collectively, these results define 2 consensus molecular subtypes across SADs: C1, driven by MK/platelet transcriptional programmes, and C2, characterised by B-cell/plasma cell-associated immune signatures.

Molecular subtypes are reproducible in an independent validation cohort

The reproducibility of both molecular subtypes was confirmed in an independent validation cohort comprising 143 patients (RA: *n* = 61; SLE: *n* = 36; pSS: *n* = 46), with 76 assigned to C1 and 67 to C2 (Fig 2A,B; Supplementary Figs S7 and S8). Both the shared molecular features and subtle disease-specific distinctions observed in the discovery cohort were recapitulated (Supplementary Figs S9–S12).

The transcriptional landscapes of these subtypes in the validation cohort closely mirrored those observed in the discovery cohort. C1 exhibited dominant enrichment of wound healing, haemostasis, coagulation, and platelet activation pathways (Fig 2C), alongside prominent MK- and platelet-associated signatures (Fig 2E), defining a MK/platelet-driven inflammatory programme. In contrast, C2 was characterised by enrichment in IgG complex assembly, IgG biosynthesis, and immune mediator production (Fig 2D), with strong B cell and plasma cell signatures (Fig 2F), reflecting a B-cell-enriched humoral immune axis.

When both cohorts were integrated (Fig 2G), C1 encompassed 135 samples (64 RA, 33 SLE, and 38 pSS), and C2 encompassed 127 samples (45 RA, 36 SLE, and 46 pSS). Both subtypes contained balanced proportions of the 3 autoimmune diseases, underscoring that these transcriptional states transcend clinical diagnoses and instead represent shared, cross-disease immune programmes—one driven by MK/platelet activity and the other by B-cell/plasma cell responses.

To distinguish between the 2 subtypes, we used 5 genes with high expression in the C1 subtype (*PPBP*, *GP9*, *PF4*, *TREML1*, and *CXCL5*). Logistic regression models based on these genes demonstrated robust performance in both cohorts, achieving an area under the receiver operating characteristic curve (AUC) > 0.8 (Supplementary Figs S13 and S14).

Distinct immune programmes and clinical phenotypes characterise the 2 subtypes

The 2 molecular subtypes exhibited divergent immune signatures and clinical characteristics. For immune feature scoring, 13 predefined immune signatures derived from a previous study were used (gene sets are listed in Supplementary Table S2). C1 displayed strong activation of C–C motif chemokine receptor (CCR) signalling and type II interferon (IFN) responses, consistent with its platelet- and MK-driven inflammatory profile. In contrast, C2 was enriched for type I IFN signalling, major histocompatibility complex I expression, and signatures of antigen-presenting cell and T cell coinhibition, accompanied by enhanced cytolytic activity (Fig 3A). This constellation reflects a B-cell-enriched immune state characterised by sustained activation, augmented antigen presentation, and compensatory regulatory or exhaustion features.

Clinically, within the validation cohort, patients classified as C1 showed higher baseline disease activity across diseases, including

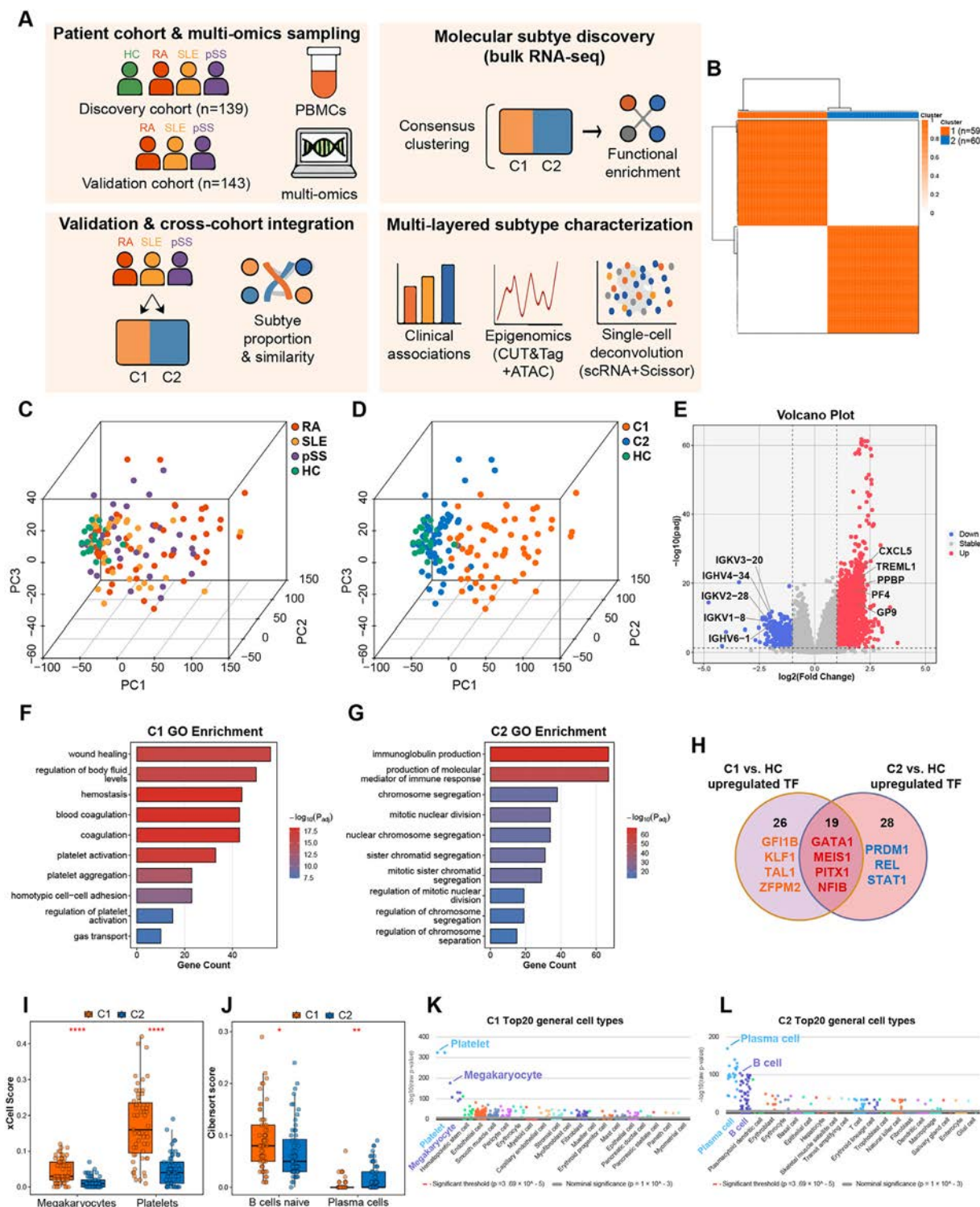


Figure 1. Study design and molecular subtype identification. (A) Schematic overview of the study design. (B) Consensus clustering heatmap dividing systemic autoimmune disease (SAD) samples into 2 subgroups. (C,D) Three-dimensional PCA plot illustrating the relationships among patients with SADs and HCs, respectively coloured by clinical diagnosis (C) and molecular subtype (D), respectively. (E) Volcano plot of differentially expressed genes (DEGs) between C1 and C2. (F) GO enrichment bar plot of DEGs upregulated in C1. (G) GO enrichment bar plot of DEGs upregulated in C2. (H) Venn diagram showing transcription factors (TFs) uniquely or commonly upregulated in C1 and C2 compared with HCs. (I) Box plots showing proportions of megakaryocytes and platelets in C1 and C2, as inferred by the xCell algorithm. (J) Box plots showing proportions of naïve B cells and plasma cells in C1 and C2, as inferred by the CIBERSORT algorithm. (K) Cell-type annotations for C1-upregulated DEGs, inferred by the WebCSEA algorithm. (L) Cell-type annotations for C2-upregulated DEGs, inferred by the WebCSEA algorithm. ATAC-seq, Assay for Transposase-Accessible Chromatin with high-throughput sequencing; CIBERSORT, Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts; CUT&Tag, cleavage under targets and tagmentation; GO, gene ontology; HC, healthy control; PBMCs, peripheral blood mononuclear cells; PCA, principal component analysis; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; scRNA-seq, single-cell RNA sequencing; SLE, systemic lupus erythematosus; TF, transcription factor; WebCSEA, Web-based Cell-type Specific Enrichment Analysis.

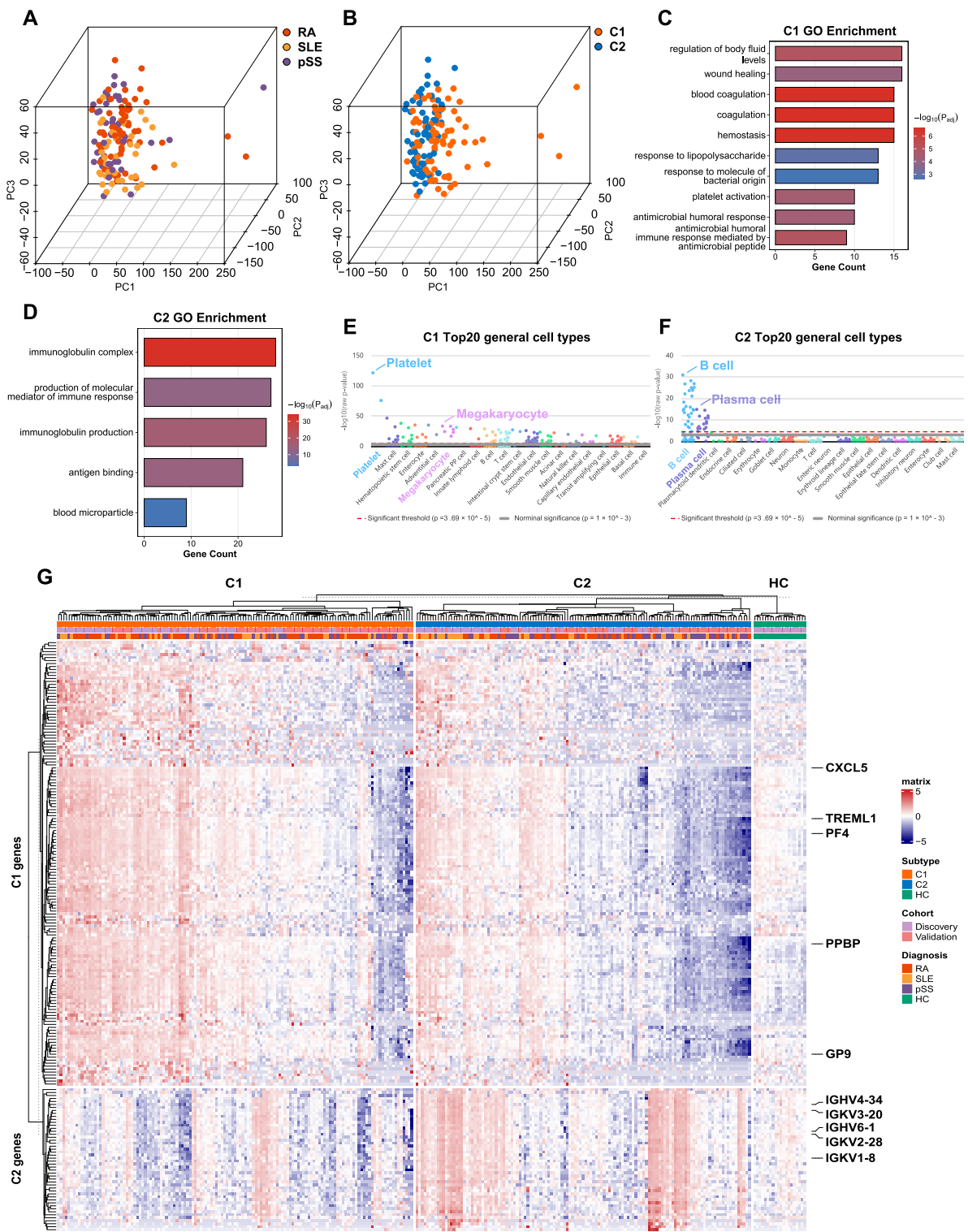


Figure 2. Independent validation cohort confirms 2 molecular subtypes with conserved transcriptomic features. (A,B) Three-dimensional PCA plots illustrating the relationships among patients with SADs, respectively coloured by clinical diagnosis (A) and molecular subtype (B), respectively. (C) GO enrichment bar plot of DEGs upregulated in C1 from the validation cohort. (D) GO enrichment bar plot of DEGs upregulated in C2 from the validation cohort. (E) Cell-type annotations for C1-upregulated DEGs, inferred by the WebCSEA algorithm in the validation cohort. (F) Cell-type annotations for C2-upregulated DEGs, inferred by the WebCSEA algorithm in the validation cohort. (G) Integrated heatmap landscape showing the distribution of C1 and C2 across the combined discovery and validation cohorts. DEG, differentially expressed gene; GO, gene ontology; HC, healthy control; PCA, principal component analysis; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; WebCSEA, Web-based Cell-type Specific Enrichment Analysis.

simple disease activity index in RA (Fig 3B), Systemic Lupus Erythematosus Disease Activity Index in SLE (Fig 3C), and EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) in pSS (Fig 3D). Conversely, analyses across the entire combined cohort demonstrated that serological profiling further distinguished the

subtypes: platelet counts (Fig 3E) and complement C3 levels (Fig 3F) were elevated in C1, whereas serum IgG concentrations were markedly higher in C2 (Fig 3G), aligning with its plasma cell/B-cell-enriched programme. Moreover, arthritis, endocrine system involvement, and multiorgan involvement were more

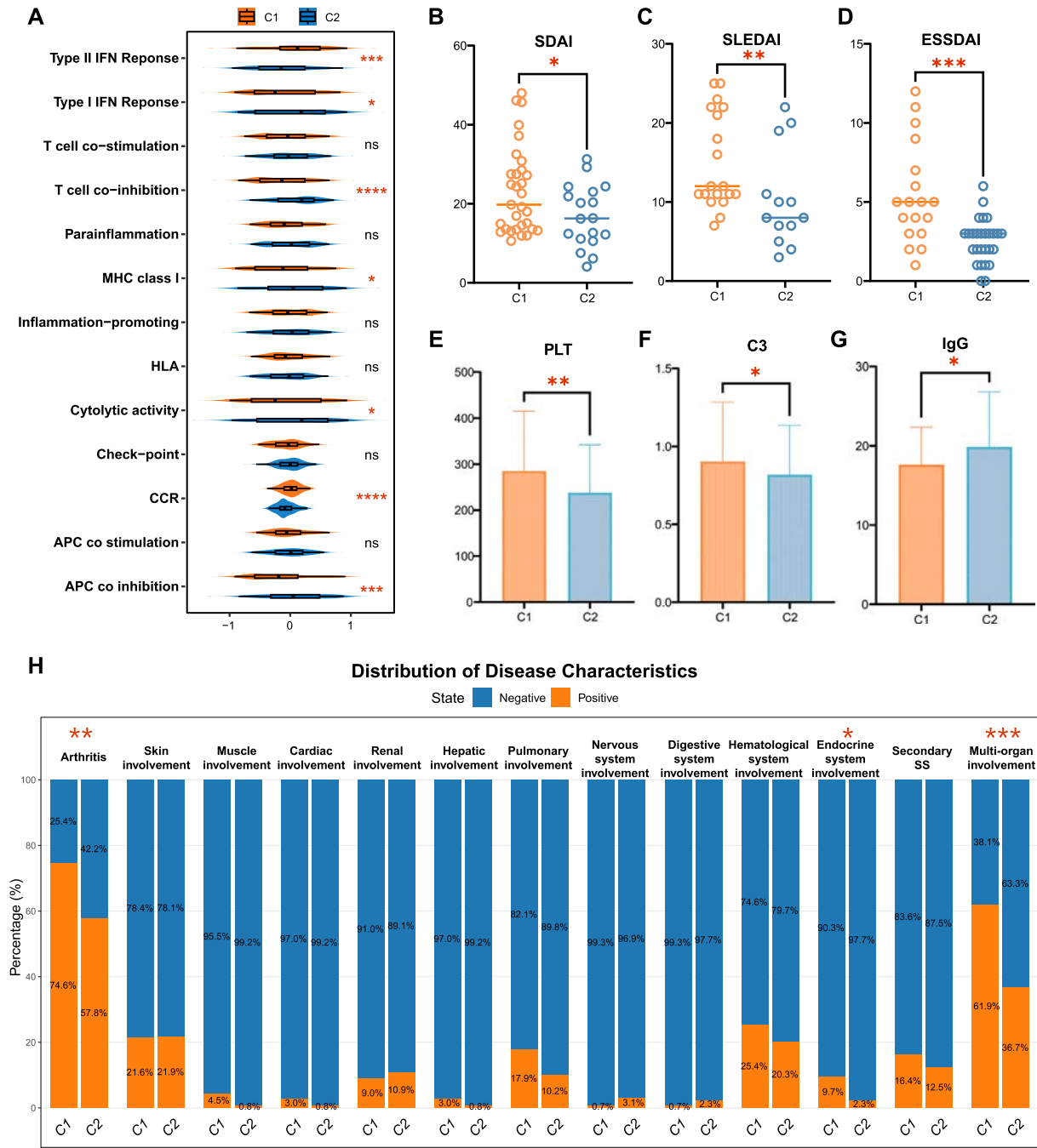


Figure 3. Molecular subtypes are associated with distinct clinical phenotypes and laboratory profiles. (A) Violin–box plots showing the distribution of 13 immune-related signatures between the C1 and C2 subtypes across all patients, as calculated by GSVA. (B,D) Dot plots comparing disease activity scores between C1 and C2 in the validation cohort for (B) RA (SDAI), (C) pSS (ESSDAI), and (D) SLE (SLEDAI). (E–G) Bar plots depicting differences in serological indicators between C1 and C2 across all patients, including (E) platelet (PLT) counts, (F) complement C3 levels, and (G) IgG concentrations. (H) Stacked bar chart illustrating the distribution of organ involvement across the 2 molecular subtypes in the combined cohort. CCR, C–C motif chemokine receptor; ESSDAI, EULAR Sjögren’s Syndrome Disease Activity Index; GSVA, gene set variation analysis; IFN, interferon; IgG, immunoglobulin G; MHC, major histocompatibility complex; APC, antigen-presenting cell; HLA, human leukocyte antigen; PLT, platelet; pSS, primary Sjögren’s syndrome; RA, rheumatoid arthritis; SDAI, Simple Disease Activity Index; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SS, Sjögren’s syndrome. * $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$.

frequent in C1 than in C2 across the full cohort (Fig 3H), underscoring its association with systemic inflammation and increased disease burden. Importantly, comprehensive baseline profiling confirmed that the distinct transcriptomic signatures between C1 and C2 were driven by intrinsic biological programmes rather than demographic or compositional confounders. The 2 subtypes were well matched in age ($P = .934$), sex distribution ($P = .350$), and immune cell compositions, including absolute lymphocyte

($P = .249$) and neutrophil ($P = .080$) counts (Supplementary Table S3). Aside from platelet counts, C3, and IgG, no other serological parameters differed significantly between C1 and C2 (Supplementary Fig S15). The proportions of C1 and C2 within each autoimmune disease (RA, SLE, and pSS) and the distribution of disease types within each subtype were generally comparable and stable across the discovery and validation cohorts (Supplementary Figs S16 and S17).

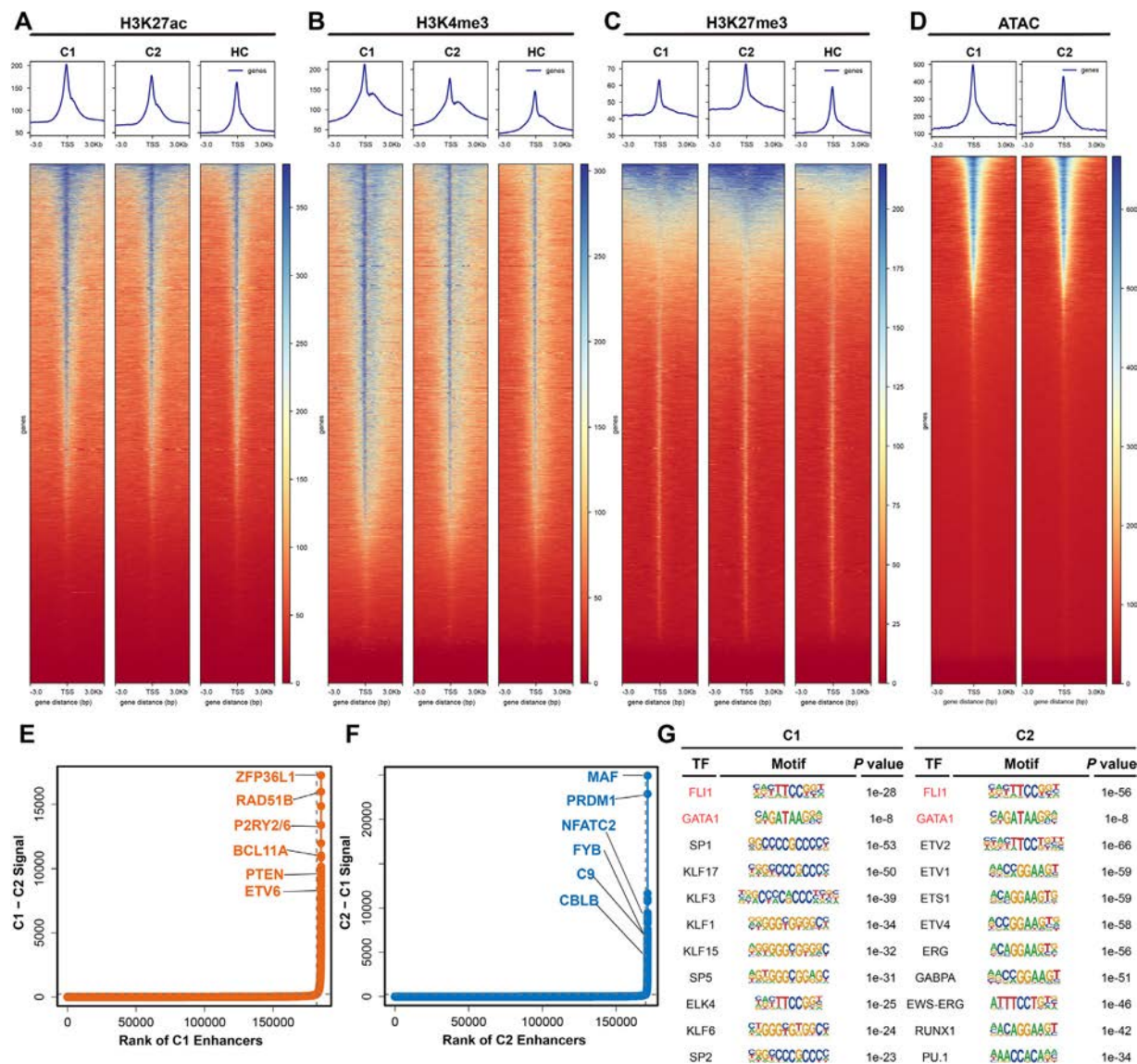


Figure 4. Epigenomic profiling reveals subtype-specific regulatory landscapes. (A) Heatmap of H3K27ac CUT&Tag signals at transcription start sites (TSS), with samples arranged from left to right as C1, C2, and HCs. (B) Heatmap of H3K4me3 CUT&Tag signals at TSS, with samples arranged from left to right as C1, C2, and HCs. (C) Heatmap of H3K27me3 CUT&Tag signals at TSS, with samples arranged from left to right as C1, C2, and HCs. (D) Heatmap of ATAC signals at TSS, with samples arranged from left to right as C1 and C2. In panels A–D, the top line plots represent the average signal intensities across all genes, highlighting the global epigenetic gradients among the subtypes, whereas the bottom heatmaps display the signal density at individual genomic loci, illustrating the broadness of the epigenetic alterations. (E) Ranked dot plot of superenhancers identified by ROSE analysis in C1. (F) Ranked dot plot of superenhancers identified by ROSE analysis in C2. (G) Enrichment of the top transcription factor binding motifs in the C1 and C2 subtypes. ATAC, Assay for Transposase-Accessible Chromatin; CUT&Tag, cleavage under targets and tagmentation; HC, healthy control; ROSE, rank ordering of superenhancers; TSS, transcription start site.

