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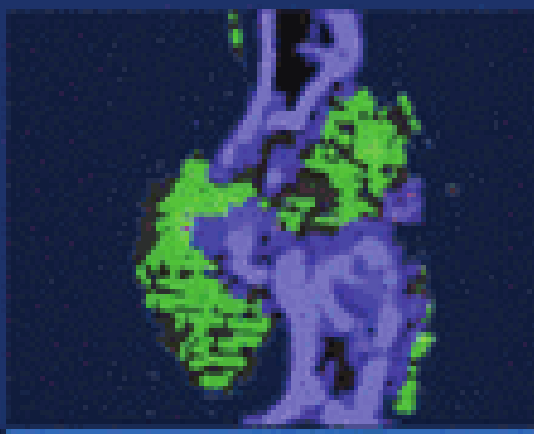

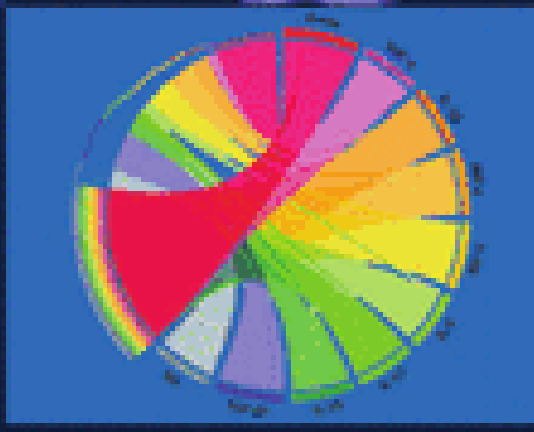

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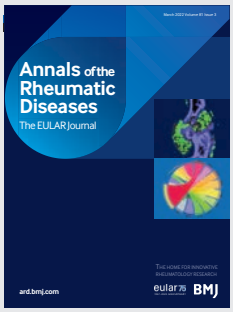




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# Hydroxychloroquine and lupus flare: a good drug, but we need to do better

Mary K Crow ,[1] Kyriakos A Kirou[2]

Most rheumatologists are well aware of the 1991 landmark study from the Canadian Hydroxychloroquine Study Group that reported results from a prospective randomised, double-blind study in which hydroxychloroquine (HCQ) was either continued or discontinued in patients with systemic lupus erythematosus (SLE) that were clinically stable for at least 3 months, although with significant disease activity (mean SLE Disease Activity Index (SLEDAI) 7.9 and 8.7, respectively).[1] The study was small, including 25 and 22 patients in the two groups, but the message was clear: over 6 months, a clinical flare (new or worse disease manifestations) occurred 2.5 times more frequently in the group that discontinued HCQ. Follow-up for three additional years demonstrated a 57% reduction in major flares (including cases of lupus nephritis and vasculitis) for those continuing HCQ, although this was not statistically significant due to the small number of study subjects.[2] The rationale for the study reflected the concerns of patients and physicians regarding the potential toxicities that might be associated with long-term use of that agent. At least in part as a consequence of the Canadian study, HCQ, first approved for treatment of SLE by the US Food and Drug Administration in 1955, is now the foundational therapy for nearly all patients with SLE. HCQ is generally considered a safe and effective medication in SLE.[3] Retinal damage is arguably the most significant toxicity of the drug and increases with cumulative exposure to HCQ. It is rare in the first 5 years of treatment (≤1%) but increases substantially after 16–20 years (8%–20%).[4] Risk factors, besides cumulative dose, include high daily dose relative to body weight, reduced renal function, older age, high body mass index and use of tamoxifen.[4 5] New recommendations have suggested a decrease of HCQ daily dosage to ≤5 mg/kg of actual body weight.[5] Of note, measuring blood levels of HCQ may help detect non-adherence and may predict both efficacy and retinal toxicity.[4–11] Assessing the benefits versus risks of continuing or stopping HCQ therapy remains an important priority for patients, and defining the features of those who might sustain low disease activity or remission after discontinuation of that drug continues to be an issue for effective management of lupus disease.[3–11]

Guidance regarding the relative benefits and risks of continuing HCQ therapy in patients with SLE is presented in a new report based on data from the Systemic Lupus International Collaborating Clinics (SLICC) inception cohort.[12] The SLICC investigators, a multinational group from 33 clinical sites, studied 1460 patients initiating HCQ therapy from among 1711 patients with SLE prospectively enrolled in the cohort from 1999 to 2019. The primary outcome of the study was time to the first of the following events indicating a flare: need for augmented therapy (including HCQ, chloroquine, glucocorticoids, immunosuppressive drugs or biologics), increase of ≥4 in SLEDAI-2000 (2K) or hospitalisation for SLE. Treatment augmentation was the most frequent flare outcome measure in all groups, while hospitalisation rates were minimal. Flares of patients decreasing or discontinuing HCQ compared with those maintaining the initial dose (for an average of 1.7 years for the groups maintaining and discontinuing HCQ and 2.0 years for those decreasing HCQ) were retrospectively analysed, and factors independently associated with flare were identified. Importantly, this large, multisite, multi-investigator study confirmed the general observation published in the original Canadian study, although with the HR for flare (1.56) in the discontinuation group compared with those who maintained HCQ somewhat less compelling than that reported in the 1991 study (table 1). As might be expected, the HR for flare in those reducing HCQ (1.20) was less than HR for flare in those discontinuing HCQ, perhaps suggesting that judicious tapering of the drug can successfully maintain a level of protection from flare. In all groups, use of glucocorticoids and immunosuppressive medications was associated with higher risks of flare. Asians (from South Korea) had a lower risk of flare if they reduced HCQ dose. Patients without a college or university education were significantly more likely to flare on discontinuation of HCQ, supporting the well-documented important contribution of socioeconomic factors to outcomes of patients with SLE.[13–16] While a recent report suggested that patients with SLE aged ≥55 years-old who are in a low disease activity state (SELENA-SLEDAI scores of ≤4) may successfully discontinue HCQ without increased risk of disease flare,[17] the SLICC group did not differentiate risk of flare in patients above or below age 50. The two studies also differed in that 36%–40% of SLICC patients had a SLEDAI-2K score of ≥4 and thus higher disease activity. SLICC patients of all ages with low lupus disease activity state (defined as SLEDAI-2K score of <4 and prednisone dose of ≤7.5 mg/day) or in remission (SLEDAI-2K score of 0 and no glucocorticoids or immunosuppressives in the last year) had lower flare rates as expected, but reduction or discontinuation of HCQ also increased their flare risk. Most notably, the flare rates for all four patient groups were ≥30 flares/100 person-years. The glass half empty interpretation is that flare rates remain unacceptably high for all groups, even when HCQ is maintained or only reduced.