Epigenetic landscape profiling uncovers subtype-specific regulatory programmes

Chromatin profiling revealed distinct regulatory architectures between the 2 subtypes. Average signal profiles and density heatmaps centred around transcription start sites provided a global view of these epigenomic alterations. Active enhancer (H3K27ac) and promoter (H3K4me3) marks (profiled via CUT&Tag; n = 15 for C1, n = 15 for C2, and n = 10 for HCs) were globally increased in both subtypes compared with HCs. Notably, signal intensities and breadth exhibited a graded pattern of C1 > C2 > HCs (Fig 4A,B). Conversely, the repressive histone mark H3K27me3 (profiled via CUT&Tag; n = 15 for C1, n = 15 for C2, and n = 10 for HCs) exhibited a distinct pattern, with the highest enrichment observed in the C2 subtype (C2 > C1 > HCs), highlighting a prominently repressed epigenetic landscape

unique to C2 (Fig 4C). Furthermore, chromatin accessibility (assessed via ATAC-seq; n = 15 for C1, n = 13 for C2) aligned with the active histone modifications, reflecting more extensive epigenetic activation and open chromatin regions in C1 (Fig 4D). Subtype-specific SEs highlighted distinct regulatory networks (Fig 4E,F). C1-specific SEs were linked to genes involved in MK differentiation, haematopoiesis, and platelet activation—such as *ZFP36L1*, *RAD51B*, *P2RY2/6*, *BCL11A*, *PTEN*, and *ETV6*—highlighting an SE network centred on MK and platelet biology. In contrast, C2-specific SEs were enriched for key regulators of B cell activation and plasma cell differentiation, including *MAF*, *PRDM1*, *NFATC2*, *FYB*, *C9*, and *CBLB*, consistent with its B-cell/plasma cell-enriched immune axis. Motif enrichment analysis of H3K27ac peaks further supported this dichotomy (Fig 4G). C1-specific motifs (eg, *SP1*, *KLF17*, *KLF3*, and *KLF1*) suggested activation of transcriptional programmes

linked to myeloid differentiation, haematopoiesis, mitochondrial metabolism, and interferon signalling, aligning with its inflammatory and MK-driven immune signature. Conversely, C2-specific motifs (eg, *ETV2*, *ETV1*, *ETS1*, and *ETV4*) were associated with transcriptional circuits that influence immune cell differentiation and function, including roles in B-cell activation, aligning with its B-cell-enriched transcriptomic profile.

Single-cell transcriptome profiling validates subtype-defining cell populations

To trace the cellular origins underlying the 2 molecular subtypes, we integrated scRNA-seq data from PBMCs of an independent cohort comprising 10 treatment-naïve patients with autoimmune diseases (3 RA, 3 SLE, and 4 pSS) and 3 matched HCs. Following strict quality control, this yielded a total of 115,848 high-quality cells. Using the Scissor algorithm, we projected bulk RNA-seq-defined subtypes onto single-cell resolution (Fig 5A), thereby linking transcriptomic subtypes to their cellular drivers. Based on canonical marker genes (Supplementary Fig S18), 9 major immune cell populations were identified, including natural killer (NK) cells, CD8⁺ T cells (CD8⁺ T), CD4⁺ T cells (CD4⁺ T), B cells (B), MKs, low-density granulocytes (LDGs), CD14⁺ monocytes (CD14⁺ Mono), CD16⁺ monocytes (CD16⁺ Mono), and dendritic cells (Fig 5B). Co-clustering analysis further confirmed that patients and HCs shared the canonical major immune cell populations (Supplementary Fig S19).

C1 and C2 exhibited distinct cellular composition profiles (Fig 5C,D). Consistent with the differential gene expression from bulk RNA-seq and cell-type deconvolution analyses, the C1 subtype was characterised by expansion of the pro-inflammatory myeloid compartment, including MKs, LDGs, and CD16⁺ monocytes, indicative of an innate and prothrombotic immune state. In contrast, C2 was enriched for the lymphoid compartment, including B cells, NK cells, and both CD8⁺ and CD4⁺ T cells.

Analysis of transcriptional activity patterns revealed divergent regulatory hierarchies. C1-MKs showed high activity of key haematopoietic regulators (*GATA1*, *KLF1*, *MAFB*, and *NFE2*), consistent with a MK-erythroid inflammatory axis. In contrast, C2-B cells exhibited strong activation of *IRF9*, *IRF3*, *EBF1*, and *BATF*, reflecting B-cell maturation, interferon signalling, and adaptive immune activation (Fig 5E,F).

To further validate the presence and expansion of MK-like cells in the C1 subtype, we performed flow cytometric analysis of PBMCs from 8 C1 patients and 10 HCs using CD41b, CD42b, and CD61 as MK markers, with the representative gating strategy shown in Figure 6A. The proportions of CD41b⁺CD42b⁺ and CD41b⁺CD61⁺ cells were significantly increased in the C1 group compared with HCs (Fig. 6B), and the frequencies of CD19⁺ B cells and CD19⁺CD38⁺ plasma cells were significantly higher in the C2 subtype than in HCs (Fig. 6C). Collectively, these findings translate our transcriptomic signatures into phenotypic evidence, providing orthogonal validation at the protein level for the 2 molecular subtypes. These results support that MK activation and expansion characterise the C1 subtype, whereas the activation of the B cell/plasma cell lineage defines the C2 subtype.

DISCUSSION

Clinical overlap among RA, SLE, and pSS is well recognised, leading to frequent use of similar immunomodulatory regimens, yet they exhibit marked variability in organ involvement and disease severity. This highlights the need for molecular

stratification that transcends traditional diagnostic boundaries. Through integrative multi-omic molecular classification across RA, SLE, and pSS, we identified 2 reproducible transcriptomic subtypes across RA, SLE, and pSS—a MK-enriched subtype and a B-cell-enriched subtype—each reflecting a divergent immune state. Unlike the study by Barturen et al [26], which classified patients with SAD into 4 subtypes, our study identified 2 major subtypes. The B-cell-enriched subtype partially resembles the fourth cluster reported by Barturen et al [26], being closer to HCs and associated with lower disease activity. In addition, the B-cell-enriched subtype is characterised by adaptive immune activation, consistent with the previously described ‘lymphoid’ cluster. In contrast, the MK-enriched subtype appears to encompass features of both the ‘inflammatory’ and ‘interferon’ clusters described by Barturen et al [26], and is characterised by predominance of innate immune activation.

Integrative epigenomic and transcriptomic analyses demonstrated that these subtypes are governed by distinct enhancer architectures and TF networks. The MK-enriched subtype is enriched for SEs linked to *ZFP36L1* and *RAD51B*, core regulators of megakaryopoiesis and thrombopoiesis, whereas the B-cell-enriched subtype features activation of *MAF* and *PRDM1*, key mediators of B-cell differentiation and plasma cell function. Single-cell transcriptomic profiling confirmed the expansion of platelet-producing MKs and innate immune cells (LDGs and CD16⁺ monocytes) in the MK-enriched subtype. In contrast, the B-cell-enriched subtype was characterised by increased activated B cells and plasma cells, along with enrichment of adaptive immune populations, including NK cells and both CD8⁺ and CD4⁺ T cells. Collectively, these lineage-specific regulatory circuits and cellular compositions provide mechanistic insights into the cellular origins of autoimmune heterogeneity. Our findings extend prior cross-disease molecular classifications by integrating transcriptomic, epigenomic, and single-cell data with clinical phenotypes [26], thereby defining mechanistically grounded immune subtypes that may inform precision therapeutic approaches. Notably, this classification may also explain the clinical presentations of thrombocytosis and thrombocytopenia observed in treatment-naïve patients with RA, SLE, and pSS [27].

The MK-enriched subtype exhibits an expansion of platelet-producing MKs, heightened inflammatory activity, and more severe multisystem involvement. In SLE, such multisystem involvement is frequently associated with thrombosis, one of the leading causes of mortality [28]. Currently, evidence-based antithrombotic guidance for high-risk patients is lacking. Our findings, which mechanistically link the MK-enriched subtype to increased platelet production and a prothrombotic state, provide a molecular basis for stratifying thrombotic risk and pave the way for developing subtype-specific antithrombotic strategies. These results are consistent with prior observations of platelet activation, MK expansion, and elevated platelet counts correlating with disease activity in SLE and RA [29–34]. Thus, MK dysregulation may serve not only as a pathogenic driver but also as a dynamic biomarker of disease activity.

Given the emerging recognition that platelets actively regulate immune and inflammatory processes, platelet-targeted strategies may have broad implications for SADs [30,35]. The therapeutic continuum spanning anti-platelet to anti-MK approaches highlights new avenues to modulate inflammatory cascades. The MK lineage therefore represents a mechanistically central effector population amenable to next-generation cell-based therapeutic interventions. In contrast, the B-cell-enriched subtype is characterised by plasma cell expansion,

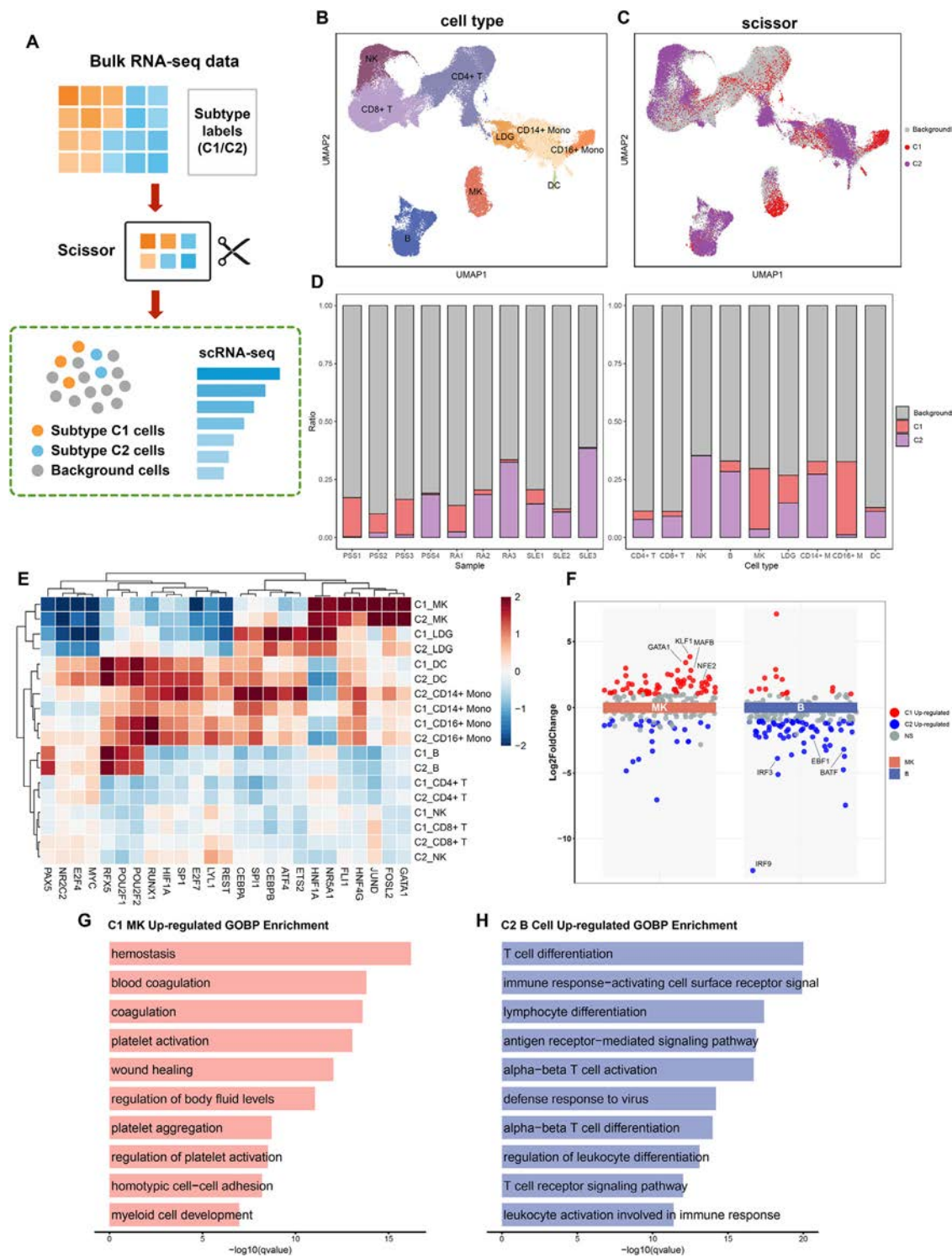


Figure 5. Single-cell RNA sequencing (scRNA-seq) confirms subtype-specific immune populations and regulatory programmes. (A) Schematic overview of the Scissor algorithm integrating bulk RNA-seq with scRNA-seq data. (B) UMAP plot showing 9 major immune cell types identified from the scRNA-seq data. (C) UMAP plot highlighting C1- and C2-associated cells inferred by Scissor. (D) Bar plots showing the proportions of C1- and C2-associated cells across patients and cell types. (E) Heatmap showing the top 25-ranked transcription factors across cell types in the 2 subtypes. (F) Volcano plots of differentially active TFs in MKs (C1) and B cells (C2). (G) GO biological process enrichment of C1-MK-specific genes. (H) GO biological process enrichment of C2-B cell-specific genes. GO, gene ontology; LDGs, low-density granulocytes; MK, megakaryocyte; TF, transcription factor; UMAP, uniform manifold approximation and projection.

type I interferon activation, and pronounced humoral immune responses. Patients within this subtype may be more responsive to B-cell-targeted therapies, such as anti-CD20 monoclonal antibodies, consistent with their enriched B-lineage activation profile.

Nevertheless, several limitations warrant consideration. First, the single-centre design may limit the representativeness of our

cohorts, and validation in independent, multicentre studies is required to establish the generalisability of these findings. Second, the immune subtyping was based on transcriptomic data from a single time point, lacking longitudinal sampling to assess subtype dynamics following treatment. Therefore, future longitudinal studies are needed to elucidate the temporal evolution of these immune subtypes and to determine their potential

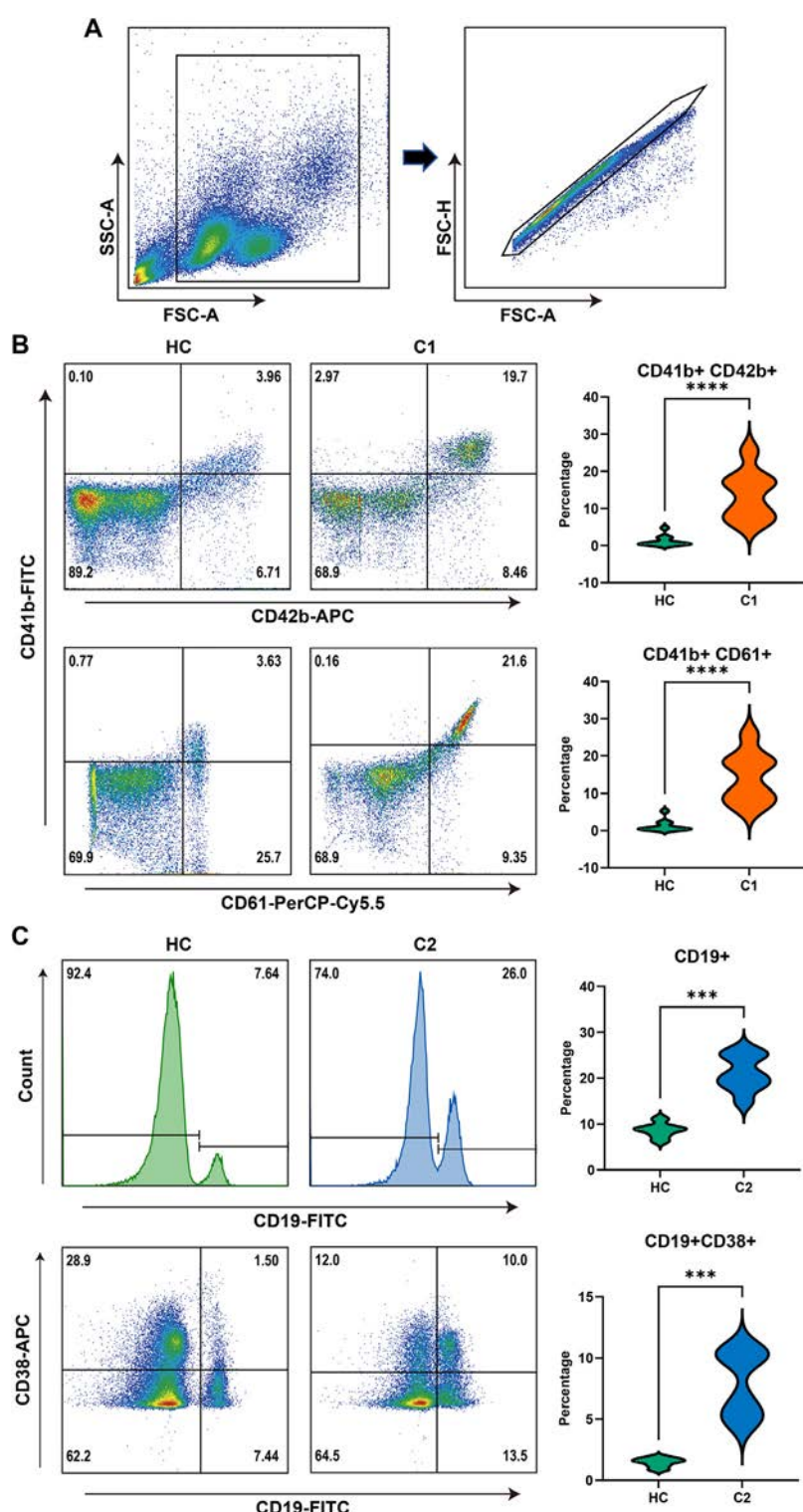


Figure 6. Flow cytometric validation of subtype-specific cellular expansions. (A) Representative gating strategy used for flow cytometric analysis of PBMCs. (B) Validation of the megakaryocyte-enriched C1 subtype. Left panels show representative flow cytometry plots for the identification of CD41b⁺CD42b⁺ cells (top) and CD41b⁺CD61⁺ cells (bottom) in patients with HCs and C1. Right panels display violin plots of the statistical quantification, confirming the significant expansion of these megakaryocyte-like populations in the C1 subtype (n = 8) compared with HCs (n = 10). (C) Validation of the B-cell-enriched C2 subtype. Left panels show representative flow cytometry plots identifying total CD19⁺ B cells (top) and activated CD19⁺CD38⁺ plasma cells/plasmablasts (bottom) in patients with HCs and C2. Right panels present violin plots of the statistical quantification, demonstrating the significant enrichment of these B-lineage populations in the C2 subtype (n = 8) compared with HCs (n = 6). APC, allophycocyanin; FITC, fluorescein isothiocyanate; FSC, forward scatter; HC, healthy control; PBMC, peripheral blood mononuclear cell; SSC, side scatter. *** $P < .001$; **** $P < .0001$.

utility in predicting treatment responses. Third, the epigenetic regulatory mechanisms underlying these transcriptional subtypes remain incompletely understood; in particular, integrative analyses focusing on specific cellular subsets and their associated epigenetic elements—including SEs, TFs, and chromatin accessibility networks—are needed to clarify their mechanistic basis.

CONCLUSION

This taxonomy, reproduced in an independent validation cohort, highlights shared molecular programmes across RA,

SLE, and pSS, supporting a cross-disease framework for patient stratification. Clinically, it suggests that the assessment of disease activity may benefit from integrating molecular subtype information, rather than relying solely on conventional markers such as autoantibody levels and B-cell activation. In particular, features associated with the MK-enriched subtype, including MK abundance and platelet counts, may provide complementary metrics for disease evaluation.