While these new data support the utility of HCQ in reducing risk of flare, the study has important limitations that call for continued analysis of this and other cohorts.[12] The SLICC cohort study benefits from access to a large number of patients but did not provide the reasons for tapering or discontinuing therapy and did not differentiate mild–moderate from severe disease flares. The occurrence of clinical flares was ascertained only once per year, so uncertainties remain in the time to flare from the indicated time zero and whether mild flares might have been missed. Degree of drug adherence was not confirmed, and HCQ blood levels, a valuable measure of adherence, were not assessed.[18–20]

Nonetheless, the data support the value of HCQ in limiting flares that resulted in increased or (re)started HCQ, prednisone, immunosuppressive or biological agents, or increased SLEDAI-2K scores by ≥4.

In addition to data indicating the benefits of HCQ therapy with regard to flare, knowledge of the mechanisms responsible for the beneficial and harmful effects of HCQ can inform shared decision making in patient management. The benefits of HCQ have been traditionally attributed

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**Table 1** Incidence rates of first flare per 100 person-years for the HCQ reduction or discontinuation groups versus HCQ maintenance group with corresponding HRs for flare (in parenthesis) from analysis of the Systemic Lupus International Collaborating Clinics cohort[12]

| | All patients | Low activity | Remission | No low activity |
|---|---|---|---|---|
| Reduce HCQ | 40.0 vs 31.9 (1.20) | 37.5 vs 27.8 (1.32) | 26.2 vs 13.2 (2.14) | 43.9 vs 39.8 (1.04) |
| Discontinue HCQ | 41.3 vs 30.0 (1.56) | 35.5 vs 26.6 (1.62) | 24.7 vs 12.2 (2.77) | 53.6 vs 36.4 (1.6) |

HCQ, hydroxychloroquine .

to its capacity to alkalinise intracellular lysosomes, limiting antigen-presenting cell function.[21] As the contributions of endosomal toll-like receptors (TLRs) in driving production of type I interferon and B-cell differentiation have gained support as key components of lupus pathogenesis, HCQ-mediated alkalinisation of those endosomal TLRs (primarily TLR7, 8 and 9) is assumed to be an important contributor to efficacy in SLE.[10 11 22 23] Recent investigation of the functions mediated by HCQ has extended understanding of its beneficial and harmful effects (figure 1).[23–27]

As weak bases, HCQ and chloroquine, derivatives of 4-aminoquinoline, accumulate in intracellular acidic endolysosomes and neutralise their pH, potentially altering protein processing and antigen presentation on major histocompaticility complex (MHC) class II molecules and inhibiting TLR signalling and the resultant production of type I interferon, proinflammatory cytokines and differentiation of autoantibody-producing B cells.[23 25 26] Beyond its effects on endosomal pH, HCQ directly binds to nucleic acids, favouring binding to the guanosine–cytosine-rich sequences in the major groove of DNA and thereby potentially blocking the interaction of DNA with TLR9.[24 27] Similarly, HCQ can bind RNA, inhibiting activation of RNA-sensing TLR7 and TLR8. The observed inhibition of cytokine secretion by HCQ may be attributable to its Golgi alkalinisation, impairing protein secretion.[25]

The potential for patients treated with HCQ to experience toxicity from that drug is primarily a function of daily dose, reflected in blood levels, and duration of treatment.[25] For patients treated with HCQ for less than 5 years, it would appear that the mechanisms that abrogate production or secretion of type I interferon and other cytokines and are purported to limit antigen presentation are likely to outweigh the mechanisms that contribute to the toxicity of HCQ, most notably those that may impact vision. The risk of retinal toxicity is estimated to be <2% in the first 10 years in patients taking HCQ of 5 mg/kg of their actual weight.[5 25] Many of the toxicities attributable to HCQ may involve its inhibition of autophagy.[28] Efficient autophagy contributes to degradation and clearance of cell organelles, and impaired autophagy can result in accumulation of damaging intracellular and extracellular aggregates. HCQ may initiate retinal damage by binding to melanin in retinal epithelium, inducing intracellular accumulation of lipofuscin (lipid-containing material derived from lysosomes) by inhibiting autophagy, followed by damage to photoreceptors.[25 28 29] Cardiac toxicity leading to conduction abnormalities and toxic myopathy may also occur and, like the ocular manifestations, appears to be dose related. In cardiac myocytes, HCQ's capacity to alkalinise lysosomal contents can inhibit enzyme function and can result in accumulation of phospholipids that are not properly degraded, with generation of lipid bodies.[25 30] Skin pigmentation at sites of bruising may be a result of HCQ promoting accumulation of cell debris, including melanin, followed by stimulation of melanogenesis.