From a prognostic perspective, the MK-enriched subtype appears to be associated with a higher frequency of multiorgan involvement, indicating its potential utility in risk stratification and monitoring. These findings raise the possibility

that molecular classification could inform more tailored surveillance strategies and, potentially, therapeutic decision-making. However, further longitudinal validation in independent, prospective, multicentre cohorts is required to establish the stability of these subtypes prior to their clinical implementation. In addition, the epigenetic mechanisms in MKs and B cells that underlie these molecular subtypes remain to be elucidated.

Competing interests

The authors declare that they have no competing interests.

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Contributors

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. YW and GZ had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conceptualisation was performed by YW and GZ. Methodology design was developed by YF, GZ, and YW. Patient recruitment and evaluation was conducted by YF, MS, LY, SC, KZ, JL, QL, WZ, SYZ, ZZ, SHZ, and XY. Investigation was conducted by YF, MS, and SC. Data analysis was performed by YF and MS. Supervision was provided by YW and GZ. Writing-original draft was performed by YF and GZ. Writing-revision and editing was performed by MM-C, DEF, YW, and GZ.

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Patient consent for publication

Not applicable.

Ethics approval

All samples were collected in accordance with the ethical requirements and regulations of the Ethics Committee of Shantou Central Hospital. Informed consent was obtained from all the subjects, and the studies were conducted under approval (approval number: [2022] KY-006).

Provenance and peer review

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Data availability statement

All sequencing data have been or will be deposited in the public, open-access repository of the Genome Sequence Archive for Human (GSA-Human, <https://ngdc.cncb.ac.cn/gsa-human/>). The accession ID for the bulk RNA-seq data of the discovery cohort is HRA000911; the accession ID for the bulk RNA-seq data of the validation cohort is HRA017486; the accession IDs for the single-cell RNA-seq data are HRA000916 and

HRA010146; the accession ID for the ATAC-seq data is HRA017409; and the accession ID for the CUT&Tag dataset is HRA017687. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

Code availability

This study did not generate any custom code. All computational tools and software packages used in the analyses are publicly available and described in the Methods section.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that generative artificial intelligence (AI) was used in the preparation of this manuscript. Specifically, OpenAI ChatGPT (version 5.0) was employed to assist in improving the clarity and grammar of the English text. All scientific content, analyses, and conclusions were conceived, generated, and verified solely by the authors.

Supplementary materials

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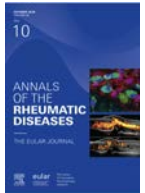
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Epidemiology

Referral pathways and duration of care for musculoskeletal complaints across Europe: an analysis of primary and secondary care

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ABSTRACT

Objectives: The objective of the study is to compare care pathways of patients with musculoskeletal (MSK) complaints—from first presentation in primary care to referral and follow-up in rheumatology—across 5 European healthcare systems, and to explore how healthcare system characteristics influence these pathways.

Methods: Routinely collected healthcare data from 5 European countries (UK, Sweden, the Netherlands, Spain, and Hungary) were analysed. Primary care data included > 4 million adults with at least 1 MSK encounter, and secondary rheumatology data included > 600,000 patients. Primary care consultation rates, referral rates to specialist care, and rheumatology follow-up duration were compared across countries. Healthcare characteristics were extracted from the literature.

Results: The distribution of MSK complaints in primary care was similar across countries, with 25% to 30% of residents visiting their general practitioner annually for an MSK complaint (UK data not available). In contrast, referral of such patients with MSK complaints to rheumatology varied considerably (5%–38%). Among those referred, 46% to 70% were discharged within 3 months, whereas only 13% to 43% remained in long-term rheumatology care. We observed that countries with a high proportion of sustained long-term rheumatology care (UK and Sweden) had lower referral rates, fewer rheumatologists per capita, and employed a selective triaging system, whereas countries where referrals more frequently resulted in short-term rheumatology care (the Netherlands, Spain, and Hungary) had higher referral rates and employed nonselective triaging.

Conclusions: Substantial cross-country variation exists in the management of MSK complaints. Although primary care MSK presentations are similar, referral rates and the patient-mix reaching rheumatology differ widely between countries—an important consideration for developing cross-national clinical guidelines and diagnostic tools.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Care for musculoskeletal (MSK) conditions faces persistent challenges: diagnostic delays, frequent referrals to rheumatology for non-inflammatory rheumatic diseases, and a shortage of rheumatologists.
- Healthcare organisation varies substantially across countries, whereas guidelines and referral tools are largely uniform, limiting their cross-national applicability.

WHAT THIS STUDY ADDS

- Unlike previous research that examined diagnostic trajectories for single diseases (eg, rheumatoid arthritis and axial spondylarthritis) within individual healthcare systems, this study is the first to analyse the full diagnostic trajectory of MSK complaints across 5 European healthcare systems, spanning both primary and secondary care.
- The distribution of MSK presentations in primary care is consistent across countries, with 25% to 30% of adults consulting their general practitioner annually for MSK symptoms.
- Referral rates to rheumatology vary considerably between countries (5%–38%). Countries with low referral rates were observed to have selective triaging, a lower number of rheumatologists per capita, and a higher proportion of referrals resulting in sustained long-term rheumatology care as compared to countries with high referral rates.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Gives insights into MSK pathways and their relation to the organisation of care across different European countries and provides helpful context to optimise diagnostic care pathways.
- Understanding country-specific differences in the patient case-mix seen in rheumatology clinics helps interpret research findings across Europe.
- The observed differences in MSK care pathways across Europe provide context for the development and implementation of cross-national clinical guidelines and diagnostic tools.

INTRODUCTION

Rheumatic and musculoskeletal (MSK) diseases are the leading cause of years lived with disability worldwide [1], significantly impacting patients' quality of life [2]. They also represent the most common reason for primary care consultations across Europe [3,4]. Notwithstanding this burden, timely and efficient diagnosis remains challenging, often leading to delayed treatment initiation, the occurrence of otherwise preventable joint damage, and patients' loss in quality of life [5,6].

At the general practitioner (GP) level, longer-than-recommended delays in the diagnosis of conditions such as rheumatoid arthritis (RA) (6–12 months) [7–10], spondylarthritis (5–8 years) [11–15], and fibromyalgia (FM) (3–6 years) [16–19] remain. Because MSK complaints are highly prevalent [3,4], whereas immune-mediated rheumatic diseases (IRDs) are relatively rare [20–22], GPs face the challenge of weighing the risk of delayed referral for IRDs against the potential for over-referral to rheumatology. A similar balance is required within rheumatology—ensuring early access for patients with potential IRDs, while relying on GPs to manage conditions that can be safely diagnosed and treated elsewhere. This balance has become increasingly important given the widespread shortage of rheumatologists [23–25], emphasising the need to direct specialist resources towards patients for whom rheumatology care adds greatest value.

To improve patients' pathways through the healthcare system, it is essential to understand how patient populations differ across stages of the healthcare trajectory. Such differences can strongly influence the discriminatory value of clinical features for diagnostic purposes. For instance, both in Germany and the Netherlands, most patients referred to rheumatology for suspected axial spondylarthritis (axSpA) had inflammatory back pain—reflecting its use as a referral criterion by GPs—thereby reducing the diagnostic value of inflammatory back pain in the rheumatology clinic compared with primary care [26,27]. This

resulted in the adjustment of the Berlin algorithm for diagnosing axSpA in secondary care [28].

Beyond differences between primary and secondary care, substantial variation likely exists between countries, driven by structural and organisational differences in healthcare systems. Factors such as GP accessibility, the number of available GPs and specialists, triaging procedures, and referral pathways vary widely across Europe [29–31]. For instance, although some systems accept nearly all referrals to rheumatology (nonselective triaging), others implement strict screening criteria (selective triaging).

Understanding these structural and organisational differences between countries is relevant for the development and implementation of international guidelines and diagnostic tools. Whenever these contextual factors are not considered, the cross-national applicability of guidelines and tools is limited [32]. In addition, healthcare system characteristics determine the patient case-mix that ultimately reaches rheumatology, meaning that study populations may differ substantially between countries—potentially reducing the generalisability of clinical research findings. Despite their potential impact, the extent to which systemic healthcare characteristics shape the pathway of MSK complaints remains insufficiently understood. There is a lack of studies that examined the full care trajectory across the broad spectrum of MSK complaints, and particularly of those that compared this trajectory across several countries. Existing research on the MSK care pathway typically focuses on individual diseases such as RA [33–36] or axSpA [37–39], examines an isolated segment of care (either primary or secondary care), and is almost exclusively conducted within single countries.

This study examined MSK pathways and healthcare structures across Europe. Specifically, we aimed to: (i) quantify the occurrence of MSK complaints in primary care; (ii) assess referral patterns to secondary care, specifically rheumatology and orthopaedic surgery; (iii) determine the proportion of referrals to rheumatology that required sustained rheumatological care; and (iv) compare these patterns across multiple European countries with different healthcare system characteristics.

METHODS

Data Sources

Routinely collected primary and secondary (rheumatology) healthcare data were obtained from 6 regions across 5 European countries: the UK (Newcastle), Sweden (Stockholm), the

Netherlands (Leiden/The Hague and Rotterdam/Randstad), Spain (Madrid), and Hungary (Budapest). The Rotterdam/Randstad data were used to validate the Dutch primary care results obtained from Leiden (Supplementary Section S1). All datasets included adult patients (aged ≥ 18 years) with at least 1 recorded healthcare encounter during the study period, and spanned 5 to 12 years in the periods 2010 to 2024 (exact time spans are provided in Supplementary Section S2). Primary care data were obtained from all countries except the UK. Detailed primary care information was available for over 4 million patients who consulted their GP with an MSK complaint, and sites provided a broader selection representative of the total population to calculate population-level rates. Secondary care data were available from all sites except for Rotterdam and comprised over 600,000 patients attending rheumatology clinics. Secondary care rheumatology data were obtained from the same geographic regions as the primary care datasets; however, inclusion in the primary care dataset was not required for inclusion in the secondary care dataset, or vice versa.

In all participating countries, GPs act as gatekeepers, controlling patient access to specialist care. However, triaging procedures for rheumatology referrals differed across regions:

- Included hospitals in the Netherlands, Spain, and Hungary have nonselective triaging: almost all primary care referrals to rheumatology are seen in secondary care.
- Included hospitals in the UK and Sweden have selective triaging: referrals that are considered to concern nonimmune mediated diseases and can be managed through other means than a rheumatology visit are returned.

Primary care data in the Netherlands and Spain were coded using ICPC (International Classification of Primary Care), whereas Sweden and Hungary used ICD (International Classification of Disease)-10. Secondary care data across all included countries were coded using ICD-10. An overview of the included datasets and healthcare characteristics of their respective regions is provided in Table 1, with additional details in Supplementary Section S2.

Nine diagnostic categories were defined based on ICD-10 and ICPC codes: 2 symptom-based categories (arthralgia and dorsalgia), 6 diagnosis-based categories (IRDs, polymyalgia rheumatica [PMR], gout, osteoarthritis [OA], FM, and mechanical problems/bursitis/tenosynovitis), and a residual category of other MSK complaints. For primary care analyses, and where

Table 1
Overview of included datasets and healthcare system characteristics by region

Country	Region	GP acts as gatekeeper	Primary care coding	Number of primary care patients with MSK complaints	Triaging system	Secondary care coding	Number of secondary care rheumatology patients
United Kingdom	Newcastle	Yes			Selective	ICD-10	10,444
Sweden	Stockholm	Yes	ICD-10	1,037,452	Selective	ICD-10	59,745
	Leiden/The Hague	Yes	ICPC	423,241	Nonselective	ICD-10	25,222
The Netherlands	Rotterdam/Randstad ^a	Yes	ICPC	1,216,828	Nonselective		
Spain	Madrid	Yes	ICPC	162,493	Nonselective	ICD-10	14,850
Hungary	Budapest	Yes	ICD-10	1,321,458	Nonselective	ICD-10	497,055

GP, general practitioner; ICD-10, International Classification of Disease-10 coding; ICPC, International Classification of Primary Care coding; MSK, musculoskeletal. Study periods differed between sites and ranged from 5 to 12 years between 2010 and 2024 (Supplementary Section S2). Secondary care rheumatology data were obtained from the same geographic regions as the primary care datasets; however, inclusion in the primary care dataset was not required for inclusion in the secondary care dataset.

^a The Rotterdam/Randstad data was used as an independent validation dataset for the Dutch primary care findings obtained from Leiden/The Hague. No overlap existed between the GP practices contributing to the 2 datasets.

available, OA was subdivided into ‘knee/hip OA’ and ‘non-knee/hip OA’, whereas arthralgia was subdivided into ‘hand pain’, ‘foot pain’, and ‘multiple joint pain’.

In ICPD coding, MSK conditions were defined as all L-codes and T92 (gout). In ICD-10 coding, MSK complaints were defined as all M-codes. Detailed definitions for each diagnostic category are provided in [Supplementary Section S3](#).

Primary Care

Primary care data were analysed at the level of individual consultations (the Netherlands, Sweden, and Hungary) or episodes of care (Spain). In episodes of care, multiple consultations for the same condition are grouped into a single episode. Only consultations or episodes involving direct patient-GP contact (in person or remote) were included. For clarity, we refer to both consultations and episodes as *GP encounters* throughout this manuscript, unless explicitly stated otherwise. As most analyses presented are based on ratios, using consultations vs episodes were not expected to significantly affect the results. Nevertheless, to assess the potential influence of using consultations vs episodes of care, a sensitivity analysis using Dutch (Leiden) data—where both formats were available—was performed ([Supplementary Section S4](#)).

A referral was defined as a GP referral that resulted in a specialist consultation. In the Netherlands, Spain, and Hungary, referral information was explicitly recorded in primary care and linked to individual GP encounters. In Sweden, where information on referrals was not available to us, referrals were inferred based on rheumatology visits within 6 months of a GP encounter listing an MSK code. Additional referral indicators, such as referring clinic ID, were used to ascertain the referral.

Primary Care analyses

We quantified primary care activity via:

- 1. Overall GP encounter rate, defined as the total number of GP encounters (irrespective of condition) divided by the total number of patient-years in the data.
- 2. MSK encounter proportion, calculated as the total number of MSK encounters divided by the total number of encounters.
- 3. Annual proportion of patients with an MSK encounters, calculated by dividing the number of patients with at least 1 MSK encounter by the total GP population in that year.
- 4. Frequency of specific diagnostic categories, defined as the proportion of GP encounters for each diagnostic category among all MSK encounters.

Referral practices were described using:

- 1. Cumulative referral rate, defined as the percentage of patients who, among all patients with at least 1 MSK-related GP encounter, were referred to rheumatology at any point during follow-up.
- 2. Referral proportion by diagnostic category, defined as the proportion of GP encounters within each diagnostic category that resulted in a referral to rheumatology or orthopaedic surgery.
- 3. Distribution of diagnostic categories among referred encounters, defined as the proportion of all referred encounters attributable to each diagnostic category.

For the latter 2 analyses, each patient could contribute only 1 referral event per speciality (rheumatology or orthopaedic

surgery) during follow-up, and GP encounters from patients previously referred were excluded from the analyses. Patients remained eligible for referral to the other speciality (eg, rheumatology followed by orthopaedic surgery, or vice versa). Therefore, by construction, re-referrals to the same speciality after discharge were not considered in these analyses.

Secondary Care

Rheumatology care episodes were defined as the period between the first rheumatologist visit (‘New Patient’ visit) and the last available follow-up visit. Episodes were categorised by follow-up duration: short-term (≤ 3 months), intermediate (4–12 months), and long-term (> 12 months). We only included patients with at least 12 months of observation time.

The diagnostic category of each episode was determined using the latest ICD-10 code assigned in the episode. For each follow-up category, we reported the age- and biological sex-distribution, and the relative proportions of diagnostic categories. Episodes without an available diagnostic category were classified as ‘missing’. A sensitivity analysis using Leiden data was performed to compare alternative approaches for defining the diagnostic category of each rheumatology care episode (eg, using the first or most common code, applying a hierarchy of codes) ([Supplementary Section S4](#)).

Healthcare Organisation

To contextualise country-level differences in healthcare organisation, we reviewed the literature for the number of GPs and rheumatologists per 100,000 inhabitants in each participating country.

Patient and public involvement statement

This work was discussed at multiple SPIDeRR (Stratification of Patients using advanced Integrative modelling of Data Routinely acquired for diagnosing Rheumatic complaints) consortium meetings in which several patient research partners as well as patient-organisation ReumaNederland participated.

Table 2
GP consultation rates across regions

	Sweden Stockholm	The Netherlands Leiden	Spain Madrid	Hungary Budapest
Mean number of GP consultations per person per year (all complaints)	2.2	5.9	NA ^a	4.8
Proportion of GP consultations for MSK complaints	11.0%	14.0%	NA ^a	22.5%
Proportion of population with ≥ 1 MSK-related complaint in a year	24.5%	30.0%	NA ^a	29.1%

GP, general practitioner; MSK, musculoskeletal; NA, not available.
Average number of consultations per year at the GP, the proportion of GP visits related to MSK complaints, and the proportion of the total population who visit the GP at least once per year with an MSK complaint, averaged over the available time spans for each region.
^a For Spain, population-level estimates were unavailable due to the absence of data representative of the general population.

Table 3
Frequency of diagnostic categories in primary care MSK encounters

			Sweden Stockholm	The Netherlands Leiden	Spain Madrid	Hungary Budapest
All MSK conditions			100%: N = 4,723,846 (F: 61%, Age: 57)	100%: N = 5,080,932 (F: 61%, Age: 56)	100%: N = 324,332 (F: 62%, Age: 58)	100%: N = 28,059,739 (F: 58%, Age: 65)
Symptom-based categories	Arthralgia	All arthralgia	36.5% (F: 60%, Age: 53)	33.4% (F: 62%, Age: 56)	37.3% (F: 64%, Age: 59)	11.2% (F: 62%, Age: 63)
		Hand/finger pain		2.8% (F: 63%, Age: 53)	2.8% (F: 63%, Age: 57)	
		Foot pain	NA ^a	4.5% (F: 63%, Age: 55)	6.7% (F: 65%, Age: 58)	NA ^a
		Multiple joints pain		1.5% (F: 77%, Age: 53)	1.4% (F: 76%, Age: 59)	
Diagnosis-based categories	Dorsalgia		21.4% (F: 59%, Age: 52)	21.4% (F: 60%, Age: 54)	31.6% (F: 63%, Age: 56)	27.0% (F: 59%, Age: 61)
	IRDs		2.8% (F: 68%, Age: 65)	1.0% (F: 73%, Age: 62)	1.0% (F: 68%, Age: 63)	6.7% (F: 66%, Age: 63)
	PMR		2.5% (F: 65%, Age: 76)	0.4% (F: 64%, Age: 75)	NA	0.1% (F: 71%, Age: 74)
	Gout		2.2% (F: 23%, Age: 71)	2.3% (F: 25%, Age: 65)	0.6% (F: 18%, Age: 68)	15% (F: 50%, Age: 70)
	OA	Hip & Knee OA	5.7% (F: 66%, Age: 71)	4.0% (F: 71%, Age: 70)	1.7% (F: 72%, Age: 76)	13.8% (F: 64%, Age: 69)
		Other OA	3.1% (F: 72%, Age: 70)	1.9% (F: 74%, Age: 67)	2.6% (F: 76%, Age: 70)	17.7% (F: 66%, Age: 68)
	FM		2.1% (F: 97%, Age: 54)	0.4% (F: 94%, Age: 49)	0.1% (F: 95%, Age: 56)	0%
	Mechanical Problems/ Bursitis/ Tenosynovitis		19.3% (F: 60%, Age: 56)	25.1% (F: 59%, Age: 54)	16.5% (F: 66%, Age: 59)	13.6% (F: 61%, Age: 59)
Other MSK complaints			4.3% (F: 76%, Age: 70)	10.2% (F: 61%, Age: 56)	8.5% (F: 67%, Age: 63)	21% (F: 76%, Age: 72)

F, female; FM, fibromyalgia; GP, general practitioner; IRDs, immune-mediated rheumatic diseases; MSK, musculoskeletal; NA, not available; OA, osteoarthritis; PMR, polymyalgia rheumatica.

Proportion of GP encounters for each diagnostic category among all MSK GP encounters, along with average patient age and sex distribution (% female) across the regions.

Bold rates indicate high similarity between countries, based on row-wise normalised z-scores, defined as: $z_i = (x_i - \text{median}(x))/(1.4826 \times \text{MAD}(x))$, with MAD being the median absolute deviation. Cells with an absolute z-score lower than 1 are bold.

^a Arthralgia subtypes (hand/finger pain, foot pain, and multiple joints pain) are not available in Hungary and Sweden due to International Classification of Disease-10 coding.

RESULTS

Primary care

MSK consultations in primary care

Information on GP consultation rates is summarised in Table 2. The mean number of GP visits per patient per year varied across countries, ranging from 2 consultations per year in Sweden to 5 in Hungary and 6 in the Netherlands. MSK complaints accounted for 11% to 23% of all GP visits, with the lowest proportion in Sweden and the highest in Hungary. The proportion of individuals who visited a GP at least once per year with an MSK-related complaint was quite similar across sites (25% to 31%), again lowest in Sweden and highest in the Netherlands.

The age- and sex-distribution of patients visiting their GP with an MSK complaint was comparable across sites (mean age: 56–65 years; 54%–62% female) (Table 3). In all countries except Hungary, arthralgia and dorsalgia together accounted for more than half of all MSK encounters (55%–69%), with arthralgia being more frequent than dorsalgia. Among diagnosis-based categories, mechanical problems/bursitis/tenosynovitis constituted the most common category (14%–25%), although this group encompasses a broad range of conditions. When focusing on specific diseases, knee/hip OA represented the most frequent single diagnostic category (2%–14%). IRDs accounted for a small share

of MSK encounters (1%–3%) in Sweden, the Netherlands, and Spain, but were notably more prevalent in Hungary (7%). A sensitivity analysis using Dutch (Leiden) data compared defining encounters as either consultations or episodes of care, and showed minimal differences between the 2 approaches (Supplementary Section S3).