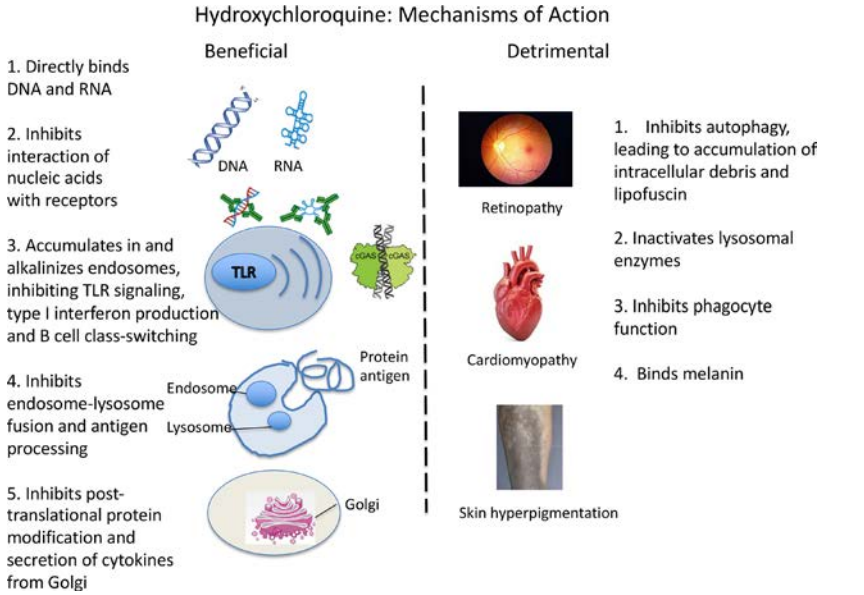


**Figure 1** Mechanisms of HCQ contributing to beneficial and detrimental effects. While additional research is required to fully elucidate the relevant mechanisms of HCQ, many of the proposed mechanisms, including direct binding to nucleic acids, alkalinisation of endosomal compartments, inhibition of endosome–lysosome fusion and Golgi alkalinisation, resulting in impaired secretion of proinflammatory cytokines, impact pathogenic mechanisms operative in systemic lupus erythematosus. Inhibition of autophagy, a cellular process responsible for degrading spent cellular components, can result in accumulation of intracellular and extracellular debris, leading to deposition of lipofuscin and damage to cells. In addition, HCQ binds to melanin, contributing to changes in skin pigmentation. cGAS, cyclic GMP-AMP synthase; HCQ, hydroxychloroquine; TLR, toll-like receptor.

As is the case for any discussion of disease management with patients, knowledge of the benefits of HCQ as well as potential for harm should be based on the most reliable data available. The study of the SLICC cohort confirms the impact of HCQ therapy on reducing risk of lupus flare and provides data that can inform discussions between patients and physicians. Knowledge of the drug's molecular mechanisms, particularly those consistent with current concepts of lupus pathogenesis, supports the case for inclusion of HCQ in therapeutic regimens. There is clear benefit in the setting of relatively modest risks, but those risks increase with duration of therapy. The message to patients will remain nuanced pending future research that might define biological predictors of flare and for patients desiring tapering or discontinuation of HCQ. As the HRs for patients decreasing HCQ in the SLICC study were lower than for those discontinuing the drug, perhaps judicious tapering of HCQ, guided by monitoring of blood levels, might optimise

the flare reduction benefits of HCQ while minimising risk of dose-related toxicities. Addressing barriers to care attributable to socioeconomic circumstances and limited educational opportunities might also improve compliance, reduce likelihood of flare and improve disease outcomes.

Perhaps the most striking and instructive message from the SLICC cohort study is that even in the context of continued HCQ therapy, flares are unacceptably high. The lupus research community has its marching orders, pointing to the need for improved understanding of underlying pathogenic mechanisms, therapeutic target identification, and development of effective and safe therapeutics that might ultimately surpass the benefits of HCQ.

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# Time to reconsider what Global Burden of Disease studies really tell us about low back pain

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## INTRODUCTION

Most of us have read publications where the introduction includes a statistic from one of the Global Burden of Disease (GBD) studies.[1 2] We may be told that musculoskeletal conditions are very common,[3] low back pain (LBP) is the leading cause of disability worldwide,[4] neck pain is most prevalent in Scandinavia[3] and the burden of osteoarthritis is increasing,[5] but have you ever stopped to think about the data underpinning these claims?

In this perspective, we considered three limitations of the GBD Study that need to be borne in mind when considering GBD Study results. We used LBP as an exemplar, but the limitations apply more generally. But first we begin with an introduction to the metrics used in the GBD Study.

### GBD 101

There are four GBD Study metrics commonly used to provide information on the societal impact of LBP: incidence, prevalence, years lived with disability (YLDs) and disability adjusted life years (DALYs). Incidence reflects the number of new cases of LBP. Prevalence describes the proportion of the population experiencing LBP and is important as it drives the final two metrics. YLD estimates the amount of healthy life that is lost due to poor health, where 1 YLD represents the equivalent of 1 full year of healthy life lost. DALYs combine years of life lost (YLL) due to poor health (YLD) and YLL due to premature mortality. One DALY represents 1 year of healthy life lost because of poor health or premature mortality.

The GBD Study estimates loss of healthy life with disability weights proportional to the severity of ill health. For LBP, there are six health states representing increasingly severe LBP (table 1). Severity distributions are used to describe the proportion of the population with LBP experiencing each of these six health states.

### GBD presents modelled estimates not real data

Many people may not realise that the numbers presented in the GBD Study are modelled estimates and not observed data. Estimates are provided at the global, regional and national level, for individual years, age bands and by gender. With 204 countries, 30 years, two genders and 20 age bands, the GBD 2019 Study needed to provide about one quarter of a million estimates. Some GBD studies are also reported at subnational level, for example, the 33 provinces/regions in China,[6] and so it is easy to see the enormous challenge the GBD Study faces in having sufficient observed data to inform the modelled estimates.

A fair question to ask would be to what extent do we have sufficient LBP studies to provide coverage across countries and years. The GBD 2017 Study provided estimates for 195 countries over 28 years (5460 country-years); however, the appendix reports that there were only studies to provide incidence data for 4 country-years and prevalence data for 741 country-years. This equates to 0.07% and 13.6% of the 5460 country-years in GBD 2017. While coverage seems poor, it is actually better than for the other musculoskeletal conditions (table 2).

We recently reviewed the prevalence reports used in GBD 2017 to gain a better idea of data coverage.[7] We found that there were only prevalence studies for 103 of the 204 countries, making it difficult to study global LBP burden. Judging whether LBP burden is changing over time is also challenging as only sixteen countries had at least one prevalence study for each of the GBD Study time periods 1987–1996, 1997–2006 and 2007–2017. The limited prevalence data also had significant limitations because quite often an appropriate survey instrument was not used, for example, measurements were of bodily pain not of LBP. Only 33 of the 204 countries had at least one report using an acceptable measure of LBP.

Unfortunately, GBD Study estimates can be presented in a way that suggests more certainty than is possible given the limited primary data. For example, Jin *et al*[8] report the number of incident cases of LBP for 195 countries for 1990 and 2017, but GBD 2017 only includes 4 country-years of incidence data. Wu *et al*[6] reported that for the year 2017, there were marked differences in LBP point prevalence across global regions; yet, our review[7] found that none of the prevalence studies in GBD 2017 provide prevalence data for the year 2017. Studies from China[9] (including their 33 regions), Brazil[10] (including their 27 states) and Iran[11] provide prevalence estimates for 1990 and 2016 or 2017, but there are no actual prevalence data for these countries in those years.