Referral patterns of MSK complaints

Referral patterns for MSK complaints varied markedly across countries. The proportion of patients with MSK complaints ever referred to and seen by a rheumatologist was lowest in Sweden (5%), followed by the Netherlands (11%) and Spain (22%), and the highest in Hungary (38%) (Table 4). When considering the proportion of MSK encounters resulting in a rheumatology referral, this was 0.7% in the Netherlands, 1.0% in Sweden, 4.2% in Spain, and 4.6% in Hungary (Table 4).

The referral rate to rheumatology per diagnostic category (as coded in primary care), relative to each country’s average MSK referral rate, is shown in Figure 1. Across all regions, IRDs (1.2–21 × average), PMR (1.1–5.1 × average, not available in Spain), arthralgia (1.1–3 × average), and non-knee/hip OA (1.1–3.3 × average) had higher-than-average MSK referral rates. Within arthralgia subtypes, hand/finger pain (1.5–3.0 × average) and multiple joint pain (7.4–15 × average) were most frequently referred. FM was also frequently referred in the Netherlands and

Table 4
Proportion of primary care patients with MSK and MSK encounters referred to rheumatology

		Sweden Stockholm	The Netherlands Leiden	Spain Madrid	Hungary Budapest
Proportion of primary care patients with MSK ever referred to and seen by:	Rheumatology (%)	5.1	11.0	21.5	37.6
Proportion of primary care MSK encounters referred to and seen by:	Rheumatology (%)	1.0	0.7	4.2	4.6
	Orthopaedic surgery (%)	11.8	3.8	15.0	2.0

MSK, musculoskeletal.

Percentage of patients with MSK complaints and primary care MSK encounters that have ever been referred to and seen by the rheumatologist. The analysis only includes patients who have, at some point, visited the general practitioner with an MSK complaint.

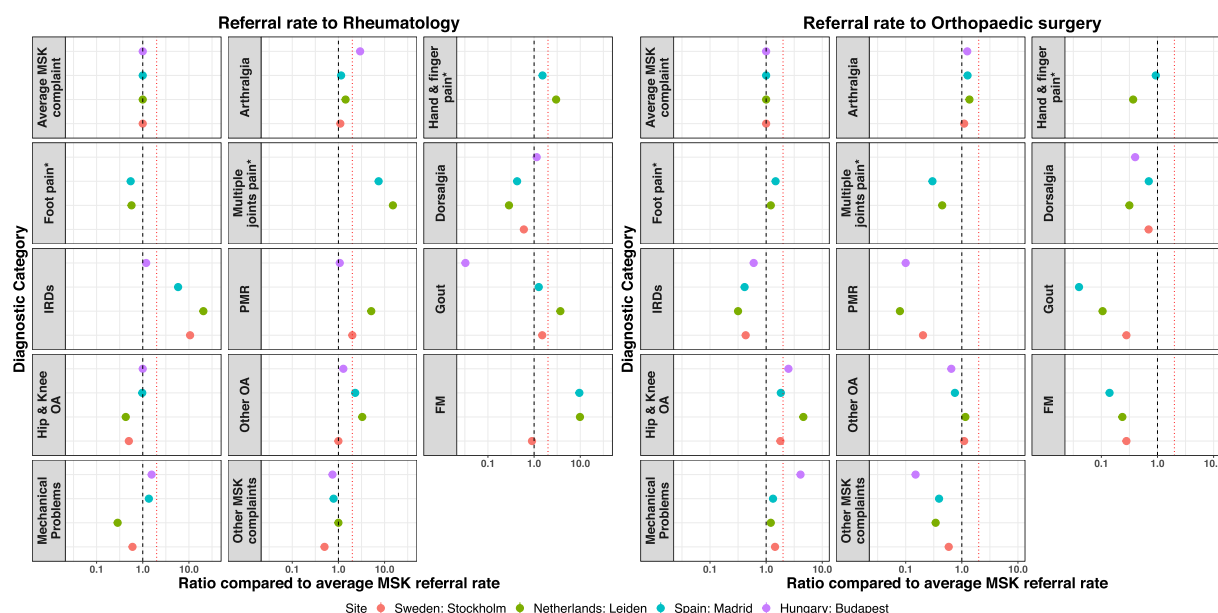


Figure 1. Proportion of GP encounters resulting in referrals to rheumatology and orthopaedic surgery by diagnostic category, compared with the average country-specific referral rate across all MSK complaints. The black dotted line indicates the country-specific average MSK referral rate, whereas the red dotted line represents twice this average. Referral rates are shown on a logarithmic scale. FM, fibromyalgia; GP, general practitioner; ICD-10, International Classification of Disease-10 coding; IRDs, immune-mediated rheumatic diseases; MSK, musculoskeletal; OA, osteoarthritis; PMR, polymyalgia rheumatica. *Arthralgia subtypes (hand/finger pain, foot pain, and multiple joints pain) are not available in Hungary and Sweden due to ICD-10 coding.

Spain ($7.3\text{--}9.9 \times$ average) but less commonly in Sweden and Hungary ($0.9 \times$ average). Mechanical problems had higher-than-average referral rates in Spain and Hungary ($1.4\text{--}1.6 \times$ average) but not in the Netherlands and Sweden ($0.3\text{--}0.6 \times$ average). Knee/hip OA ($0.4\text{--}1.0 \times$ average), dorsalgia ($0.3\text{--}0.6 \times$ average; $1.1 \times$ in Hungary), and foot pain ($0.3\text{--}0.6 \times$ average) were referred to the rheumatologist less frequently than average.

Referral to orthopaedic surgery was more frequent than referral to rheumatology across all sites except for Hungary (Table 4), with the highest referral rates for knee/hip OA ($1.8\text{--}4.6 \times$ average) and mechanical problems ($1.1\text{--}4.1 \times$ average) (Fig 1).

The distribution of complaints (as coded in primary care) of the population referred to and seen by the rheumatologist is shown in Table 5. Across all regions, arthralgia was the most common reason for referral (36%–47%), except in Hungary, where dorsalgia predominated (26%). IRDs accounted for a substantial proportion of those referred in the Netherlands and Sweden (10%–13%), but less so in Spain and Hungary (6%). OA also represented a notable share of referrals in all sites, particularly non-knee/hip OA (3%–15%). Gout referrals were relatively more frequent in the Netherlands (8%) compared with other countries (1%–3%). In Spain and Hungary, mechanical problems constituted a larger proportion of referred cases (17%–22%) as compared with the Netherlands and Sweden (6%–12%).

The Rotterdam/Randstad data were used to validate the Dutch primary care findings and showed very similar results regarding MSK consultation rates at the GP, referral rates across diagnostic categories, and the distribution of complaints among referred patients (Supplementary Section S1).

Secondary care

The duration of care in specialised rheumatology clinics is shown in Table 6. The proportion of patients remaining under long-term rheumatology care (> 12 months) varied widely

between regions. In the Netherlands, Spain, and Hungary, 13% to 19% of patients stayed under specialist care beyond 1 year, compared with 43% to 44% in Sweden and the UK. Long-term care was primarily driven by IRDs in all countries (the Netherlands: 76%, Sweden: 65%) except in Hungary, where IRDs accounted for only 7% of long-term care and OA represented the largest group (30%).

Short-term care (≤ 3 months) dominated in the Netherlands, Spain, and Hungary (65%–70%), substantially higher than in Sweden and the UK (45%–46%). This group was driven by arthralgia in the Netherlands (43%) and Sweden (19%), whereas OA and mechanical problems accounted for a large share in Hungary (each 28%–29%). In the Netherlands, FM contributed a notable proportion of short-term care (6%), much higher than the other countries (0%–1%).

Results from the UK need to be interpreted with care, as a large proportion of episodes (76%) have a missing diagnostic code. A sensitivity analysis using Leiden data compared alternative approaches for defining the diagnostic category of each episode (eg, using the first or most common code, applying a hierarchy of codes) and showed no meaningful differences (Supplementary Section S2). In the Netherlands, Sweden, Hungary, and Spain, no major changes in the proportion of short/intermediate/long-term care were detected over the included time span.

Healthcare organisation

To quantify differences in healthcare organisation, the literature was reviewed. The number of GPs per 100,000 inhabitants was highest in the Netherlands (183), followed by Spain (95), Hungary (69), and lowest in Sweden (62) [29]. The annual mean number of doctor visits per capita was highest in Hungary (11), followed by the Netherlands (10), Spain (8), and again lowest in Sweden (2.3) [29]. The number of rheumatologists per 100,000 inhabitants was highest in Hungary (8.3) [31],

Table 5
Characteristics of primary care MSK encounters by rheumatology referral status (referred vs nonreferred)

			Sweden		The Netherlands		Spain		Hungary	
			Stockholm		Leiden		Madrid		Budapest	
			Not referred	Referred	Not referred	Referred	Not referred	Referred	Not referred	Referred
Total number of primary care MSK encounters			4,249,493 (99%)	40,963 (1%)	4,911,786 (96.3%)	34,803 (0.7%)	260,124 (95.8%)	64,208 (4.2%)	8,742,742 (94.4%)	515,208 (4.6%)
Age (average)			57	57	56	55	59	62	63	59
Sex (% female)			62	64	60	68	65	73	56	62
Codes in primary care										
Symptom-based categories	Arthralgia	All arthralgia	37.6%	44.4%	33.5%	47.4%	37.1%	42.2%	9.5%	11.9%
		Hand/finger pain			2.8%	8.2%	2.8%	4.2%		
		Foot/toe pain		NA ^a	4.6%	2.5%	6.8%	3.6%		NA ^a
		Multiple joints pain			1.2%	19.6%	1.0%	10.7%		
Diagnosis-based categories	Dorsalgia		22.3%	13.1%	21.8%	7.3%	32.4%	13.6%	23.4%	25.6%
	IRDS		1.1%	13.3%	0.4%	9.5%	0.8%	5.6%	5.2%	5.8%
	PMR		2%	4.4%	0.3%	1.7%	NA		0.1%	0.1%
	Gout		2%	3.2%	2.0%	7.8%	0.6%	0.8%	13.4%	2.4%
	OA	Hip & Knee OA	5.8%	2.9%	4.0%	1.8%	1.7%	1.6%	10.2%	10.3%
		Other OA	3.1%	3.3%	1.8%	5.8%	2.5%	6.0%	11.8%	15.0%
	FM		1.7%	1.5%	0.2%	2.0%	0.1%	1.3%	0%	0%
Other MSK complaints	Mechanical Problems/Bursitis/Tenosynovitis		20%	11.7%	25.8%	6.0%	16.2%	22.3%	11.6%	17.3%
			4.3%	2.2%	10.2%	10.6%	8.6%	6.6%	14.9%	11.8%

FM, fibromyalgia; GP, general practitioner; IRD, immune-mediated rheumatic disease; MSK, musculoskeletal; NA, not available; OA, osteoarthritis.

Percentage and characteristics of MSK encounters at the GP that are referred to the rheumatologist. A single patient can contribute to multiple encounters. The diagnosis is defined as the code assigned by the GP at the moment of referral.

^a Arthralgia subtypes (hand/finger pain, foot pain and multiple joints pain) are not available in Hungary and Sweden due to International Classification of Disease-10 coding.

Table 6
Episodes in rheumatology care, stratified by length of follow-up and diagnosis

In Rheumatology care for (months)	United Kingdom Newcastle (N = 10,444)			Sweden Stockholm (N = 54,090)			The Netherlands Leiden (N = 25,222)			Spain Madrid (N = 14,850)			Hungary Budapest (N = 493,078)		
	≤3	4-12	>12	≤3	4-12	>12	≤3	4-12	>12	≤3	4-12	>12	≤3	4-12	>12
Percentage of episodes	46%	11%	43%	47%	9%	44%	67%	14%	19%	65%	2%	14%	70%	17%	13%
Age (average)	54	51	52	51	50	52	53	56	56	62	61	65	56	62	65
Sex (% female)	71	65	66	61	58	61	71	55	60	73	74	76	61	66	74
Diagnoses in specialised Rheumatology clinics															
Arthralgia (%)	0.2 ^a	1.4 ^a	2.9 ^a	19.1	14.2	7.5	42.9	30.0	11.9				10.0	7.2	5.0
Dorsalgia (%)	0.0 ^a	0.6 ^a	2.3 ^a	2	1.9	0.9	4.0	2.5	0.7				18.8	16.4	13.1
IRD (%)	3.7 ^a	10.2 ^a	25.0 ^a	8	23.7	58.7	13.8	34.4	69.0				2.1	3.2	7.0
PMR (%)	0.0 ^a	0.0 ^a	0.1 ^a	1.7	2.6	2.2	0.6	3.5	6.5				0.1	0.2	0.4
Gout (%)	0.8 ^a	1.2 ^a	1.0 ^a	2.7	3.2	1.9	4.2	15.2	5.4	NA ^b			0.5	0.5	0.6
OA (%)	7.4 ^a	5.3 ^a	2.8 ^a	3.9	2.1	1.1	15.9	7.3	3.0				28.1	30.1	29.9
FM (%)	1.0 ^a	0.5 ^a	0.1 ^a	0.5	0.4	0.2	6.2	1.0	0.4				0	0	0
Mechanical problems/Bursitis/Tenosynovitis (%)	0.0 ^a	0.4 ^a	2.4 ^a	1.4	1.4	0.9	4.8	3.4	0.8				29.4	29.5	23.1
Other (%)	0.0 ^a	0.4 ^a	0.3 ^a	55.4	42.4	24.2	12.8	3.1	2.0				6.3	9.2	18.0
Missing code (%)	86.9	80.0	63.1	5	7.8	2.1	0.0	0.3	0.4				4.7	3.7	2.9

FM, fibromyalgia; IRDs, immune-mediated rheumatic diseases; NA, not available; OA, osteoarthritis; PMR, polymyalgia rheumatica. Episode seen in rheumatology care, stratified by diagnostic category and length of follow-up: episodes that remained in care for ≤ 3 months, between 4 and 12 months and > 12 months. All episodes referred to specialised rheumatology clinics within regular care were included. The last available code was used to define the disease classification of each episode.

^a Results from the UK regarding diagnostic categories need to be interpreted with care, as a large proportion of episodes (76%) have a missing diagnostic code.
^b Secondary care coding is unavailable in Spanish data.

followed by Sweden (2.7) [40], Spain (2.2) [41], the Netherlands (2.1), and lowest in the UK (1.2) [42].

An overview of healthcare system characteristics and key MSK trajectory features per region is summarised in Table 7. The UK and Sweden had fewer referrals from GPs to rheumatology, fewer rheumatologists per capita, and employed selective triaging. The Netherlands, Spain, and Hungary showed the opposite pattern, with more referrals to rheumatology, more rheumatologists per capita and employed non-selective triaging.

DISCUSSION

This study is the first to compare the MSK care pathway—from initial presentation at the GP to referral and follow-up in rheumatology—across 5 European healthcare systems. We observed a broadly similar distribution of MSK complaints in primary care, with around 25% to 30% of adults visiting their GP annually for an MSK complaint. In contrast, referral patterns varied widely between countries: 5% to 38% of patients consulting their GP for an MSK complaint were at some point referred to rheumatology.

Among those referred, 46% to 70% stayed under rheumatology care for less than 3 months, indicating that many referrals were for diagnostic clarification rather than ongoing specialist management, whereas only 13% to 43% received long-term rheumatology care. We observed that countries with a high proportion of sustained long-term rheumatology care (UK and Sweden) had lower referral rates, fewer rheumatologists per capita, and selective triaging, whereas countries with a low proportion of sustained long-term rheumatology care (the Netherlands, Spain, and Hungary) had higher referral rates, larger rheumatology workforces, and nonselective triaging. These associations reflect observed patterns and do not prove causal effects.

GP consultation rates differed substantially between countries, with the Netherlands showing the highest and Sweden the lowest rate. Despite these systemic differences, the proportion of patients visiting their GP with an MSK complaint at least once a year (25%-30%) and their age and sex distribution was remarkably consistent across sites. MSK complaints prompting visits were also generally similar, except in Hungary.

Table 7
Overview of healthcare system features and MSK trajectory characteristics across participating countries

	GP acts as gatekeeper	Annual number of GP visits with MSK complaints per person	Proportion of MSK patients ever referred to rheumatology	Triaging system	Number of rheumatologist per 100,000 inhabitants	Proportion of short-term rheumatology care
UK	Yes			Selective	1.2	46%
Sweden	Yes	0.2	5.1%	Selective	2.7	47%
Netherlands	Yes	0.8	11.0%	Non-selective	2.1	67%
Spain	Yes		21.5%	Non-selective	2.2	65%
Hungary	Yes	1.1	37.6%	Non-selective	8.3	70%

Rates are color-coded based on similarity using column-wise normalized z-scores, calculated as: $z = (x - \text{mean}(x)) / (\text{SD}(x))$, with SD being the standard deviation. Values with $z < -0.5$ were categorized as low (green), those with $-0.5 \leq z \leq 0.5$ as average (orange), and those with $z > 0.5$ as high (red). No primary care information was available for the UK, as the UK only provided secondary care data.
GP: General Practitioner, MSK: Musculoskeletal.

Referral rates to rheumatology varied considerably between countries. Hungary had the highest proportion of patients with MSK complaints referred (38%), followed by Spain (22%), the Netherlands (11%), and Sweden (5%). Considering the estimated 2% to 3% population prevalence of IRDs [43], the majority of rheumatology referrals in Hungary, Spain, and the Netherlands likely involved non-IRD conditions. This interpretation was supported by secondary care data: in Hungary, Spain, and the Netherlands, 65% to 70% of patients remained in rheumatology care for less than 3 months, most with non-IRD diagnoses, suggesting their referrals were primarily for diagnostic clarification. In contrast, this short-term care group comprised only 45% of referrals in Sweden and the UK. These findings align with earlier literature showing that > 50% of referrals to rheumatology are for diagnostic confirmation only [44,45]. The composition of the population seen in secondary care appears closely tied to triaging systems. Selective triaging systems (Sweden and UK), in which referrals are prescreened, seem to effectively filter out or prevent referrals of non-IRDs. However, other factors are also likely to influence referral patterns, including the availability of orthopaedic surgeons and rehabilitation physicians, and the availability and reimbursement procedures for physiotherapy. In Hungary, we observed the highest referral rate to rheumatology, the highest number of rheumatologists per capita, and a substantially higher proportion of mechanical complaints and OA in rheumatology care as compared with other countries (as evident from Table 6, where OA accounted for 30% of diagnoses among those receiving long-term rheumatology care). Also, Hungary was the only country where referrals to rheumatology exceeded those to orthopaedic surgery. Together, these findings suggest that Hungarian rheumatologists manage a broad range of non-IRD cases, thereby acting as key providers for mechanical and degenerative conditions. This is consistent with system features in Hungary: only rheumatologists can prescribe physiotherapy for mechanical and degenerative complaints, a dedicated subspecialty exists for immune-mediated diseases, and targeted therapies can be prescribed only in specialised centres.

Referral rates also appear related to rheumatology workforce size. Hungary, with the highest referral rate, has by far the highest number of rheumatologists per 100,000 inhabitants (8.3). The UK, with strict triaging, has the lowest number of rheumatologists (1.2). Spain (2.2), the Netherlands (2.1), and Sweden (2.7) fall in between. Sweden's comparatively high rheumatologist density despite a low referral rate and selective triaging may reflect longer consultation slots, part-time work patterns, and time spent on triage, although detailed data on time allocation are lacking. In Sweden, the proportion of MSK encounters resulting in referral is higher as compared with the Netherlands (1.0% against 0.7%). However, this encounter-based metric is poorly suited for comparing referral rates across countries, as it is strongly influenced by GP consultation frequency (eg, 3-fold higher in the Netherlands than Sweden), and by the use of episode-based coding in Spain (Supplementary Section S2). Therefore, the proportion of patients with MSK complaints ever referred provides a more robust comparison of referral rates across countries.

Although absolute referral percentages varied between countries, some diagnostic categories consistently showed high referral rates across sites—particularly IRDs, PMR, multiple joint pain, and non-knee/hip OA. In contrast, conditions such as FM, gout, and mechanical problems showed greater variation, with high referral rates in some countries but low in others. These findings have direct relevance for the development of clinical

referral support tools [46–50]. They highlight both universal targets (eg, arthralgia, PMR, and non-knee/hip OA) and the need for country-specific adaptation that considers system-level factors such as triaging procedures and national expectations of rheumatology care.

Strengths and limitations

Key strengths of this study include its multinational scope, the inclusion of both primary and secondary care data, and the large sample size comprising > 4 million primary care and > 600,000 secondary rheumatology care patients, allowing for a broad, comparative assessment of MSK care pathways across healthcare systems.

This study relied on routinely collected healthcare data, requiring site-specific cleaning and harmonisation of definitions across sites. Primary care coding systems differed between countries (ICPC in the Netherlands and Spain; ICD-10 in Hungary and Sweden), and even within ICPC coding, national differences existed (eg, a specific PMR code in the Netherlands but not in Spain). Although these discrepancies may introduce minor bias, they reflect real-world practice and enable inclusion of large, population-based datasets. We minimised inconsistency by using simple, interpretable definitions and validated them with local clinicians. Lastly, although residual bias likely persists, it is unlikely to affect the overarching patterns observed.

Episode duration in secondary care was used as a proxy for whether patients required ongoing rheumatology care or were referred for diagnostic confirmation only. Although alternative measures (eg, disease-modifying antirheumatic drugs initiation or the number of follow-up visits) were considered, these suffer from high cross-country variation, whereas episode duration allowed for easy and consistent application across sites.

The included regions may not fully represent their national healthcare systems, as the datasets do not have nationwide coverage but focus on specific urban areas (Newcastle, Stockholm, Leiden/The Hague, Rotterdam, Madrid, and Budapest). Nevertheless, these regions encompass substantial proportions of their national populations. Furthermore, 2 non-overlapping Dutch primary care datasets (Leiden and Rotterdam) yielded comparable results, supporting national representativeness. Secondary care rheumatology data from the UK, Sweden and Hungary included all hospitals in their respective regions (encompassing both regional and tertiary hospitals). Although Dutch and Spanish data originated from a single university hospital (Leiden University Medical Centre and Clínic San Carlos respectively), both also function as regional providers of general rheumatology care. Overall, although results may be somewhat biased towards urban populations, their findings likely reflect broader national patterns.

Future perspectives

Although selective triaging appears to be associated with a higher number of referrals resulting in sustained long-term rheumatology care, an important remaining question is whether patients receiving chronic care across triage settings are comparable in terms of diagnosis and disease severity. Our data did not allow comparison of delay in IRD diagnoses or disease activity across sites. However, available evidence does not suggest longer diagnostic delays [30] or higher disease activity measures (including disease activity score-28, swollen joint count, tender joint count, and health assessment questionnaire scores) [51] in systems with selective triaging (UK and Sweden) as

compared to those with nonselective triaging (the Netherlands, Spain, and Hungary).

Our findings highlight opportunities for countries to learn from each other's healthcare organisation and how these shape patient pathways. However, country-specific circumstances influence which healthcare system is most appropriate, and different choices are made regarding the balance between health gains and costs. It is therefore unlikely that there is a single universal best way to structure healthcare. Future work could quantify the direct effect of specific organisational features on care trajectories, diagnostic accuracy, and referral efficiency, and relate these to patient outcomes and costs. As mechanical and degenerative complaints represent a large proportion of MSK care needs, understanding where and by whom these conditions are managed—whether in rheumatology, orthopaedic surgery, primary care or elsewhere—will be important for optimising care delivery and resource allocation. These aspects need to be considered when comparing healthcare system characteristics. Within the SPIDeRR consortium [52], we are actively pursuing this work, enabled by the integration of primary care information with secondary care outcomes across multiple countries.

Conclusion

In conclusion, this multicountry study reveals substantial variation in the care pathways of patients with MSK complaints across Europe. Although the spectrum of MSK complaints presenting in primary care was broadly similar between countries, referral rates to rheumatology differed by up to 5-fold. In countries with high referral rates, a large proportion of patients seen in secondary rheumatology care did not have an IRD and were discharged within 3 months. Overall, systems with a high proportion of short-term rheumatology care (the Netherlands, Spain, and Hungary) were observed to have higher referral rates, more rheumatologists per capita, and employed nonselective triaging, whereas systems with more sustained long-term rheumatology care (UK and Sweden) showed the opposite pattern, with lower referral rates and selective triaging. These structural differences shape the patient case-mix in rheumatology clinics and are relevant to consider when developing diagnostic guidelines and referral tools intended for cross-national use.

CRedit authorship contribution statement

Georgy Gomon: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maxime Raffray:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Carlos Betancort Rodríguez:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Ádám Misák:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Laura Struik:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Peta le Roux:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Inés Perez-Sancristobal:** Writing – review & editing, Methodology, Data curation. **Sara Curiel Manzanás:** Writing – review & editing, Formal analysis, Data curation. **Elena Polentinos-Castro:** Writing – review & editing, Data curation. **María Isabel Del Cura González:** Writing – review & editing, Data curation. **Tamás Kováts:** Writing – review & editing, Data curation. **Daniel Prieto-Alhambra:** Writing – review & editing, Data curation.

Dennis Mook-Kanamori: Writing – review & editing, Conceptualization. **Helga Westerlind:** Writing – review & editing, Supervision, Methodology, Data curation. **Judit Majnik:** Writing – review & editing, Supervision, Data curation. **Gyorgy Nagy:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Arthur Pratt:** Writing – review & editing, Funding acquisition, Data curation, Conceptualization. **Dieuwke Schiphof:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Luis Rodriguez-Rodriguez:** Writing – review & editing, Validation, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Saskia le Cessie:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Johan Askling:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Rachel Knevel:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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Competing interests

All authors declare they have no competing interests.

Patient consent for publication

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Ethics approval

Not required.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ard.2026.05.011.

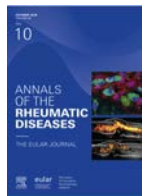
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Epidemiology

Mapping inequalities in rheumatology care in Europe: the first edition of the EULAR RheumaFacts project

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ABSTRACT

Objectives: To describe inequalities in health indicators relevant for quality of care to people with rheumatic and musculoskeletal diseases (RMDs) across Europe by comparing RMD health system indicators across European Alliance of Associations for Rheumatology (EULAR) member countries.

Methods: RheumaFacts is an EULAR initiative to improve the quality of care across Europe by monitoring access to and outcomes of care for people living with RMDs. In this study, we present the first edition of this longitudinal mixed-sources study initiated in 2024. A standardised form including indicators on RMD health resources and organisation, national workforce, and access to care was sent to the 40 National Scientific Rheumatology Societies who were EULAR members in 2024 was sent to the EULAR-member National Scientific Rheumatology Societies of 40 countries. Complementary data on the demographic and economic status of the various countries were extracted from open-source databases such as the WHO and World Bank datasets. Analyses were descriptive.

Results: Standardised report forms were returned by 36 of 40 countries (90%). The density of rheumatologists ranged from 0.8 to 6.6 per 100,000 inhabitants (median, 2.9; IQR, 2.1–3.5) across the different countries. The reimbursement of nonpharmacological care on a chronic basis was limited: physiotherapy was reimbursed in 26 of 36 countries (72%), whereas psychological support was reimbursed in only 14 of 36 countries (39%).

In 94% of the countries, all conventional synthetic disease-modifying antirheumatic drugs (DMARDs) were available. Despite all countries indicating the availability of at least 1 biologic DMARD, only 12 countries (34%) had access to all biologic DMARDs, and only 18 of 35 (51%) had access to all targeted synthetic DMARDs.

Conclusions: Wide cross-national inequalities exist in workforce capacity and reimbursed care for people with RMDs. RheumaFacts delivers the first harmonised basis for monitoring these gaps, enabling EULAR and national societies to track progress and inform health policy makers to advocate for improving the quality of life of people with RMDs.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Although rheumatic and musculoskeletal diseases (RMDs) are major contributors to disability and healthcare burden in Europe, no harmonised, RMD-specific dataset was available to allow for systematic comparison of the rheumatology workforce, health resources, and access to care across European countries.

WHAT THIS STUDY ADDS

- RheumaFacts provides the first multinational results on RMD health-related resources, rheumatology workforce, and access to care across 36 European Alliance of Associations for Rheumatology (EULAR) countries.
- It reveals large disparities in workforce density, availability of nonpharmacological care, and access to biologic disease-modifying antirheumatic drugs and targeted synthetic disease-modifying antirheumatic drugs.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- RheumaFacts established a benchmark for systematic cross-country comparisons, allowing inequities and progress in rheumatology care to be monitored over time.
- Rheumafacts generates robust, comparable evidence to strengthen advocacy, inform workforce planning, and guide investment in equitable access to pharmacological and non-pharmacological care.
- This study positions EULAR at a central place in shaping musculoskeletal health policy in Europe and underscores the need for coordinated action.

INTRODUCTION

Rheumatic and musculoskeletal diseases (RMDs) are among the leading causes of disability worldwide, accounting for more than 149 million disability-adjusted life-years (DALYs) in the most recent Global Burden of Disease update—an estimate that has increased steadily since 1990 despite advances in therapeutics in some rheumatic conditions [1,2]. In Europe, these diseases are responsible for 1 in 5 years lived with disability, and their societal cost is amplified by long-term work loss and early retirement: musculoskeletal pain remains a top driver of sickness absence in European Union labour-force surveys [3]. However, compared to cardiovascular or oncological diseases, the public health response has been hindered by a lack of convincing insight into how this increasing burden is covered by healthcare systems across Europe and into the need for healthcare to halt or reverse this burden. The lack of these metrics results in RMDs not receiving the attention commensurate with their impact and limits the ability of clinicians and patient representatives to advocate for the allocation of essential resources. Increasing evidence indicates that a significant portion of the RMD burden is preventable [4–8]. Early diagnosis, prompt and effective treatment strategies, and access to interdisciplinary care can prevent joint damage, maintain work capacity, and reduce subsequent expenditures.

Unfortunately, optimal management is not distributed evenly. Comparative studies have already documented more than tenfold differences in the uptake of biologic and targeted synthetic disease-modifying antirheumatic drugs (DMARDs)

between European countries of differing income, with lower access clustering in settings where reimbursement criteria are restrictive, or specialist density is low [9]. Similar gradients exist for nonpharmacological care: fewer than half of countries apply national standards for chronic physiotherapy funding, and only a minority offer structured psychological support for people living with persistent pain [10].

There are numerous reasons for differences in the resources allocated to RMD care in Europe. The organisation of health systems varies significantly, as do the financing mechanisms for specialist consultations and rehabilitation. The mismatch between increasing demands and response in workforce planning has resulted in workforce shortages in most of the countries [11–13]. At the same time, fragmented budgeting often ignores the importance of multidisciplinary pain management and vocational rehabilitation. Social determinants, such as low socioeconomic status, educational attainment, and occupational exposures, further exacerbate inequities in disease incidence and outcomes [14–17].

These mismatches between need and access can translate directly into excess disease burden, which is well recognised in other specialties: for example, The European Society of Cardiology Atlas has shown that cardiovascular DALY rates were high across all countries, but 3 times higher in middle-income than in high-income member states, reflecting clear differences in workforce density of cardiologists, revascularisation capacity and preventive drug use [18]. These precedents demonstrate the power of granular, comparable indicators: once published, they have enabled benchmarking, stimulated targeted investment and provided a baseline against which progress can be measured. In cardiovascular medicine, this has contributed to a sustained decline in premature mortality across most of Europe over the past 3 decades—a public health success that rheumatology should emulate [19].

In the absence of regular and standardised measurement, these structural drivers remain unacknowledged, perpetuating a cycle in which insufficient data serves as an excuse for limited action. The European Alliance of Associations for Rheumatology (EULAR), as the umbrella organisation for rheumatology in Europe, is uniquely positioned to fill this gap.

The EULAR RheumaFacts initiative has been established to create a longitudinal repository of RMD indicators spanning health resources and organisation, national workforce and access to care, with regular updates. Importantly, the project incorporates representatives from all EULAR pillars—patient organisations (PARE), health professionals in rheumatology (HPR), and national scientific societies—into its governance structure, thereby ensuring that the chosen indicators are relevant to clinicians and to policy. By integrating newly collected national data with harmonised global sources, RheumaFacts seeks to provide a comprehensive overview of the burden of RMDs and available resources, as a preliminary measure towards addressing inequities in RMD care, ultimately striving to bridge Europe's musculoskeletal health gap.

METHODS

Study design

RheumaFacts is designed as a longitudinal, multinational study. Here, we present the cross-sectional results of the first round of data collection.

Participants

A working group (including epidemiologists, patient representatives, health professionals' representatives, and EULAR staff) was appointed by the Epidemiology subcommittee from the EULAR research committee to lead the project and establish the relevant indicators to be included in RheumaFacts.

Thereafter, all National Scientific Rheumatology Societies from the 40 EULAR countries were invited to participate in the project. The RheumaFacts group included the working group and a representative of each national scientific society. The chair of the Paediatric Rheumatology Committee of EULAR was also invited to be part of the group.

Procedure

In this project, 2 types of indicators were selected and defined by the steering group: 'global' and 'national—RMD' indicators. The 'global' indicators are not related to RMD; they measure global demographics and socioeconomics and are accessible as open data from various sources such as the United Nations (UN), World Bank, Eurostat, and the World Health Organisation. Concerning the set of 'national RMD' indicators to be provided by the national scientific societies, the steering committee drafted a proposal of indicators divided into the following categories: health resources and organisation, national workforce, and access to care. Afterwards, a standardised report form was prepared, including the name, definition, units of measure, year of collection and source for each indicator; also, a 'comments' section was available for national societies to include any relevant information concerning an indicator. This form was sent to all national scientific societies to review, and all indicators and their definitions were discussed during a face-to-face meeting in January 2024 with the whole RheumaFacts group. During this meeting, the definitions of national RMD indicators were extensively discussed, and it was also agreed to deprioritise some of the initially selected indicators due to the high challenges anticipated in data generation or collection. A consensus was achieved on the final set of national RMD indicators, including their agreed-upon definitions, to be incorporated in this first round of RheumaFacts data collection. Following a 12-month data collection period, the RheumaFacts group convened again in February 2025. During this meeting, the results for all indicators were presented, and the group concurred on the final set of indicators to be featured in the present edition.

Data collection

Global indicators

For this edition, the 2023 population by country, including children (ages 0–19 years) and adults (ages 20–100 years), was obtained from the UN database [20]. The definition of 'children' also included 18 and 19-year-olds because the UN data provides 5-year age categories (0–4, 5–9, 10–14, and 15–19), and we wanted to include in this definition patients aged 15 to 17 years old.

The 2023 gross national income (GNI) expressed in U.S. dollars (USD), using the Atlas method conversion factor, was obtained from the World Bank [21]. GNI represents the sum of value added by all resident producers, along with any product taxes (less subsidies) that are not included in the valuation of output, in addition to net receipts of primary income (compensation of employees and property income) from abroad. The purpose of the GNI Atlas conversion factor is to reduce the impact

of exchange rate fluctuations in cross-country comparisons of national incomes. Countries are immediately assigned on July 1 of each year, based on their estimate of Atlas GNI per capita for the previous calendar year. This classification remains constant throughout the fiscal year (from July 1 to June 30), even if country's income data experience fluctuations. In 2023, low-income countries were those characterised by a GNI (Atlas method) per capita of 1145 USD or less; lower-middle-income countries ranged from 1146 to 4515 USD; upper-middle-income ranged from 4516 to 14,005 USD; and high-income countries for GNI above 14,005 USD in 2023.

For the current analysis, as only 1 participating country (ie, Lebanon) was classified as lower-middle-income, it was decided to reclassify it with the upper-middle-income countries for stratification purposes, resulting in the establishment of a 'Middle-Income' category [22]. The 2023 Atlas GNI used for this analysis is presented in [Supplementary Figure S1](#).

National RMD indicators

Three groups of indicators were defined: RMD health resources and organisation, National workforce, and access to care.

1. RMD health resources and organisation:

1.1 Rheumatology Departments were defined as hospital units, led by a specialist in rheumatology, with autonomy from other departments to manage their resources, and delivering care for patients with RMDs

- Academic rheumatology departments were defined as rheumatology departments that have both research and teaching activities linked to a university.

- Nonacademic rheumatology departments were defined as rheumatology departments within the general hospitals in the country.

1.2 Number of associations of patients with RMD: defined as the number of nonprofit institutions primarily representing the interests and needs of patients with RMDs, their families or caregivers.

2. National workforce

2.1 Rheumatologists (physical persons, ie, including both people working full-time and part-time) were defined as specialist physicians with a registered Rheumatology specialisation and the qualifications to be able to prescribe biologic DMARDs (bDMARDs; this latter part of the definition, ie, referring to the bDMARD prescription, was added by the group to allow the distinction from specialists in Physical Medicine and Rehabilitation working in Rheumatology Departments). The proportion of female/male rheumatologists was also collected.

2.2 Rheumatology trainees were defined as physicians registered (at the time of the collection) in the training programme for the Rheumatology specialisation.

2.3 Rheumatology full professors (physical persons) were defined as the professors with the highest rank in the university actively working in the field of Rheumatology. The proportion of female/male rheumatology full professors was also collected.

2.4 Specialists taking care of children with RMD (physical persons): for these indicators, the data provided by national rheumatology societies were complemented by the Paediatric Rheumatology Committee chairs of EULAR, who ran a survey using the Paediatric Rheumatology International Trials Organisation [23], an international research network that includes 731 centres in 95 countries worldwide. Because of the different requirements to be a specialist in paediatric rheumatology across the EULAR countries, 3 indicators were collected: (i) the total number (physical persons) of paediatric rheumatologists

(ie, specialists [either paediatricians or rheumatologists] with a specific 'paediatric rheumatology' certificate), (ii) the total number of adult rheumatologists working in paediatric rheumatology (with no specific official certificate for paediatric rheumatology), and (iii) paediatricians (with no specific official certificate for paediatric rheumatology) working in paediatric rheumatology. Altogether, the aggregated numbers in these groups were collectively considered the workforce of specialists taking care of children with RMD.

2.5 Availability of a Certified Rheumatology training for HPR: the availability of an official certification recognising the 'specialisation' in rheumatology for nurses, physiotherapists, occupational therapists, and psychologists was collected in all countries.

3. Access to care

3.1 Possibility of self-referral by the patient to the rheumatologist in the public sector: Initially, the 'average time to referral to a rheumatologist' was included in the standardised form. However, retrieving this information at the country-level proved too challenging. Instead, 'self-referral', defined as the possibility for a patient to self-refer to the rheumatologist (ie, without needing to be referred by another physician) in the public sector (either partially or fully reimbursed), was collected.

3.2 Availability of reimbursed nonpharmacological treatment:

- Physical therapy: defined as the possibility in the country for patients with RMD to be reimbursed on a chronic basis (ie, not only at referral, but as part of chronic care) for physical therapy for their RMD.

- Psychological care: defined as the possibility in the country for patients with RMD to be reimbursed on a chronic basis (ie, not only at referral, but as part of chronic care) for psychological care for their RMD.

3.3 Availability of pharmacological treatments: defined as the availability of DMARDs in the country for physicians to legally prescribe such DMARDs, because of formal approval by the national authorities, regardless of the reimbursement policy.

The DMARDs included those drugs that were approved for at least 1 RMD indication by the European Medicines Agency at the time of data collection were: conventional synthetic DMARDs (csDMARDs: azathioprine, ciclosporin, hydroxychloroquine, leflunomide, methotrexate, and sulfasalazine), bDMARDs, either bio-original or biosimilar (abatacept, adalimumab, anakinra, canakinumab, certolizumab pegol, etanercept, golimumab, guselkumab, infliximab, ixekizumab, rituximab, risankizumab, sarilumab, secukinumab, tocilizumab, and ustekinumab), and targeted synthetic DMARDs (tsDMARDs: apremilast, baricitinib, filgotinib, tofacitinib, and upadacitinib).

Data presentation and analysis

Analyses were descriptive

Country-level indicators are presented graphically and stratified by income level (ie, by GNI) to provide contextual information, but no formal statistical comparisons between or within income groups were performed. Because several indicators were skewed and included extreme values, medians and IQR ranges were used as descriptive summaries rather than means.

These summaries should be interpreted alongside the full distribution of country-level values shown in the figures, particularly for the middle-income group, which included a limited number of countries.

The working group decided not to include formal inferential analyses in this first edition; this decision was made because RheumaFacts is intended to serve as an initial landmark and benchmark dataset for future longitudinal comparisons. Given that the analyses are based on country-level aggregated indicators, the limited number of participating countries, and the heterogeneity of national healthcare systems, regression coefficients could be overinterpreted as explanatory or causal estimates. Therefore, country-level indicators are presented graphically and stratified by income level, without formal testing of associations. For adult rheumatology indicators, the ‘adult’ population (ie, from 20 to 100 years) was used, whereas for paediatric rheumatology indicators, the paediatric population (aged 0 to 19 years) was used.

Patient and public involvement statement

Patients are actively involved in the design and governance of the RheumaFacts project. Patient Research Partners representatives of PARE were full members of the RheumaFacts working group from the outset. They contributed to the selection and prioritisation of indicators, ensuring that the chosen measures reflect aspects of care that are meaningful to people living with RMDs, including access to multidisciplinary care and treatment availability. PRPs participated in the workshops where indicator definitions and feasibility were discussed, and they contributed to the interpretation of results and dissemination strategy.

RESULTS

From the 40 EULAR current member countries, 36 (90%) participated in this first data collection for RheumaFacts (Supplementary Fig S1).

RMD health resources and organisation

Rheumatology departments

The median number of rheumatology departments was 0.37 per 100,000 adults (IQR = 0.19–0.48) and ranged from 0.02 in Slovakia to 1.14 in Belgium.

Figure 1 shows the number of rheumatology departments per 100,000 adults by GNI, whereas Supplementary Figures S2 and S3 represent the number of departments per 100,000 adults in high-income and middle-income countries; the median number of departments was 0.38 and 0.24 in high- and middle-income countries, respectively.

Academic and nonacademic rheumatology departments

For two-thirds (24/36, 66%) of the countries, rheumatology departments were mainly located in nonacademic centres. In some countries (Albania, Denmark, Israel, Serbia, and Slovakia), rheumatology departments were solely situated in academic centres. In contrast, in only 1 country (Cyprus), the 4 rheumatology departments were in nonacademic centres (Supplementary Fig S4).

Number of associations of patients with RMD

Only 2 countries (Albania and Armenia) had no official association of patient with RMD. Among those with at least one association of patient with RMD, the number ranged from 1 to 40 (the latter in the United Kingdom), with no difference regarding the income level of the country (Supplementary Fig S5).

National workforce

Rheumatologists

The median (IQR) number of rheumatologists (physical persons) per 100,000 adults was 2.9 (2.1–3.5), ranging from 0.8 (Ukraine) to 6.6 (Georgia) (Supplementary Fig S6). Figure 2 illustrates the number of rheumatologists per 100,000 adults by GNI, showing that overall, high-income countries had a larger number of rheumatologists, but not systematically, for example, high-income countries like Germany reported fewer rheumatologists than Georgia or Albania (belonging to the middle-income category). Data from Hungary and Switzerland were missing for this indicator.

The proportion of female vs male rheumatologists per country ranged from 33% in Austria (from a total of 183 rheumatologists) to 93% in Armenia (from a total of 54 rheumatologists) (Fig 3).