The GBD Study prevalence data are sparse, both across countries and years. The practice of delving into the GBD Study to provide country-specific estimates of prevalence is often unwise because frequently there will be no real data to inform the estimates. The more you cut and slice the GBD data, the worse the problem of completeness becomes. The same caveat would also apply to YLD and DALYs as both are computed from prevalence data. There are so little LBP incidence data that any GBD Study report of LBP incidence is best ignored.

**Table 1** Low back pain (LBP) disability weights[12]

| Severity level | Lay description | Disability weight |
|---|---|---|
| Mild LBP | This person has mild back pain, which causes some difficulty in dressing, standing and lifting things. | 0.020 |
| Moderate LBP | This person has moderate back pain, which causes difficulty in dressing, sitting, standing, walking and lifting things. | 0.054 |
| Severe LBP, without leg pain | This person has severe back pain, which causes difficulty in dressing, sitting, standing, walking and lifting things. The person sleeps poorly and feels worried. | 0.275 |
| Severe back pain, with leg pain | This person has severe back and leg pain, which causes difficulty in dressing, sitting, standing, walking and lifting things. The person sleeps poorly and feels worried. | 0.325 |
| Most severe LBP, without leg pain | This person has constant back pain, which causes difficulty in dressing, sitting, standing, walking and lifting things. The person sleeps poorly, is worried and has lost some enjoyment in life. | 0.372 |
| Most severe LBP, with leg pain | This person has constant back and leg pain, which causes difficulty in dressing, sitting, standing, walking and lifting things. The person sleeps poorly, is worried and has lost some enjoyment in life. | 0.384 |

### GBD ignores the LBP severity information in the original prevalence studies and instead uses a separate approach to estimate disease severity

The original prevalence studies used in the GBD Study provide information on how many people in the population have LBP, but rarely the severity of these cases. To get around this limitation, the GBD Study uses an alternative approach to estimate back pain severity.

The disability weights used to compute DALYs are derived from six LBP vignettes or health states that represent increasingly severe LBP presentations. These LBP health states, and health states for other diseases, have been presented to members of the public to judge how healthy each health state is. This process yields disability weights ranging from 0 (perfect health) to 1 (health state equivalent to death). For LBP, the disability weights range from 0.02 to 0.384.[12] The proportion of LBP cases in each of the six LBP health states is estimated using US Health Service data of people who received care for LBP.[12]

There are a few reasons why this approach may be contested. The first is that severity distributions are derived from distributions of SF-12 scores, not from distributions of the GBD disability weights. More crucially, the severity distributions are from people receiving care for LBP which may not generalise to the general population with LBP, many of whom do not seek healthcare. A review of population-based surveys of LBP found that those who sought care had higher levels of pain and disability than those that did not.[13] This suggests that generalising from the care-seeking subpopulation to the general population may overestimate the proportion experiencing more severe LBP, thereby potentially inflating LBP burden metrics. The final limitation is that the distributions are derived from US Health Service data that may not generalise to other countries. A 2019 review[14] of care seeking found that the prevalence of care seeking for LBP varied across regions: 67% in the USA versus 47% in the UK.

**Table 2** Completeness of prevalence data across musculoskeletal conditions for the 195 countries and 5460 country-years in Global Burden of Disease 2017[1]

| Condition | Countries | Country-years |
|---|---|---|
| Low back pain | 102 (52.3%) | 741 (13.6%) |
| Gout | 29 (14.9%) | 507 (9.3%) |
| Rheumatoid arthritis | 42 (21.5%) | 499 (9.1%) |
| Knee osteoarthritis | 26 (13.3%) | 395 (7.2%) |
| Neck pain | 23 (11.8%) | 388 (7.1% |
| Hip osteoarthritis | 24 (12.3%) | 350 (6.4%) |
| Other musculoskeletal conditions | 18 (9.2%) | 348 (6.4% |

### LBP severity distributions are assumed to be constant over time and location

The GBD Study uses the same LBP severity distributions over time and location. The assumption is that the relative proportion of people with LBP who are suffering, for example, severe health loss, is the same across time and countries.[15] This practice results in a linear relationship between YLD and prevalence, both over time and across countries.[15] That means that the only driver in differences in YLD across time or location is prevalence.

The convention of using the same LBP severity splits may limit our ability to appreciate the societal burden of LBP if the severity of LBP is changing over time or differs by location. It is well accepted that the impact of an episode of LBP can be influenced by factors such as work, health and social systems which can vary substantially between countries,[16] but those influences will be invisible within the GBD Study. Some have argued that LBP should be portrayed as a normal life experience[17] and that the role of healthcare should be to reduce the consequences of LBP, particularly disabling chronic LBP. The call to action paper in the *Lancet* LBP series[17] argued that by improving health and social systems, over time we could reduce LBP burden. With the current modelling approach we will never be able to see if that is happening as differences in YLDs and DALYs, over time and across locations are just driven by prevalence. This means the GBD Study methods preclude examination of temporal changes or regional differences in the burden of LBP.

## CONCLUSION

The GBD Study aims to measure, among other things, the global burden of LBP. Lack of primary data and some of the approaches taken to modelling mean that the GBD Study estimates need to be interpreted with caution. It is possible that the high profile LBP enjoys in disease league tables created with GBD Study metrics and has blinded LBP researchers to the limitations of the GBD Study.

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# 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology Classification Criteria for Eosinophilic Granulomatosis with Polyangiitis

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## ABSTRACT

**Objective** To develop and validate revised classification criteria for eosinophilic granulomatosis with polyangiitis (EGPA).

**Methods** Patients with vasculitis or comparator diseases were recruited into an international cohort. The study proceeded in five phases: (1) identification of candidate criteria items using consensus methodology, (2) prospective collection of candidate items present at the time of diagnosis, (3) data-driven reduction of the number of candidate items, (4) expert panel review of cases to define the reference diagnosis and (5) derivation of a points-based risk score for disease classification in a development set using least absolute shrinkage and selection operator logistic regression, with subsequent validation of performance characteristics in an independent set of cases and comparators.