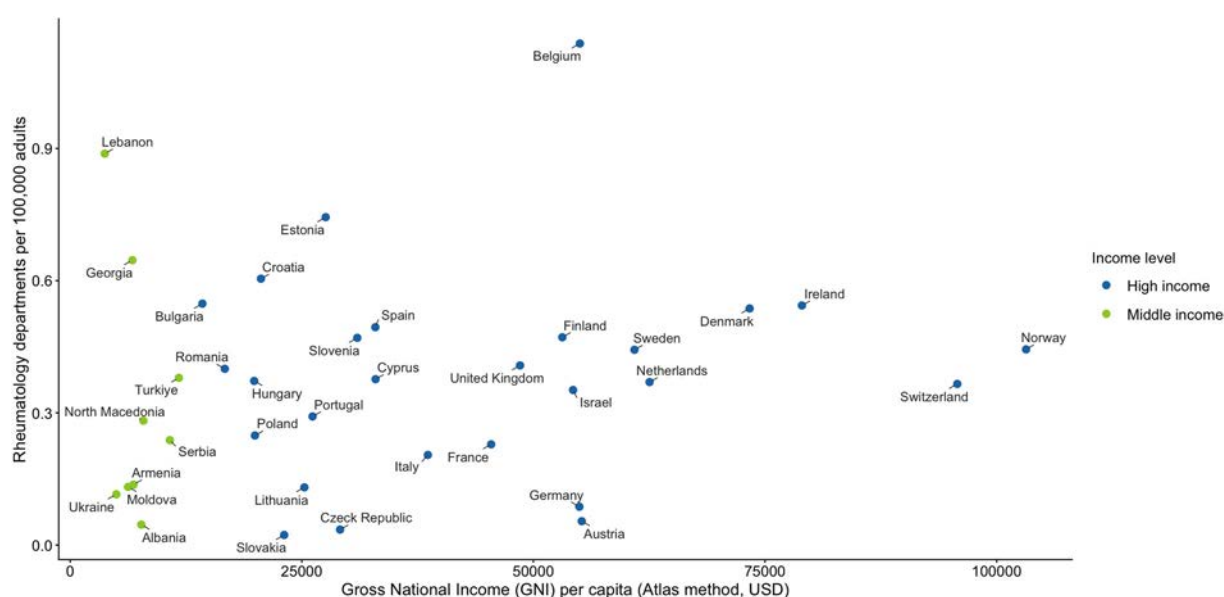


Figure 1. Rheumatology departments per 100,000 adults per country by income level (GNI, Atlas method, USD). GNI, gross national income.

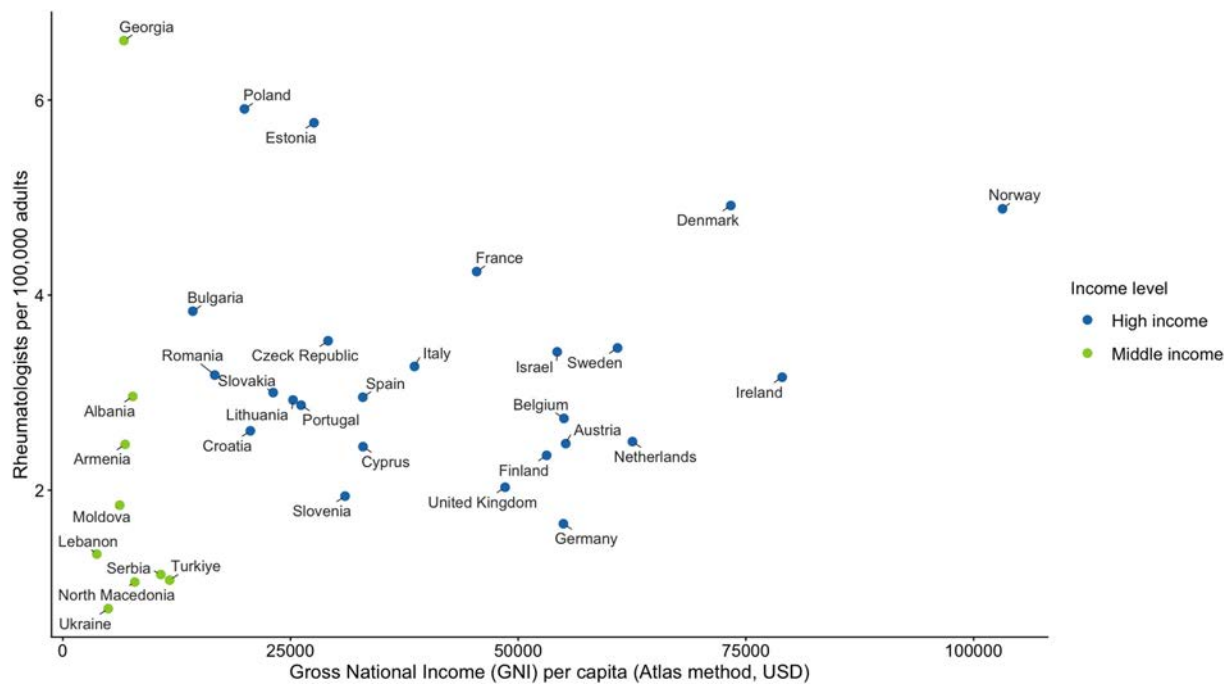


Figure 2. Rheumatologists (physical persons) per 100,000 adults per country by income level (GNI, Atlas method, USD). Data missing: Hungary and Switzerland. GNI, gross national income.

Rheumatology trainees

Among the 35 countries that provided data on that indicator, Cyprus was the only country with no Rheumatology trainees; among the other countries, the median (IQR) number of trainees per 100,000 adults was 0.64 (0.43-0.96), ranging from 0.13 in Lebanon to 2.47 in Romania. The median number of trainees was higher in high-income countries (0.76) compared with middle-income countries (0.46) (Supplementary Figs S7 and S8).

Rheumatology full professors

Among the 35 countries that provided data on that indicator, Cyprus was the only country without any Rheumatology full professors; among the rest of the countries, the median (IQR) number of Rheumatology full professors per 100,000

adults per country was 0.09 (0.05-0.16), ranging from 0.03 in Lebanon to 0.75 in Israel (Supplementary Fig S9). When looking at the proportion of women among the full professors, the median (IQR) proportion was 42% (21.1-71.9), with 6 countries in which 100% of rheumatology professors were women (Albania, Armenia, Estonia, Lithuania, Moldova, and Slovenia) and 1 country with no female professors (Lebanon) (Supplementary Fig S10).

Specialists taking care of children with RMD (physical persons)

Among the 31 countries that provided data on this indicator, the median and IQR number of specialists taking care of children with RMDs per 100,000 children was 1.33 (0.54-1.85), and higher in the high-income countries (1.40 [0.71- 1.86] vs 0.55 [0.37-0.61]) (Fig 4).

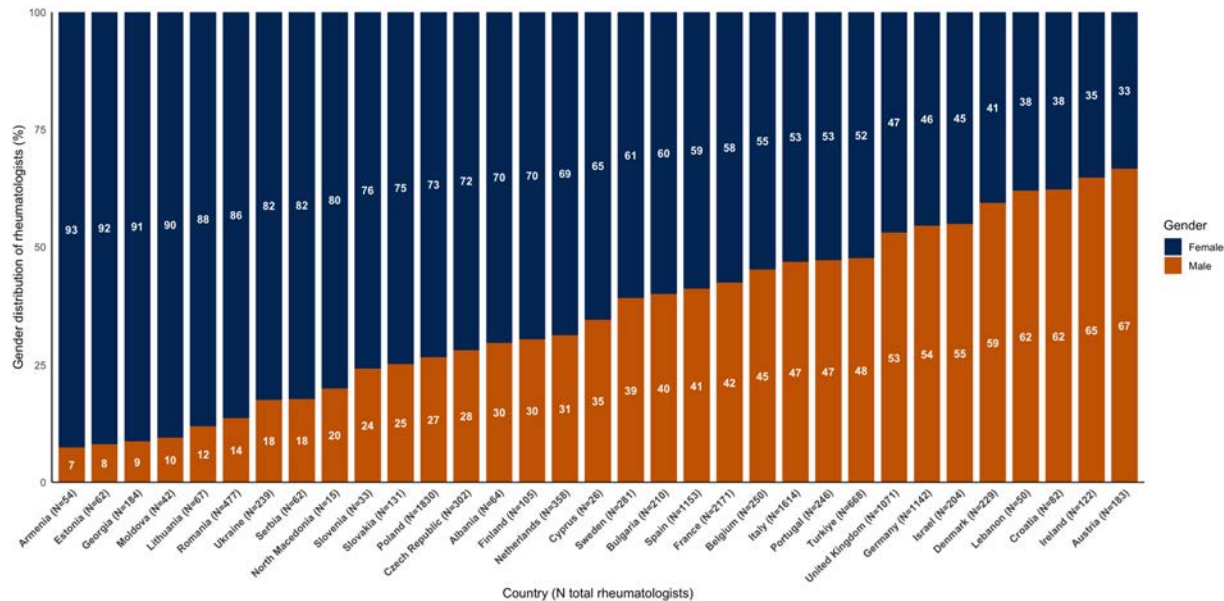


Figure 3. Gender distribution among rheumatologists (physical persons) per country.

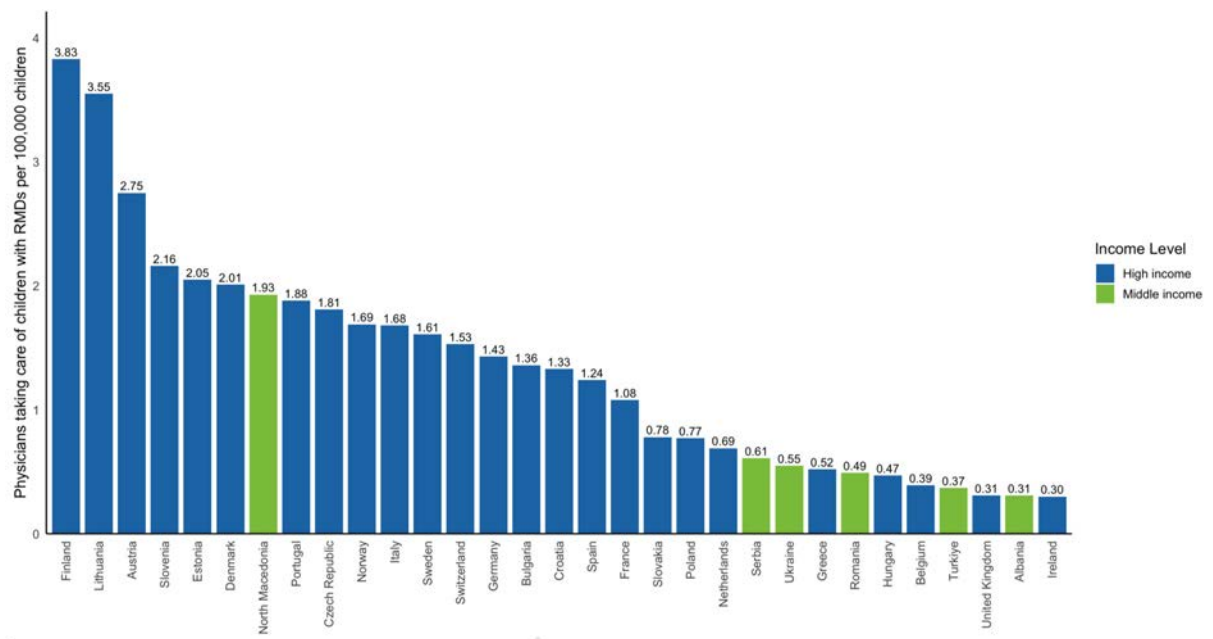


Figure 4. Number of specialists (physical persons: paediatric rheumatologists or paediatricians or adult rheumatologists) taking care of children with rheumatic and musculoskeletal diseases, per 100,000 children by income (GNI, Atlas method, USD). Data missing: Armenia, Cyprus, Georgia, Israel, Lebanon and Moldova. GNI, gross national income.

Certified rheumatology training for health professionals in the country

An official certification for rheumatology nurses was only available in 5 of 36 (14%) of the countries (Germany, Hungary, Ireland, the Netherlands, and Finland); only 4 (11%) countries (Germany, Hungary, the Netherlands and Sweden) had an official diploma for physiotherapists, and 3 (8%) countries (Germany, Hungary, and the Netherlands) for occupational therapists. No country had certified training in rheumatology for psychologists (Fig 5). Figure 6

Access to care

Self-referral

Among the 36 countries that provided data, self-referral by the patient to the rheumatologist in the public sector was possible in 18 (50%) of the countries; this was more frequent in the middle-income countries (37% vs 89%; Supplementary Fig S11).

Access to reimbursed nonpharmacological treatment

Among the 36 countries that provided information, reimbursement for physiotherapy on a chronic care basis for the RMD was reported in 26 (72%) of the countries; access was more frequent in the high-income countries group (78% of high-income countries vs 56% of middle-income countries).

Reimbursed psychological care on a chronic basis for patients with RMD was less common across countries (ie, only in 39% of countries), with no difference based on the country’s income level observed (33% vs 41% for middle vs high-income countries) (Fig 7).

Availability of pharmacological treatments

All the 36 participating countries provided information on these indicators.

- csDMARDs: Thirty-five (97%) countries had all 6 csDMARDs available; except for North Macedonia, where only salazopyrine and azathioprine were available (Fig 8 and Supplementary Table S1).



Figure 5. Availability of Certified Rheumatology training for health professionals.

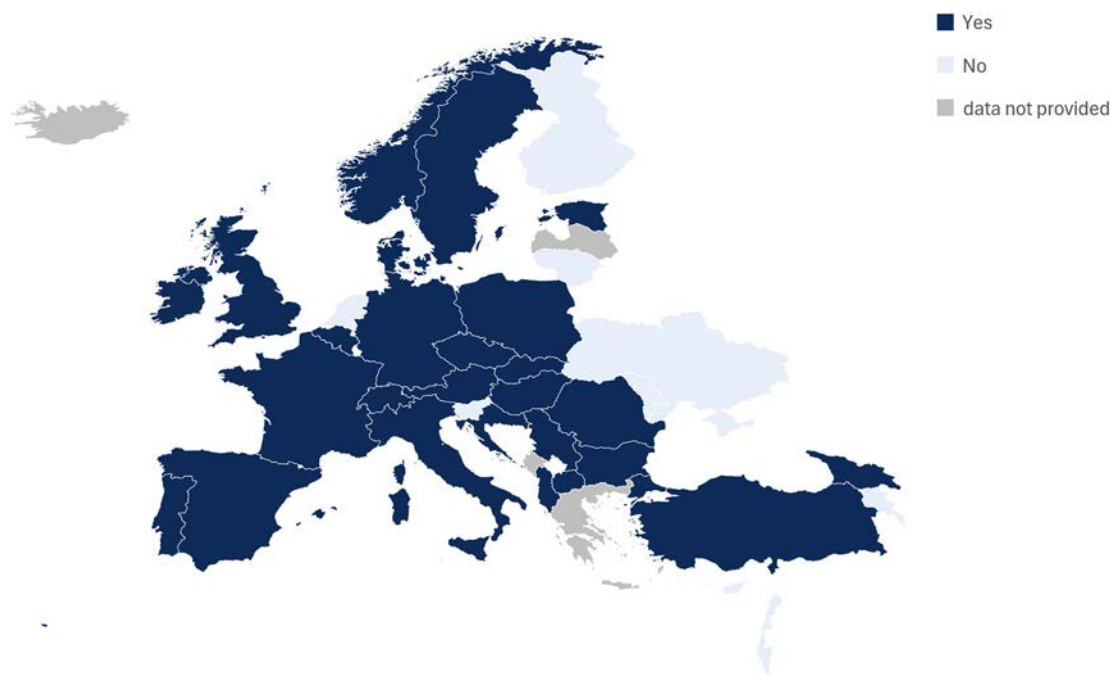


Figure 6. Possibility to access reimbursed physiotherapy on a chronic care basis for patients with RMD. RMD, rheumatic and musculoskeletal diseases.

- bDMARDs: At least 1 bDMARD was available in all countries, and 12 (34.3%) had all 20 bDMARDs available. Adalimumab and rituximab were the most frequently available bDMARDs across the countries; all countries had access to at least 1 TNFi, whereas all except North Macedonia had access to tocilizumab. Interestingly, more recent bDMARDs, such as risankizumab and guselkumab, were available in a large proportion of countries (ie, 65% and 81%, respectively) (Fig 8 and [Supplementary Table S2](#)). At the time of the data collection, the only biosimilars available were adalimumab, etanercept, infliximab, and rituximab. All 4 biosimilars were available in more than 70% of the countries: adalimumab biosimilars were

available in all countries, whereas rituximab, infliximab, and etanercept biosimilars were available in 89%, 86%, and 78% of the countries, respectively. Conversely, only in a minority of countries was the biosimilar the only available biologic treatment: 5 (14%) for infliximab, 3 (9%) for adalimumab and rituximab, and 1 (3%) for etanercept.

- tsDMARDs: All countries except 1 (Moldova) had access to at least 1 tsDMARD, and all but 2 (Moldova and Georgia) had access to at least 1 JAK inhibitor. Tofacitinib was the most frequently available JAK inhibitor. Overall, only 18 of 35 (51%) had access to all targeted synthetic DMARDs (Fig 8 and [Supplementary Table S3](#)).

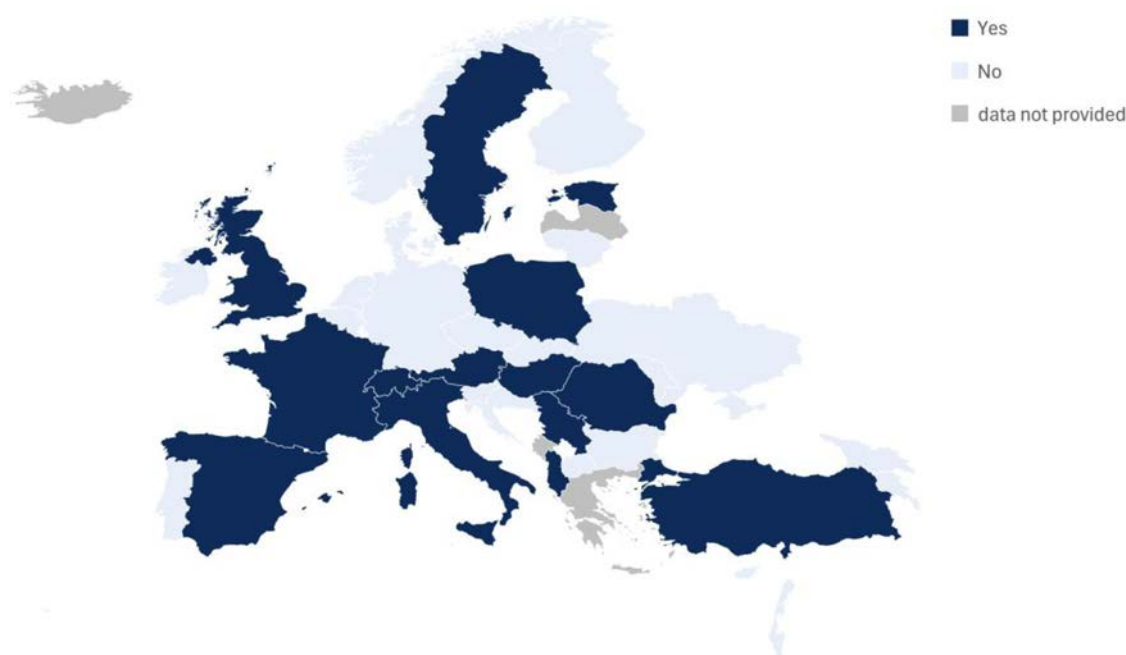


Figure 7. Possibility to access reimbursed psychological care on a chronic care basis for patients with RMD. RMD, rheumatic and musculoskeletal diseases.

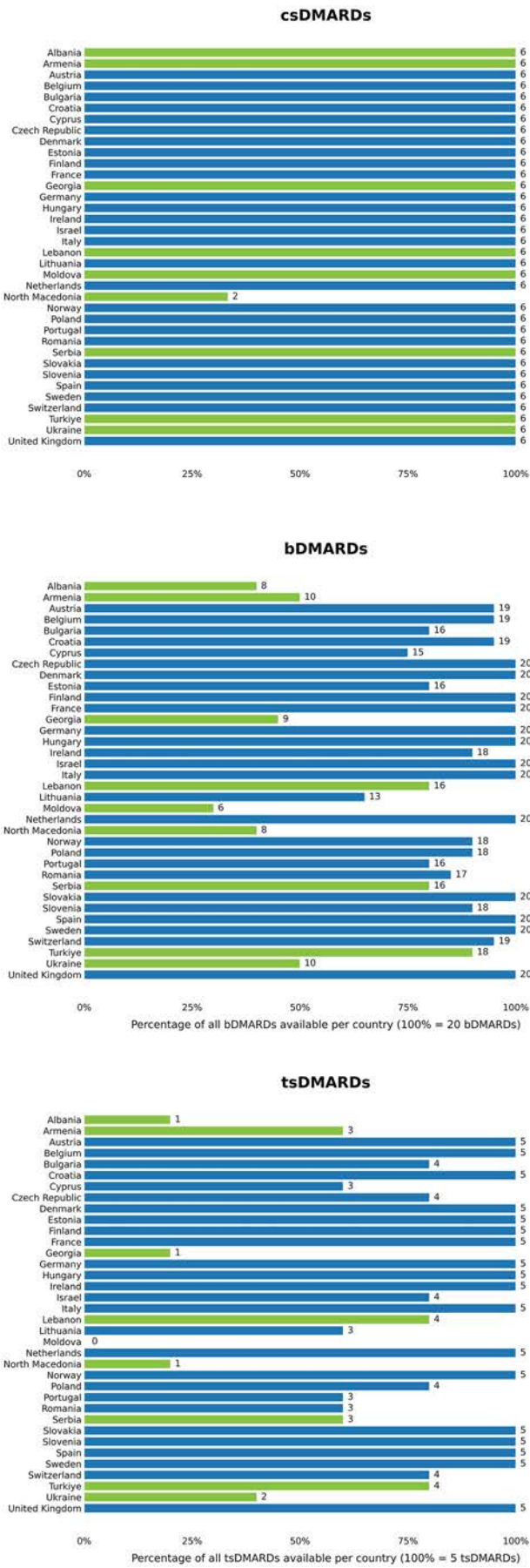


Figure 8. Availability of DMARDs per country. For bDMARD: 20 different bDMARDs = 16 bio-original bDMARDs (Adalimumab, Etanercept, Infliximab, Certolizumab pegol, Golimumab, Tocilizumab, Sarilumab, Ustekinumab, Abatacept, Rituximab, Canakinumab, Anakinra, Secukinumab, Risankizumab, Ixekizumab, Guselkumab) + 4 biosimilar bDMARDs (Adalimumab, Etanercept, Infliximab, and Rituximab). bDMARD, biologic DMARD; csDMARD, conventional synthetic DMARD; DMARD, disease-modifying antirheumatic drug; tsDMARD, targeted synthetic DMARD.