**Results** The development set for EGPA consisted of 107 cases of EGPA and 450 comparators. The validation set consisted of an additional 119 cases of EGPA and 437 comparators. From 91 candidate items, regression analysis identified 11 items for EPGA, 7 of which were retained. The final criteria and their weights were as follows: maximum eosinophil count ≥$1\times10^9$/L (+5), obstructive airway disease (+3), nasal polyps (+3), cytoplasmic antineutrophil cytoplasmic antibody (ANCA) or anti-proteinase 3–ANCA positivity (−3), extravascular eosinophilic predominant inflammation (+2), mononeuritis multiplex/motor neuropathy not due to radiculopathy (+1) and haematuria (−1). After excluding mimics of vasculitis, a patient with a diagnosis of small- or medium-vessel vasculitis could be classified as having EGPA if the cumulative score was ≥6 points. When these criteria were tested in the validation data set, the sensitivity was 85% (95% CI 77% to 91%) and the specificity was 99% (95% CI 98% to 100%).

**Conclusion** The 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology Classification Criteria for Eosinophilic Granulomatosis with Polyangiitis demonstrate strong performance characteristics and are validated for use in research.

## INTRODUCTION

Eosinophilic granulomatosis with polyangiitis (EGPA), formerly known as Churg-Strauss syndrome, is a form of vasculitis that is histologically defined by eosinophil-rich, necrotising granulomatous inflammation primarily involving the respiratory tract, along with necrotising vasculitis of small- to medium-sized arteries.[1] EGPA is considered a form of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), along with granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA). ANCAs are detected in ∼40% to 60% of patients with EGPA and are typically directed against myeloperoxidase (MPO).[2 3]

In 1990, the American College of Rheumatology (ACR) published classification criteria for EGPA.[4] By current standards, these criteria have never been validated because they were developed using data from only 20 patients with EGPA without independent test and validation sets. Furthermore, the criteria were derived by comparing clinical data from patients with EGPA to data from 787 patients with other forms of vasculitis. Many of these comparators were patients with giant cell arteritis, a form of large-vessel vasculitis that is typically not difficult to readily distinguish from EGPA based on obvious clinical differences. Despite these methodological weaknesses, the 1990 ACR criteria for EGPA have existed unchanged for several decades and have been useful to advance clinical research in these diseases. This article outlines the development and validation of the new ACR/European Alliance of Associations for Rheumatology (EULAR)-endorsed classification criteria for EGPA.

## METHODS

A detailed and complete description of the methods involved in the development and validation of the classification criteria for EGPA is provided in online supplemental appendix 1. Briefly, an international steering committee comprising clinician investigators with expertise in vasculitis, statisticians and data managers was established to oversee the overall Diagnostic and Classification Criteria in Vasculitis (DCVAS) project.[5] The steering committee established a five-stage plan using data-driven and consensus methodology to develop the criteria for each of six forms of vasculitis.

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

Candidate classification items were generated by expert opinion and were reviewed by a group of

vasculitis experts across a range of specialties using a nominal group technique.

### Stage 2: DCVAS prospective observational study

A prospective, international, multisite observational study was conducted (see collaborators for study investigators and sites). Consecutive patients representing the full spectrum of disease were recruited from academic and community practices. Patients were included if they were 18 years or older and had a diagnosis of vasculitis or a condition that mimics vasculitis. Patients with AAV could only be enrolled within 2 years of diagnosis. Only data present at diagnosis were recorded.

### Stage 3: refinement of candidate items specifically for AAV

The steering committee conducted a data-driven process to reduce the number of candidate items of relevance to cases and comparators for AAV. Items were selected for exclusion if they had a prevalence of <5% within the data set and/or they were not clinically relevant for classification criteria (eg, related to infection, malignancy or demographic characteristics). Low-frequency items of clinical importance could be combined, when appropriate.

### Stage 4: expert review to derive a gold standard-defined final set of cases of AAV

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

### Stage 5: derivation and validation of the final classification criteria for EGPA

The DCVAS AAV data set was randomly split into development (50%) and validation (50%) sets. Comparisons were performed between cases of EGPA and a comparator group randomly selected from the DCVAS cohort in the following proportions: another type of AAV (including GPA and MPA), 60%; and another form of small-vessel vasculitis (eg, cryoglobulinemic vasculitis) or medium-vessel vasculitis (eg, polyarteritis nodosa), 40%. Least absolute shrinkage and selection operator (lasso) logistic regression was used to identify items from the data set and to create a parsimonious model including only the most important items. The final items in the model were formulated into a clinical risk-scoring tool with each factor assigned a weight based on its respective regression coefficient. A threshold that best balanced sensitivity and specificity was identified for classification.

In sensitivity analyses, the final classification criteria were applied to an unselected population of cases and comparators from the DCVAS data set based on the submitting physician diagnosis. Comparison was also made between the measurement properties of the new classification criteria for EGPA and the 1990 ACR classification criteria for EGPA using pooled data from the development and validation sets.

## RESULTS

### Generation of candidate classification items for the systemic vasculitides

The steering committee identified >1000 candidate items for the DCVAS case report form (see online supplemental appendix 2, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41982/abstract).

### DCVAS prospective observational study

Between January 2011 and December 2017, the DCVAS study recruited 6991 participants from 136 sites in 32 countries. Information on the DCVAS sites, investigators and participants is listed in online supplemental appendices 3–5, available on the *Arthritis & Rheumatology* website (http://onlinelibrarywileycom/doi/101002/art41982/abstract).

### Refinement of candidate items specifically for AAV

Following a data-driven and expert consensus process, 91 items from the DCVAS case report form were retained for regression analysis, including 45 clinical (14 composite), 18 laboratory (two composite), 12 imaging (all composite) and 16 biopsy (one composite) items. Some clinical items were removed in favour of similar but more specific pathophysiological descriptors. For example, 'hearing loss or reduction' was removed, and the composite item 'conductive hearing loss/sensorineural hearing loss' was retained. See online supplemental appendix 6, available on the *Arthritis & Rheumatology* website (http://onlinelibrary.wiley.com/doi/10.1002/art.41982/abstract), for the final candidate items used in the derivation of the classification criteria for GPA, MPA and EGPA.

### Expert review to derive a gold standard-defined final set of cases of AAV

Fifty-five independent experts reviewed vignettes derived from the case report forms for 2871 cases submitted with a diagnosis of either small-vessel vasculitis (90% of case report forms) or another type of vasculitis or a mimic of vasculitis (10% of case report forms). The characteristics of the expert reviewers are shown in online supplemental appendix 7. A flowchart showing the results of the expert review process is shown in online supplemental appendix 8. A total of 2072 cases (72%) passed the process and were designated as cases of vasculitis; these cases were used for the stage 5 analyses.

After expert panel review, 226 of 315 cases of EGPA were retained for subsequent analysis. Compared with patients who were retained, patients who were excluded from further analysis had significantly higher serum creatinine levels (mean±SD 102.8±88.7 vs 85.0±53.6 $\mu$mol/L, p=0.03) and lower rates of MPO-ANCA positivity (22% vs 43%, p<0.01), and were less likely to have maximum eosinophil counts $\geq 1\times10^9$/L (62% vs 92%, p<0.01). There were 887 comparators randomly selected for analysis. Table 1 shows the demographic and disease features of the 1113 cases included in this analysis (226 patients with EGPA and 887 comparators), of which 557 (50%, 107 patients with EGPA and 450 comparators) were in the development set and 556 (50%, 119 patients with EGPA and 437 comparators) were in the validation set.

### Derivation and validation of the final classification criteria for EGPA

Lasso regression of the previously selected 91 items yielded 11 independent items for EGPA (online supplemental appendix 9A). Each item was then adjudicated by the DCVAS Steering Committee for inclusion based on clinical relevance and specificity to EGPA, resulting in seven final items. Weighting of an individual criterion was based on logistic regression fitted to the seven selected items (see online supplemental appendix 10A).

**Table 1** Demographic and disease features of cases of EGPA and comparators*

| | EGPA (n=226) | Comparators (n=887)* | P value |
|---|---|---|---|
| Age (years), mean±SD | 52.9±14.4 | 56.2±17.6 | 0.009 |
| Sex: female, n (%) | 113 (50.0) | 445 (50.2) | 1.000 |
| Maximum serum creatinine, mean±SD | | | <0.001 |
| μmol/L | 85.0±53.6 | 205.90±237.0 | |
| mg/dL | 0.96±0.6 | 2.33±2.7 | |
| cANCA positive, n (%) | 17 (7.5) | 251 (28.3) | <0.001 |
| pANCA positive, n (%) | 83 (36.7) | 289 (32.6) | 0.271 |
| Anti-PR3-ANCA positive, n (%) | 7 (3.1) | 264 (29.8) | <0.001 |
| Anti-MPO-ANCA positive, n (%) | 98 (43.4) | 323 (36.4) | 0.065 |
| Maximum eosinophil count ≥1×10$^9$/L, n (%) | 208 (92.0) | 53 (6.0) | <0.001 |

*Diagnoses of comparators for the classification criteria for EGPA included granulomatosis with polyangiitis (n=300), microscopic polyangiitis (n=291), polyarteritis nodosa (n=51), non-ANCA-associated small-vessel vasculitis that could not be subtyped (n=51), Behçet's disease (n=50), IgA vasculitis (n=50), cryoglobulinemic vasculitis (n=34), ANCA-associated vasculitis that could not be subtyped (n=25), primary central nervous system vasculitis (n=19) and antiglomerular basement membrane disease (n=16).
cANCA, cytoplasmic antineutrophil cytoplasmic antibody; EGPA, eosinophilic granulomatosis with polyangiitis; MPO-ANCA, myeloperoxidase–antineutrophil cytoplasmic antibody; pANCA, perinuclear antineutrophil cytoplasmic antibody; PR3-ANCA, proteinase 3–antineutrophil cytoplasmic antibody.

### Model performance

Use of a cut-off of ≥6 for total risk score (see online supplemental appendix 11A for different cut points) yielded a sensitivity of 84.9% (95% CI 77.2% to 90.8%) and a specificity of 99.1% (95% CI 98.3% to 99.8%) in the validation set. The area under the curve (AUC) for the model was 0.98 (95% CI 0.97 to 1.00) in the development set and 0.99 (95% CI 0.97 to 1.00) in the validation set for the final EGPA classification criteria (online supplemental appendix 12A). The final classification criteria for EGPA are presented in figure 1 (for the slide presentation version, see online supplemental figure 1.

### Sensitivity analyses

The classification criteria for EGPA were applied to 2871 patients in the DCVAS database using the original physician-submitted diagnosis (n=315 EGPA and 2556 randomly selected comparators). Use of the same cut point of ≥6 points for the classification of EGPA yielded a similar specificity of 99% but a lower sensitivity of 75%. This upheld the a priori hypothesis that specificity would remain unchanged, but sensitivity would be reduced in a population of patients that included fewer clear-cut diagnoses of EGPA (ie, cases that did not pass expert panel review).

When the 1990 ACR classification criteria for EGPA were applied to the DCVAS data set, the criteria performed poorly due to low sensitivity (44%) but retained excellent specificity (99%), with an AUC of 0.72 (95% CI 0.68 to 0.75).

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## CLASSIFICATION CRITERIA FOR EOSINOPHILIC GRANULOMATOSIS WITH POLYANGIITIS

**CONSIDERATIONS WHEN APPLYING THESE CRITERIA**

- These classification criteria should be applied to classify a patient as having eosinophilic granulomatosis with polyangiitis when a diagnosis of small- or medium-vessel vasculitis has been made
- Alternate diagnoses mimicking vasculitis should be excluded prior to applying the criteria

**CLINICAL CRITERIA**

| | |
|---|---|
| Obstructive airway disease | +3 |
| Nasal polyps | +3 |
| Mononeuritis multiplex | +1 |

**LABORATORY AND BIOPSY CRITERIA**

| | |
|---|---|
| Blood eosinophil count ≥ 1 x10$^9$/liter | +5 |
| Extravascular eosinophilic-predominant inflammation on biopsy | +2 |
| Positive test for cytoplasmic antineutrophil cytoplasmic antibodies (cANCA) or antiproteinase 3 (anti-PR3) antibodies | -3 |
| Hematuria | -1 |

**Sum the scores for 7 items, if present. A score of ≥ 6 is needed for classification of EOSINOPHILIC GRANULOMATOSIS WITH POLYANGIITIS.**

**Figure 1** 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology Classification Criteria for Eosinophilic Granulomatosis with Polyangiitis.

## DISCUSSION

Presented here are the final 2022 ACR/EULAR EGPA classification criteria. A five-stage approach has been used, underpinned by data from the multinational prospective DCVAS study and informed by expert review and consensus at each stage. The comparator group for developing and validating the criteria was patients with other forms of AAV and other small- and medium-vessel vasculitides, which are the clinical entities where discrimination from EGPA is difficult but important. The new criteria for EGPA have excellent sensitivity and specificity and incorporate ANCA testing. The criteria were designed to have face and content validity for use in clinical trials and other research studies.