DISCUSSION

This first edition of the EULAR RheumaFacts initiative offers the first harmonised, multinational assessment of healthcare system capacity, workforce, and access to care for people living with RMD across Europe. Using data from 36 EULAR countries, our findings reveal significant cross-national disparities across the entire spectrum of rheumatology care. Notable differences were identified in the density of rheumatologists and rheumatology departments, the availability of rheumatology-certified health professionals, and the organisation of referral pathways, with only 50% of countries allowing patient self-referral within the public sector. Access to reimbursed nonpharmacological treatments—particularly psychological support—was limited in a substantial proportion of countries.

Differences in access to advanced pharmacological therapies remain 1 of the most visible and policy-relevant dimensions of inequity in RMD care. RheumaFacts builds on previous European work showing marked inequalities in access to biologic and synthetic DMARDs across countries [9] and provides a sustainable framework to monitor whether such differences persist or improve over time. In the present edition, almost all countries reported the availability of the full range of conventional synthetic DMARDs, and all countries had access to at least 1 bDMARD. However, only a minority of countries reported the availability of the complete range of bDMARDs, and only around half had access to all targeted synthetic DMARDs. These findings suggest that the existence of at least 1 advanced therapy in a country does not necessarily imply equitable access to the full therapeutic armamentarium recommended for contemporary RMD care. Importantly, in RheumaFacts, drug availability was defined as the possibility for physicians to legally prescribe the treatment following national approval, regardless of reimbursement policy. Therefore, these results should be interpreted as a first layer of access: they do not capture reimbursement restrictions, affordability for patients, prescribing criteria, administrative barriers, or real-world uptake. Future RheumaFacts editions should allow these indicators to be monitored longitudinally and may provide a platform for more detailed analyses of reimbursement policies and effective access to DMARDs across Europe.

The collection of sex distribution among rheumatologists and full professors was intended to inform 2 distinct dimensions of workforce and academic capacity. First, sex distribution may be relevant to workforce planning, as the increasing feminisation of rheumatology is sometimes proposed as 1 explanation for challenges in replacing retiring rheumatologists, based on the assumption that women may more often work part-time. However, this explanation is likely overly simplistic, as changing work patterns may also reflect broader generational shifts in work-life balance expectations and career organisation among younger physicians, irrespective of sex. In this first edition, rheumatologists were counted as physical persons rather than full-time equivalents, and age distribution was not collected, which limits interpretation. Future RheumaFacts editions will aim to collect full-time equivalent workforce data and, if feasible, age distribution stratified by sex, to better assess effective specialist capacity and distinguish sex-related from generational effects. Second, the sex distribution among full professors was included as a marker of women's representation at the highest levels of academic rheumatology. This indicator may help describe potential gender disparities in access to senior academic positions across

countries, although causal explanations cannot be inferred from these descriptive data.

The indicators related to rheumatology trainees and full professors also deserve consideration as markers of future capacity. The number of trainees may reflect the ability of a country to renew and expand its rheumatology workforce, whereas an academic background of professors may secure the broader scientific and societal relevance of healthcare and the quality of training of next-generation rheumatology care providers. These indicators should not be interpreted as direct measures of unmet needs, as they are shaped by national academic systems, training pathways, and healthcare organisation. Nevertheless, they are highly relevant for workforce planning. Countries with limited current rheumatology workforce, few trainees, and low academic capacity may be particularly vulnerable to persistent or worsening gaps in access to care.

Workforce planning is an important issue for all countries, particularly in the context of population ageing and the associated increase in demand for rheumatology care. In future editions of RheumaFacts, the workforce is expected to remain a key area of interest, with continued data collection enabling the analysis of changes over time.

When considering the rheumatology workforce, it is also important to account for care delivered to patients with RMDs by nonrheumatologists, such as nurses specialised in rheumatology or physician assistants. These professionals may play an important role in addressing, at least in part, shortages in the rheumatologist workforce. This first edition of RheumaFacts revealed that the vast majority of countries do not have a specific certification for health professionals working in rheumatology. A next step will therefore be to quantify these additional professionals involved in the care of patients with RMDs across countries in order to also consider them in workforce analyses.

Although countries with higher GNI generally reported greater capacity and access, economic status alone did not fully account for the extent and heterogeneity of the disparities observed, as considerable disparities persisted within income groups. The differences observed across countries are likely to reflect a complex interplay of upstream determinants, including demographic structure, population ageing, gender distribution within the medical workforce, training capacity, health system organisation, financing mechanisms, and the broader socioeconomic context. However, this first edition of RheumaFacts was not designed to identify the causal drivers of between-country differences. The analyses were based on country-level aggregated indicators and should therefore be interpreted as descriptive and hypothesis-generating. Future editions, once repeated measurements are available, will provide an opportunity to examine temporal trends and to explore, in dedicated ancillary analyses, how demographic, socioeconomic, and health system determinants may contribute to inequalities in RMD care across Europe.

Also, not all differences documented in RheumaFacts should be interpreted as indicators of suboptimal care. Some between-country variation may reflect legitimate differences in health system organisation, geography, care pathways, professional roles, academic structures, and national policy choices. For example, there is no universal benchmark for the optimal number of rheumatology departments or patient organisations, and the absence of formal rheumatology certification for health professionals does not necessarily imply poor quality of care if alternative training and care models

are in place. In this first edition, RheumaFacts was not intended to define normative thresholds or to rank countries, but rather to provide a harmonised descriptive benchmark. Observed differences should therefore be interpreted as signals that may require further contextualisation. They may become particularly relevant when they concern indicators likely to influence access, quality of care or patient outcomes; when they are large, persistent or unexplained; when they cluster with other markers of limited capacity; or when they are identified by national societies, HPR or patient representatives as barriers to care.

Before RheumaFacts, no comparable dataset existed focusing specifically on RMDs across the EULAR region. Earlier initiatives, such as EuMusc.net [24], had a similar aim but lacked longitudinal structure. Other international databases, including Eurostat, OECD, and WHO, provide information on health system capacity and activity, but do not include RMD-specific indicators. Similarly, platforms such as the WHO Health Systems and Policy Observatory offer policy-level data, yet without any dedicated focus on RMDs.

RheumaFacts addresses this gap by collecting primary data through EULAR's national scientific societies and its constituent pillars—HPR and PARE.

The dataset consolidates comprehensive information on RMD service provision, along with country-level demographic and macroeconomic indicators obtained from validated international sources.

The global indicators incorporated—sourced from the WHO and the World Bank—constitute the most authoritative estimates accessible. Their credibility is founded on stringent methodological standards; however, as is customary with such data, adjustments have been undertaken by the source institutions to compensate for missing data and reporting heterogeneity, leading to variable precision across countries [9].

To ensure the reliability of the data on national RMD indicators collected, an expert group developed the list of indicators and the associated reporting form, which were then validated through 2 structured workshops involving the national rheumatology societies. The template incorporated internationally harmonised definitions, and each country's data were reviewed and formally approved by the respective national society. All sources were documented to ensure traceability and transparency.

An important limitation was the lack of data on within-country inequalities, but also that comparisons between high- and middle-income countries, as classified by the World Bank, were based on national averages, which naturally mask within-group differences. As a result, the substantial heterogeneity observed within each income category could not be formally explored in this first descriptive edition.

All country and aggregated data have been shared with the EULAR Council and national rheumatology societies, with plans for wider dissemination through interactive digital tools on the EULAR website. Controlled access via a dedicated scientific committee is also planned for academic institutions, public health authorities, and government agencies wishing to carry out advanced analyses of the dataset, such as identifying systemic trends, gaps, and disparities in RMD care delivery.

Furthermore, data from RheumaFacts could be linked to the EULAR RMD Impact Survey [25] to investigate how system-level characteristics affect patient burden and disease impact. This integrated approach will provide a valuable foundation for comparative health system research.

This unique dataset is expected to serve as a cornerstone in advancing EULAR's mission in both research and advocacy. As a

provider of capacity in the field of RMDs, RheumaFacts enables the generation of robust, harmonised data that may support epidemiological surveillance, comparative health systems research, and health services analysis across Europe. More importantly, it establishes a credible, evidence-driven platform for advocacy, empowering EULAR and its member societies to engage effectively with national and European-level decision-makers. By documenting inequalities and potential inequities, healthcare gaps, and system inefficiencies in RMD care, RheumaFacts strengthened EULAR's capacity to influence health policy, secure appropriate resource allocation, and elevate the profile of RMDs within broader public health agendas.

Through this initiative, EULAR signalled its strategic intent to play a leading role in health policies and healthcare organisation in the field of RMDs. This objective was supported by a deliberate investment in internal expertise, data infrastructure, and research capacity, complemented by the development of strategic partnerships.

Until now, no single dataset had brought together economic indicators, healthcare system variables, and RMD-specific metrics. The launch of RheumaFacts created, for the first time, an integrated platform for monitoring, benchmarking, and informing policy in rheumatology. This represents a major step forward in EULAR's ability to support high-quality, data-driven decision-making aimed at reducing inequities and improving care delivery for patients with RMDs across Europe.

Competing interests

All authors declare they have no competing interests.

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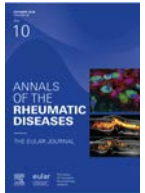
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Images in rheumatology

Seven years of destructive mono-arthritis: a radiographic clue from 1924

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THE CHALLENGE

A 62-year-old woman with Sjögren syndrome and chronic kidney disease presented with 7 years of progressive right knee mono-arthritis. Serial arthrocenteses consistently revealed inflammatory synovial fluid without crystals or bacterial growth, effectively excluding microcrystalline and pyogenic arthritis. Despite multiple therapeutic trials, the joint relentlessly evolved towards severe structural destruction with persistent hypertrophic synovitis.

Radiographs (Fig. A, B) demonstrated a striking triad: marked juxta-articular osteopenia contrasting sharply with preserved bone density distant from the joint, poorly defined peripheral erosions with irregular margins, and progressive joint-space narrow-

ing. Magnetic resonance imaging (Fig. C) revealed extensive bone marrow oedema, florid synovial hypertrophy, and subchondral destruction with intraosseous signal abnormality—features consistent with a chronic granulomatous process.

Synovial biopsy showed fibrous tissue containing well-formed nonnecrotising granulomas with <2 neutrophils per high-power field, a histological pattern incompatible with pyogenic infection. Mycobacterial cultures of synovial fluid remained negative despite prolonged incubation. Chest radiography revealed calcified apical granulomas suggestive of prior pulmonary exposure.

The combination of chronic mono-articular destruction, granulomatous histology, and this particular radiographic constellation—first described a century ago—points towards a specific diagnosis.



Figure. Imaging of the right knee. (A) Anteroposterior radiograph demonstrating the Pheemister triad: juxta-articular osteopenia, poorly defined marginal erosions, and joint-space narrowing. (B) Lateral radiograph confirming extensive osteolysis with subchondral destruction. (C) Coronal proton density fat-saturated magnetic resonance imaging scan showing diffuse bone marrow oedema, florid synovial hypertrophy, and intraosseous cavitation consistent with chronic granulomatous infection.

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What unifying diagnosis does this classic triad suggest, and what confirmatory investigation would you request?

THE ANSWER

The radiographic constellation represents the Phemister triad, described in 1924 as pathognomonic for osteoarticular tuberculosis: juxta-articular osteoporosis, peripheral erosions, and gradual joint-space narrowing. Polymerase chain reaction (GeneXpert MTB/RIF) performed on synovial tissue was positive for *Mycobacterium tuberculosis*, confirming the diagnosis. Both interferon-gamma release assay (QuantiFERON-TB Gold) and tuberculin skin test were positive, supporting tuberculous aetiology. Antituberculous therapy was initiated with clinical improvement.

This case delivers 2 teaching points. First, paucibacillary skeletal tuberculosis in immunocompromised patients frequently evades microbiological confirmation, demanding clinicopathological correlation. Second, the Phemister triad—although a century old—retains remarkable diagnostic utility. Rheumatologists confronting chronic, treatment-refractory destructive monoarthropathies should remember: sometimes the oldest signs illuminate the diagnosis that modern investigations miss.

CRediT authorship contribution statement

Mohammad Yassine Chérif: Formal analysis, Conceptualization. **Xavier Cabrol:** Writing – review & editing, Resources, Methodology, Formal analysis, Conceptualization. **Florent Lhotellerie:** Writing – review & editing, Formal analysis, Conceptualization. **Muhammad Shahnawaz Soyfoo:** Writing – review

& editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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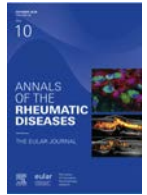
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Letter

Anti-CD19/CD22 CAR T cells in refractory immune thrombocytopenia of paediatric systemic lupus erythematosus

Immune thrombocytopenia (ITP) is a common haematological manifestation of systemic lupus erythematosus (SLE), which occurs in 10% to 40% of patients with SLE. The incidence of ITP is ~30% in paediatric lupus patients [1]. Severe ITP is not only associated with a long disease course but also implies a poor prognosis and a high mortality rate of SLE [2]. A proportion of SLE-ITP patients fail to respond to preliminary treatments such as glucocorticoids and immunoglobulins. These patients often receive repeated high doses of glucocorticoids, aggressive immunosuppressants, and even anti-CD20 antibodies, which may still be not effective and leads to increased risk of infection. The use of chimeric antigen receptor (CAR) T-cell therapy has seldomly reported in refractory SLE-ITP, and has never been reported in paediatric population [3].

Here, we report the successful use of autologous CD19/CD22 CAR T-cell therapy for the treatment of refractory paediatric SLE-ITP. The female patient was born in November 2014 and was diagnosed with ITP at the age of 6 years. Previous treatments with glucocorticoids, intravenous immunoglobulins (IVIGs), eltrombopag, and hetrombopag did not alleviate thrombocytopenia. Peripheral platelet counts could only sustain transiently with IVIGs or high doses of glucocorticoids. She was tested positive of anti-nuclear antibody (ANA) (titre 1:1000), anti-Smith (anti-Sm), anti-Ribonucleoprotein (anti-RNP) antibody in April, 2023. Low serum complement (both C3 and C4) levels were also detected (Table). Hence, she was diagnosed with SLE-ITP, and immunosuppressants were added. Mycophenolate mofetil, cyclosporin A, tacrolimus, sirolimus, rituximab, telitacicept, eculizumab, and obinutuzumab did not alleviate thrombocytopenia. Despite these aggressive treatments, platelet counts were $<10,000/\text{mm}^3$ most of the time. She presented with repeated purpura and ecchymoses and suffered from frequent gingival bleeding and epistaxis. At her worst time, she even received red blood cells transfusion due to haemorrhagic shock. Long-term use of glucocorticoids leads to ocular hypertension, high blood pressure, hyperglycaemia, hyperlipidaemia, hepatic steatosis, obesity, osteoporosis, and short stature.

Prednisone and hydroxychloroquine were discontinued in the month before CAR T-cell infusion. Autologous fresh leukapheresis was used to produce CAR T cells. A total of 6.45×10^6 CD19/CD22 CAR T cells per kilogram of body weight were administered after the patient had undergone lymphodepleting chemotherapy with fludarabine on days –5, –4, and –3 and cyclophosphamide on days –5 and –4 before CAR T-cell therapy. A second dose (4.5×10^6 per kilogram of body weight) of CD19/CD22 CAR T cells was administered 10 days after the first infusion. CAR T-cell numbers rapidly reached the expansion peak on day 19, followed by a typical decrease, with continuous

Table

Laboratory parameters at the time of diagnosis of SLE-ITP

Variable	Value
White cell count ($/\text{mm}^3$)	9170
Differential count (%)	
Neutrophils	75.9
Lymphocytes	14.6
Platelet count ($/\text{mm}^3$)	5000
Haemoglobin (g/dL)	10.5
D-dimer (mg/L)	0.23
Activated partial thromboplastin time (s)	44.8
Prothrombin time (s)	14.7
International normalised ratio for prothrombin time	1.17
Fibrinogen (g/L)	1.94
Creatinine ($\mu\text{mol/L}$)	31.05
Alanine aminotransferase (U/L)	12.42
Aspartate aminotransferase (U/L)	19.67
Albumin (g/L)	38.09
C-reactive protein (mg/L)	<5
Erythrocyte sedimentation rate (mm/h)	2
Antinuclear antibody	1:1000
Anti-RNP antibody (U/mL)	Positive
Anti-Smith antibody (U/mL)	Positive
Anti-SS-A antibody (U/mL)	Negative
Anti-SS-B antibody (U/mL)	Negative
Anti-double-stranded DNA	Negative
Anticardiolipin antibody (U/mL)	Negative
Complement (g/L)	
C3	0.79
C4	0.142
Urine	
Screening-dipstick test	
Protein	Negative
White blood cells	Negative
Red blood cells	Negative
Coombs test	
Direct Coombs test	Negative
Indirect Coombs test	Negative

anti-RNP, anti-ribonucleoprotein antibody; ITP, immune thrombocytopenia; SLE, systemic lupus erythematosus.

Chen-xing Zhang, Jing Zhang, and Jia He are cofirst authors and contributed equally to this work.

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detection of circulating CAR T cells during the next 9 months. The patient had transient fever from day 1 to day 4, associated with cytokine release syndrome (grade 1). Her temperature returned to normal with antipyretics and tocilizumab. Vital signs were stable, with normal blood pressure and oxygen saturation.

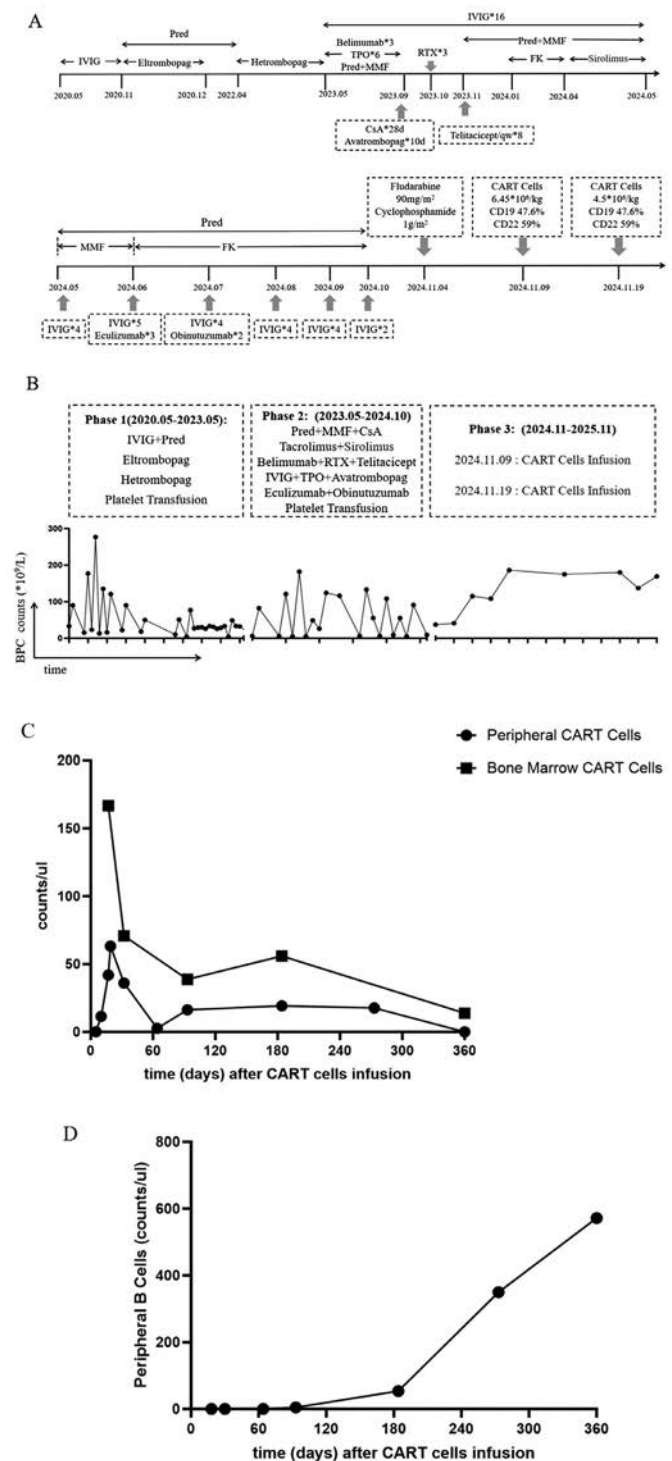


Figure. A, Graphical overview of the disease course and therapeutic regimen of the paediatric systemic lupus erythematosus (SLE) patient with immune thrombocytopenia (ITP). B, Peripheral platelet counts of the paediatric SLE-ITP patient before and after chimeric antigen receptor T-cell (CART) infusion. C, Peripheral and bone marrow CART counts following CART infusion. D, Peripheral B-cell counts following CART infusion. BPC, blood platelet count; CsA, cyclosporin A; FK, tacrolimus; IVIG, intravenous immunoglobulin; MMF, mycophenolate mofetil; Pred, prednisolone; qw, quaque week; TPO, thrombopoietin; RTX, rituximab.