These criteria are validated and intended for the purpose of *classification* of vasculitis and are not appropriate for use in establishing a *diagnosis* of vasculitis. The aim of the classification criteria is to differentiate cases of EGPA from similar types of vasculitis in research settings. Therefore, the criteria should only be applied when a diagnosis of small- or medium-vessel vasculitis has been made and all potential 'vasculitis mimics' have been excluded. The exclusion of mimics is a key aspect of many classification criteria, including those for Sjögren's syndrome[6] and rheumatoid arthritis.[7] The 1990 ACR classification criteria for vasculitis perform poorly when used for diagnosis (ie, when used to differentiate between cases of vasculitis vs mimics without vasculitis),[8] and it is expected that the 2022 criteria would also perform poorly if used inappropriately as diagnostic criteria in people in whom alternative diagnoses, such as infection or other non-vasculitis inflammatory diseases, are still being considered. Specifically, the criteria were not developed to differentiate patients with EGPA from those with other related hypereosinophilic syndromes or eosinophilic malignancies.[9]

The 2022 ACR/EULAR EGPA classification criteria reflect the collaborative effort of the international vasculitis community to delineate the salient clinical features that differentiate EGPA from other forms of vasculitis. The final criteria include seven clinical items that are easily assessed during routine clinical evaluation of patients with EGPA. The criteria highlight the importance of blood eosinophilia, asthma and eosinophilic inflammation to classify EGPA among other forms of vasculitis and specify additional features (eg, nasal polyps and mononeuritis multiplex) that function as important disease classifiers. Classification criteria are intended to define a homogeneous group of patients with a particular disease for inclusion into clinical research studies. By maximising specificity, the revised criteria for EGPA ensure that few cases will inappropriately meet the criteria threshold of $\geq 6$ points; thus, these criteria will function to facilitate the conduct of future clinical trials and other studies in EGPA.

The negative items included in the final criteria underscore that these criteria are intended for use as classification, not diagnostic, criteria to differentiate EGPA from other forms of vasculitis in research settings. Both haematuria and antiproteinase 3–antineutrophil cytoplasmic antibody (anti-PR3-ANCA) function as negative items in the new EGPA classification criteria, yet glomerulonephritis and ANCA are features of disease that, when present, can be useful to diagnose EGPA. When compared with other forms of AAV, however, biopsy-proven glomerulonephritis was significantly less common in the DCVAS cohort in patients with EGPA (4.9%) compared with those with GPA (27.8%) or MPA (48.5%). Similarly, anti-PR3-ANCAs have been reported in few patients with EGPA but are much more prevalent in GPA.[10] For these reasons, haematuria and anti-PR3-ANCAs work against a patient with small-vessel vasculitis being classified as having EGPA. Although anti-MPO-ANCAs can be detected in 40%–60% of patients with EGPA, anti-MPO-ANCA positivity was not included in the final criteria because these antibodies are significantly more prevalent in diseases like MPA and thus are not discriminant classifiers for EGPA.[11]

There are some study limitations to consider. Although this was the largest international study ever conducted in vasculitis, most patients were recruited from Europe, Asia and North America. The performance characteristics of the criteria should be further tested in African and South American populations, which may have different clinical presentations of vasculitis. These criteria were developed using data collected from adult patients with vasculitis. Although the clinical characteristics of EGPA and the other vasculitides which these criteria were tested against are not known to differ substantially between adults and children, these criteria should be applied to children with some caution. The scope of the criteria is intentionally narrow and applies only to patients who have been diagnosed as having vasculitis. Diagnostic criteria are not specified. The criteria are intended to identify homogeneous populations of disease and, therefore, may not be appropriate for studies focused on the full spectrum of clinical heterogeneity in these conditions. To maximise relevance and face validity of the new criteria, study sites and expert reviewers were recruited from a broad range of countries and different medical specialties. Nonetheless, the majority of patients were recruited from academic rheumatology or nephrology units, which could have introduced referral bias.

There are several strengths to the new 2022 ACR/EULAR EGPA classification criteria. The criteria were developed within a large cohort reflecting international expertise in systemic vasculitis according to ACR guidance for classification criteria development.[11] The criteria represent several important methodological advancements compared with the original 1990 ACR classification criteria for EGPA. Expert review rather than submitting physician diagnosis was used as the diagnostic reference standard to minimise investigator bias. Second, while the 1990 ACR criteria were entirely derived from 20 patients with EGPA and were not validated, the new criteria were developed in 107 patients with EGPA and were validated in an independent test set that contained an additional 119 patients with EGPA. Third, unlike the 1990 ACR criteria, the new ACR/EULAR EGPA criteria are weighted to reflect the relative importance of specific items (eg, eosinophil counts). Finally, when both criteria sets were tested within the DCVAS cohort, the performance characteristics of the 1990 ACR criteria were suboptimal when compared with the 2022 revised ACR/EULAR EGPA criteria.

The 2022 ACR/EULAR classification criteria for EGPA are the product of a rigorous methodological process that used an extensive data set generated by the work of a remarkable international group of collaborators. These criteria have been endorsed by the ACR and EULAR and are now ready for use to differentiate one type of vasculitis from another to define populations in research studies.

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# 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for granulomatosis with polyangiitis

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## ABSTRACT

**Objective** To develop and validate revised classification criteria for granulomatosis with polyangiitis (GPA).

**Methods** Patients with vasculitis or comparator diseases were recruited into an international cohort. The study proceeded in five phases: (1) identification of candidate criteria items using consensus methodology, (2) prospective collection of candidate items present at the time of diagnosis, (3) data-driven reduction of the number of candidate items, (4) expert panel review of cases to define the reference diagnosis and (5) derivation of a points-based risk score for disease classification in a development set using least absolute shrinkage and selection operator logistic regression, with subsequent validation of performance characteristics in an independent set of cases and comparators.