Levels of cytokines, including interleukin (IL)-5, IL-6, IL-8, and interferon (IFN)- γ , were increased within 6 days of the first CAR T-cell infusion. No other adverse events related to CAR T-cell therapy were observed. Circulating CD19 B cells were eliminated by day 18, and B-cell reconstitution occurred at about 3 months. The platelet count rose from 14,000/mm³ at screening to 51,000/mm³ at 1 month, 108,000/mm³ at 2 months, 196,000/mm³ at 3 months, 175,000/mm³ at 6 months, 186,000/mm³ at 9 months, and 132,000/mm³ at 12 months (Fig). In addition, antinuclear, antiglycoprotein (GP)Ib, anti-GPIIb, and anti-GMP 140 antibodies were negative. She has discontinued all medications, including prednisone and immunosuppressants, since CAR T-cell therapy. Her height has increased by 10 cm over the past year, and her body mass index has dropped to normal.

CD19-directed CAR T-cell therapy has shown remarkable success in the treatment of autoimmune diseases. The emergence of CD19-negative B-cell escape may be a frequent cause of relapse. One strategy to prevent CD19-negative B-cell escape is the simultaneous targeting of CD19 and a second B-lineage antigen, CD22, which is expressed early in B-cell ontogeny and continues to be expressed until differentiation into plasma cells. This case suggests that CD19/CD22 CAR T-cell therapy may be effective for managing severe and refractory SLE-ITP. Primary ITP is an autoimmune disease in which autoreactive B cells play a crucial role in pathogenesis by producing autoantibodies primarily directed against platelet surface glycoproteins. By deeply depleting autoreactive B cells, CD19/CD22 CAR T-cell therapy may also apply to the treatment of primary ITP.

Competing interests

The authors declare no potential conflicts of interest with respect to the authorship and publication of this letter.

Contributors

C-XZ, JZ, and JH contributed equally to this paper. B-SL and LY conceived the idea and prepared the treatment protocol. C-XZ and JZ performed the data analysis and wrote the letter. KA and MS prepared the CAR T cells. Y-YM, JH, J-YS, WZ, JQ, and Y-JT were responsible for follow-up, patient assessments, and clinical data collection. JH revised the manuscript. All authors approved the final version of the letter to be published.

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Patient consent for publication

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Ethics approval

This study involves human participants and was approved by the Ethics Committee of Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine.

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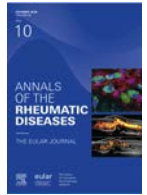
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Correspondence

Correspondence on 'Frequency and characteristics of axial involvement in psoriatic arthritis: results from the International Multicentre AXIS Study' by Torgutalp et al.

We read with interest the Axial Involvement in Psoriatic Arthritis Study (AXIS) study by Torgutalp et al [1] that provides a comprehensive assessment of axial involvement in psoriatic arthritis (PsA). However, 2 critical methodological flaws undermine the validity of its prevalence estimates and associated comparisons.

First, the study contains irreconcilable numerical discrepancies between the parallel-set plots and the reported text. In Figure 1, the local initial assessment identified 153 patients (37.4%) with axial involvement and 256 without. The final assessment yielded 112 and 297, respectively, representing a net shift of 41 patients from 'Yes' to 'No'. The authors state that '44 (28.7%) had their diagnosis revised to no axial involvement ... whereas 43 (28.1%) remained identified as having axial involvement despite negative central imaging' (page 6). These 2 groups alone account for 87 of 153 initially positive patients, leaving 66 patients unaccounted for. More importantly, the text claims only 8 patients (3.1%) were reclassified from 'No' to 'Yes' (page 6). Simple arithmetic dictates that if 41 more patients ended as 'No' than started, the number reclassified from 'Yes' to 'No' must exceed those reclassified from 'No' to 'Yes' by exactly 41. A 44-to-8 ratio gives a difference of 36, not 41. The 43 'remained positive despite negative imaging' further complicates the flow because these patients should not have contributed to the net shift. The presented numbers do not add up, rendering the diagnostic cascade uninterpretable. Such internal inconsistency violates basic data integrity and calls into question all derived prevalence figures.

Second, the study performs dozens of hypothesis test without any adjustment for multiple comparisons. At $\alpha = 0.05$, one would expect 2 to 3 false positives by chance alone. For example, leflunomide use differs between groups in the final assessment (12.5% vs 9.8%; $P = .42$; not significant), but

sulfasalazine use shows $P = .010$. Given the number of tests, this isolated significance is likely spurious. The authors also report 'higher frequency of Human Leukocyte Antigen B27 (HLA-B*27) positivity' $P < .001$ —a well-known association—but then claim 'peripheral enthesitis was less frequent in participants with axial involvement by central clinical review (37.7% vs 51.8%, P not shown)' from Table 2. Without multiplicity correction, the risk of type I errors across the 20+ clinical and imaging variables undermines any conclusion about distinctive features of axial PsA [2].

In summary, the AXIS study suffers from uncorrected mathematical inconsistencies and unadjusted multiple testing. We recommend that the authors provide a corrected flow diagram with coherent numbers and reanalyse all comparative data using a prespecified false discovery rate procedure (eg, Benjamini–Hochberg). Until then, the reported prevalence and phenotype of axial involvement in PsA remain unreliable.

Competing interests

All authors declare they have no competing interests.

CRediT authorship contribution statement

Chong Luan: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Yiwen Ding:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Shuo Dong:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Pengyun Wang:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

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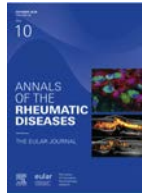
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Response

Response to correspondence on 'Frequency and characteristics of axial involvement in psoriatic arthritis: results from the International Multicentre AXIS Study' by Luan et al

Dear Editor,

We thank Luan et al. [1] for their interest in our recently published Axial Involvement in Psoriatic Arthritis (AXIS) study [2] and for the opportunity to clarify the points raised in their correspondence.

In our view, neither point identifies an error in the published paper [2] nor warrants correction of the figure, revision of the prevalence estimates, or reanalysis of the comparative data.

The first point concerns the numerical consistency of Figure 1 [2]. The numbers in Figure 1 [2] are correct and internally consistent when the full 3-step structure of the parallel-set plot is taken into account. The figure displays transitions from the local investigator's initial assessment to the central imaging assessment and then to the local investigator's final assessment. Therefore, the flow cannot be reconstructed by considering only 1 direct transition from the initial to the final assessment.

Among the 153 participants initially classified as having axial involvement, 44 followed the pathway initial 'Yes' → central imaging 'No' → final 'No'. However, this is not the total number moving from initial 'Yes' to final 'No'. An additional 5 participants followed the pathway initial 'Yes' → central imaging 'Yes' → final 'No'. Thus, the total number moving from initial 'Yes' to final 'No' is $44 + 5 = 49$.

Conversely, 8 participants moved from initial 'No' to final 'Yes'; 5 followed the pathway initial 'No' → central imaging 'Yes' → final 'Yes'; and 3 followed the pathway initial 'No' → central imaging 'No' → final 'Yes'. The net change is therefore $49 - 8 = 41$, which exactly corresponds to the decrease in participants classified as having axial involvement, from 153 at the initial local assessment to 112 at the final local assessment. The same calculation explains the increase in the number of participants classified as having no axial involvement, from 256 to 297.

The remaining pathways are also coherent: 61 participants remained 'Yes' after positive central imaging, and 43 remained 'Yes' despite central imaging not indicating axial involvement. These 43 participants did not contribute to the net shift from 'Yes' to 'No'; rather, they remained in the 'Yes' category at the final assessment. Therefore, the flow diagram does not contain a numerical discrepancy, and no correction is needed.

The second point concerns the absence of multiplicity adjustments to *P* values. No correction for multiple testing was required because AXIS was not designed, analysed, or interpreted as a confirmatory hypothesis-testing study. The study was a prospective, multicentre, cross-sectional descriptive study. Its main purpose was to estimate the frequency of axial involvement in psoriatic arthritis (PsA) across predefined diagnostic assessment layers and to describe the associated clinical and imaging phenotype.

The primary results of the study were prevalence estimates and diagnostic assessment flows. These results are descriptive and not derived from statistical testing. Therefore, they cannot be rendered unreliable by the absence of multiplicity adjustments.

The *P* values presented in the tables were included as descriptive aids to contextualise the observed group differences. They were not used to define axial involvement, select variables, construct a prediction model, establish independent predictors, or support causal conclusions. Accordingly, there was no predefined family of confirmatory hypotheses for which control of the family-wise error rate or false discovery rate would have been required [3].

We agree with the general principle that isolated nominal *P* values should not be overinterpreted when multiple comparisons are presented. This is precisely why the interpretation in the article was not based on isolated statistically significant findings but on the consistency, magnitude, clinical plausibility, and stability of patterns across assessment layers. Features such as HLA-B*27 positivity, inflammatory back pain, elevated C-reactive protein, and imaging abnormalities were interpreted as part of a coherent clinical and imaging phenotype, rather than as independent findings from multiple unadjusted statistical tests.

Applying a Benjamini–Hochberg or similar false discovery rate procedure post hoc across all tabulated variables would therefore not be appropriate for the aims of this study. It would impose a confirmatory testing framework for analyses that were explicitly descriptive. More importantly, such an adjustment would not affect the prevalence estimates or the diagnostic flow results, which represent the central findings of the AXIS study.

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For these reasons, the points raised in the correspondence do not constitute valid errors in the study. The flow diagram is numerically consistent, and the absence of multiplicity corrections is appropriate for the descriptive analytical framework used in AXIS.

Competing interests

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Murat Torgutalp: Conceptualization, Writing – original draft. **Denis Poddubnyy:** Conceptualization, Writing – review & editing.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used DeepL in order to assist with proofreading the manuscript text. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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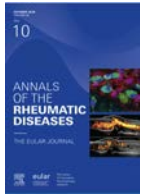
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Correspondence

Correspondence on 'Progression from symptom onset to rheumatoid arthritis: is the trajectory similar in anticitrullinated protein antibody-positive and -negative disease? A comparative longitudinal study' by Malekroudi et al

We read with interest the longitudinal comparison of anti-citrullinated protein antibody (ACPA)-positive vs ACPA-negative rheumatoid arthritis (RA) development by Malekroudi et al [1]. The authors are to be commended for assembling a well-characterised clinically suspect arthralgia (CSA) cohort. However, some flaws undermine the study's core conclusions.

First, the study equates clinical tenderness with inflammation without justification, directly contradicting the authors' own magnetic resonance imaging (MRI) data. ACPA-negative patients had significantly more tender hand joints at CSA presentation (median 3 vs 1; $P < .001$) and throughout follow-up, yet hand MRI inflammation did not differ between groups over time (incidence rate ratio (IRR), 1.03; $P = .28$, Supplementary S5). This dissociation of higher tenderness without parallel imaging-confirmed inflammation is a classic signature of central sensitisation or fibromyalgia, both known to be overrepresented in seronegative arthralgia populations [2]. The authors briefly mention 'non-inflammatory mechanisms' in the discussion but do not accept that this possibility invalidates their core conclusion that ACPA-negative RA has 'more hand joint inflammation'. The abstract and conclusion repeatedly attribute the clinical differences to 'underlying inflammatory mechanisms' and 'different preventive strategies', yet imaging evidence of hand inflammation is absent. A substantial body of literature has shown that tender joint counts correlate poorly with MRI-detected synovitis, especially in ACPA-negative disease [3]. By ignoring their own negative imaging data, the authors overinterpret clinical symptoms as proof of differential inflammatory trajectories.

Second, the study's reliance on morning stiffness as a distinguishing feature between ACPA groups is undermined by the very data presented. At symptom onset, morning stiffness was reported in 70% of ACPA-negative patients vs 54% of ACPA-positive patients ($P = .024$). However, 2 separate independent studies have reported nearly identical proportions: 52% in ACPA-positive patients and 80% in ACPA-negative patients, indicating that while the direction of the association may be real, its magnitude is highly variable across cohorts and its diagnostic specificity is poor, with specificity for predicting RA as low as 48% to 52% at a 30-minute cutoff [4]. The authors do not cite these previous contrasting findings, nor do they discuss why a visual analogue scale (VAS)-based measure (0-10 scale) was used instead of the standardised 'morning stiffness ≥ 1 hour' criterion recommended by the American College of Rheumatology/European League Against Rheumatism classification criteria, raising concerns about comparability with prior literature [5].

Moreover, the authors commit a direct contradiction between their narrative and their own figure. In the Results section (page 5), they state: 'ACPA-positive patients showed ... a significantly greater increase in forefoot inflammation ($P = .043$).' Yet the legend of Figure 5B reads: '... subclinical inflammation in the feet ... was significantly higher in ACPA-negative patients (IRR: 1.08; 95% CI: 1.00-1.16; $P = .043$).' This is not a typographical nuance; it reverses the direction of the main finding for foot inflammation.

In conclusion, these contradictions and overinterpretations leave the study's central claim unsupported. Without resolving the figure-text reversal or accounting for negative imaging data, the observed clinical differences cannot be confidently attributed to distinct inflammatory mechanisms.

Competing interests

None of the authors has any conflicts of interest.

CRediT authorship contribution statement

Jia Yu: Writing – original draft. **Jingjing Pan:** Writing – original draft. **Xiaowei Zhang:** Writing – review & editing. **Ming Zhou:** Writing – review & editing.

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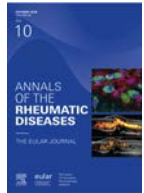
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Response

Response to: Correspondence on ‘Progression from symptom onset to rheumatoid arthritis: is the trajectory similar in anticitrullinated protein antibody-positive and -negative disease? A comparative longitudinal study’ by Yu et al

With great interest, we read the correspondence by Yu et al about our recent study that compared the trajectory from symptom onset to rheumatoid arthritis (RA) development between anticitrullinated protein antibody (ACPA)-positive and ACPA-negative disease [1]. They raised 3 issues.

First, Yu et al expressed surprise about the discrepancy between tender joint counts and imaging findings. In the predisease phase, ACPA-negative patients exhibit more tender joints than ACPA-positive patients, yet they do not show greater subclinical joint inflammation on magnetic resonance imaging scans. This appears paradoxical. However, it is not a novel observation. A recent study similarly reported a higher disease burden—based on patient-reported outcomes—in the predisease phase of ACPA-negative RA, alongside a weaker association with subclinical inflammation on magnetic resonance imaging scans [2]. Taken together, the previous and current findings suggest that different mechanisms underlie the symptoms and signs in the predisease phase of ACPA-negative and ACPA-positive RA. In particular, local joint inflammation seems to be a less prominent explanation in ACPA-negative pre-RA. Yu et al proposed that the tender joint counts in ACPA-negative RA may be driven by central sensitisation or fibromyalgia. While this possibility is also mentioned in our discussion, there is currently no direct evidence to support it. Other explanations should also be considered. For instance, systemic inflammatory processes may differ between ACPA-negative and ACPA-positive RA during the predisease phase. We fully agree with Yu et al that these findings are intriguing and warrant further investigation. However, attributing them primarily to central sensitisation may be an overinterpretation. The key processes underlying symptoms in the predisease phase of ACPA-negative RA—including both

central sensitisation and inflammatory pathways—remain to be elucidated.

Second, there may be a misunderstanding about what is presented in Table 1 of the original manuscript to which this correspondence refers. The proportions of 70% and 54% for stiffness refer to the symptoms reported by patients at the time their first complaints occurred; for stiffness, it referred to any stiffness rather than to morning stiffness with a duration of ≥ 60 minutes. Therefore, the proportion is not directly comparable to the proportion of patients who had morning stiffness lasting

Table 1

Patient characteristics at symptom onset and at presentation with CSA

Characteristic	ACPA-negative (n = 87)	ACPA-positive (n = 86)	P value
Age, y (mean $\pm$ SD)	46 $\pm$ 13	49 $\pm$ 13	.23.96
Female, n%	72%	72%	
Characteristics at symptom onset			
Symptom started with (%):			
• Pain	95	97	1.000
• Loss of function	38	36	.800
• Stiffness	70	54	.024
Localisation at symptom onset (%):			
• Small joints (hand/foot)	79	62	0.045
• Large joints	9	14	
• Both	12	23	
Localisation at symptom onset ^a (%):			
• Upper extremities	69	52	.073
• Lower extremities	12	13	
• Both	16	29	
Patients' characteristics at presentation with CSA			
VAS morning stiffness (mean, SD)	6 (2.4)	5 (2.7)	0.006
TJC 68 (median, IQR)	5 (3-10)	3 (1-6)	< .001
• Hand joints (including wrist)	3 (1-6)	1 (0-3)	< .001
• Foot joints	0 (0-3)	0 (0-2)	.28
• Large joints ^b	0 (0-2)	0 (0-1)	.018
^c Positive RF, %	18	92	< 0.001
CRP (median, IQR)	3 (3-8)	4 (3-8)	0.52
MRI total inflammation (median, IQR)	5 (2-10)	6 (3-13)	0.35

ACPA, anticitrullinated protein antibodies; CRP, C-reactive protein; CSA, clinically suspect arthralgia; MRI, magnetic resonance imaging; RF, rheumatoid factor; TJC, tender joint count; VAS, visual analogue scale.

^a Totals may not equal 100% because of missing values.

^b Large joints are shoulder, elbow, hip, knee, and ankle.

^c Positive RF is defined as ≥ 3.5 IU/mL.

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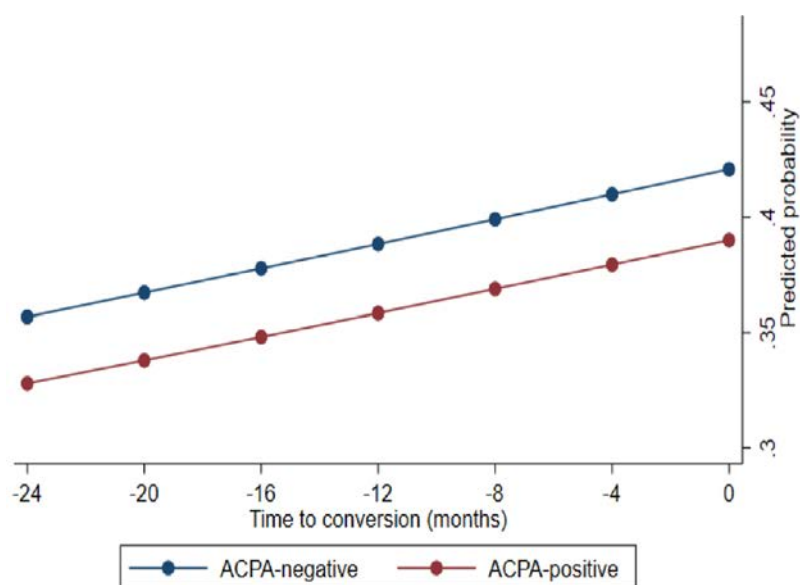


Figure 1. Frequency of patients with morning stiffness ≥ 60 minutes (predicted probability) in the pre-RA phase of patients with ACPA-negative and ACPA-positive disease. For comparability, the time of clinical arthritis was set as time 0. ACPA, anticitrullinated protein antibodies; RA, rheumatoid arthritis.

≥ 60 minutes at the time of first presentation with clinically suspect arthralgia at the outpatient clinic. The frequency of morning stiffness ≥ 60 minutes at onset of clinically suspect arthralgia in our data is 36.4% (46.2% of which are ACPA-negative and 37.5% ACPA-positive patients) and is comparable with the baseline characteristics reported in the previous work from our group [3]. Another issue is the measurement of morning stiffness in duration (expressed in minutes) or in severity (measured using a visual analogue scale). There is evidence that severity is more sensitive to change than the morning stiffness duration [4]. With respect to the trajectory of morning stiffness prior to the development of clinical arthritis, we also examined the frequency of patients reporting morning stiffness lasting ≥ 60 minutes. Although this dichotomised measure is less sensitive than severity assessment using a visual analogue scale, the data presented in the Figure 1 below suggests a higher frequency of morning stiffness during the predisease phase among ACPA-

negative patients compared with ACPA-positive patients. In other words, the finding of more morning stiffness in the development of ACPA-negative RA seems consistent. Of note, the authors also cite their reference 5, which was not included in our references. As this study does not concern a predisease population, it is not directly relevant here.

Third, the authors identified an error in the legend of Figure 5B [1]. In fact, ACPA-positive patients (not ACPA-negative patients) exhibit more subclinical joint inflammation in the feet in the pre-RA phase of the disease, which is also mentioned in the abstract and in the text. This will be corrected, and we thank the authors for bringing this to our attention.

Competing interests

The authors declare no conflicts of interest related to the work under consideration for publication.

CRediT authorship contribution statement

Mitra Hosseini Malekroudi: Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis. **Hanna W van Steenberg:** Writing – review & editing, Resources. **Elise van Mulligen:** Writing – review & editing, Visualization, Methodology. **Annette H M van der Helm-van Mil:** Writing – review & editing, Visualization, Supervision, Methodology.

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Ethics approval

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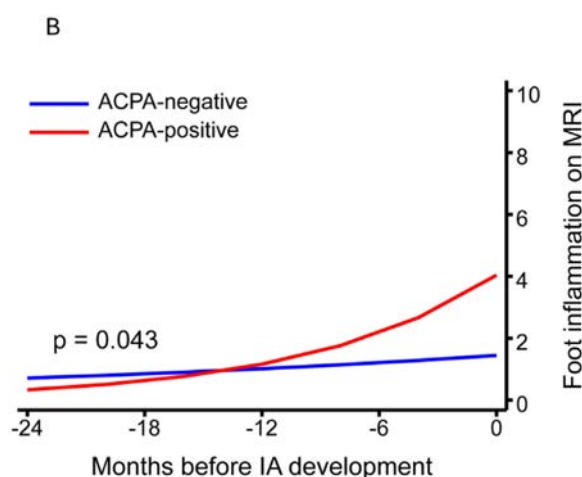


Figure 5B. Course of subclinical joint inflammation until development of clinical arthritis, presented for the feet only [1]. The data presented were collected at the presentation with clinically suspect arthralgia (CSA) until the development of clinically apparent inflammatory arthritis. For comparability, the time of clinical arthritis development was set as time 0. In B, subclinical inflammation in the feet is presented, which was significantly higher in anticitrullinated protein antibodies (ACPA)-positive patients (incidence rate ratio, 1.08; 95% confidence interval [CI], 1.00–1.16; $p = 0.043$).

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