**Results** The development set for GPA consisted of 578 cases of GPA and 652 comparators. The validation set consisted of an additional 146 cases of GPA and 161 comparators. From 91 candidate items, regression analysis identified 26 items for GPA, 10 of which were retained. The final criteria and their weights were as follows: bloody nasal discharge, nasal crusting or sino-nasal congestion (+3); cartilaginous involvement (+2); conductive or sensorineural hearing loss (+1); cytoplasmic antineutrophil cytoplasmic antibody (ANCA) or anti-proteinase 3 ANCA positivity (+5); pulmonary nodules, mass or cavitation on chest imaging (+2); granuloma or giant cells on biopsy (+2); inflammation or consolidation of the nasal/paranasal sinuses on imaging (+1); pauci-immune glomerulonephritis (+1); perinuclear ANCA or antimyeloperoxidase ANCA positivity (−1); and eosinophil count ≥$1\times10^9$/L (−4). After excluding mimics of vasculitis, a patient with a diagnosis of small- or medium-vessel vasculitis could be classified as having GPA if the cumulative score was ≥5 points. When these criteria were tested in the validation data set, the sensitivity was 93% (95% CI 87% to 96%) and the specificity was 94% (95% CI 89% to 97%).

**Conclusion** The 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for GPA demonstrate strong performance characteristics and are validated for use in research.

## INTRODUCTION

The antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) are multisystem disorders involving inflammation of the small blood vessels and include granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA).[1] GPA is characterised by necrotising granulomatous inflammation involving the ears, nose and upper and lower respiratory tracts, and necrotising vasculitis affecting predominantly small- to medium-sized vessels, often including necrotising glomerulonephritis.[1]

Unlike diagnostic criteria, the purpose of classification criteria is to ensure that a homogeneous population is selected for inclusion in clinical trials and other research studies of GPA. In 1990, the American College of Rheumatology (ACR) published criteria for the classification of GPA (then named Wegener's granulomatosis).[2–4] The 1990 criteria were effective and widely accepted, facilitating coordinated approaches to international randomised controlled trials.[5 6] In 2011 it was proposed to change the name 'Wegener's granulomatosis' to 'granulomatosis with polyangiitis' with subsequent wide adoption of the new terminology.[7–9] The 1994 and 2012 publications of the International Chapel Hill Consensus Conference (CHCC) nomenclature for vasculitis clarified and standardised the nomenclature of the systemic vasculitides.[1 10] The CHCC is a nomenclature system based on expert consensus rather than a classification system.[1]

There are several important reasons for the development of revised classification criteria for the vasculitides, including a decline in the sensitivity of the 1990 ACR classification criteria, particularly for AAV[11]; a consensus that any such criteria must now incorporate testing for ANCA; increased and widespread use, since 1990, of cross-sectional diagnostic imaging tools, including MRI and CT[12 13]; and the introduction and adoption of the classification of patients with MPA, a term not in use in the 1990 ACR classification criteria.

There have been methodological advances in the derivation of classification criteria, moving from the 'number of criteria' rule, as used in the ACR 1990 criteria,[3] toward weighted criteria with threshold scores, as demonstrated in the 2010 classification criteria for rheumatoid arthritis.[14] Weighted criteria improve measurement properties of classification

criteria because certain items within a criteria list may be more discriminative. The previous 1990 criteria for vasculitis collected retrospective data from patient files, without specification of which items were relevant at the time of diagnosis compared with those that were important later in the disease process. Criteria based on prospectively collected data sets from newly diagnosed patients should have higher face validity as inclusion criteria for future clinical trials of early-stage disease. This article outlines the development and validation of the revised ACR/European Alliance of Associations for Rheumatology (EULAR)—endorsed classification criteria for GPA.

## METHODS

A detailed and complete description of the methods involved in the development and validation of the classification criteria for GPA is provided in online supplemental appendix 1. Briefly, an international Steering Committee comprising clinician investigators with expertise in vasculitis, statisticians, and data managers was established to oversee the overall Diagnostic and Classification Criteria in Vasculitis (DCVAS) project.[15] The Steering Committee established a 5-stage plan using data-driven and consensus methodology to develop the criteria for each of six forms of vasculitis.

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

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

### Stage 2: DCVAS prospective observational study

A prospective, international, multisite observational study was conducted (see collaborators for study investigators and sites). Consecutive patients representing the full spectrum of disease were recruited from academic and community practices. Patients were included if they were 18 years or older and had a diagnosis of vasculitis or a condition that mimics vasculitis. Patients with AAV could only be enrolled within 2 years of diagnosis. Only data present at diagnosis were recorded.

### Stage 3: refinement of candidate items specifically for AAV

The Steering Committee conducted a data-driven process to reduce the number of candidate items of relevance to cases and comparators for AAV. Items were selected for exclusion if they had a prevalence of <5% within the data set and/or they were not clinically relevant for classification criteria (eg, related to infection, malignancy or demographic characteristics). Low-frequency items of clinical importance could be combined, when appropriate.

### Stage 4: expert review to derive a gold standard—defined set of cases of AAV

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

### Stage 5: derivation and validation of the final classification criteria for GPA

The DCVAS AAV data set was randomly split into development (80%) and validation (20%) sets. Comparisons were performed between cases of GPA confirmed by expert review and a comparator group randomly selected from the DCVAS cohort in the following proportions: another type of AAV (including MPA and EGPA), 64%; another form of small-vessel vasculitis (eg, cryoglobulinaemic vasculitis) or medium-vessel vasculitis (eg, polyarteritis nodosa), 36%. Least absolute shrinkage and selection operator (lasso) logistic regression was used to identify items from the data set and create a parsimonious model including only the most important items. The final items in the model were formulated into a clinical risk-scoring tool with each factor assigned a weight based on its respective regression coefficient. A threshold that best balanced sensitivity and specificity was identified for classification.

In sensitivity analyses, the final classification criteria were applied to an unselected population of cases and comparators from the DCVAS data set based on the submitting physician diagnosis. Comparison was also made between the measurement properties of the new classification criteria for GPA and the 1990 ACR classification criteria for GPA using pooled data from the development and validation sets.

## RESULTS

### Generation of candidate classification items for the systemic vasculitides

The Steering Committee identified >1000 candidate items for the DCVAS case report form (see online supplemental appendix 2.

### DCVAS prospective observational study

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

### Refinement of candidate items specifically for AAV

Following a data-driven and expert consensus process, 91 items from the DCVAS case report form were retained for regression analysis, including 45 clinical (14 composite), 18 laboratory (2 composite), 12 imaging (all composite) and 16 biopsy (1 composite) items. Some clinical items were removed in favour of similar but more specific pathophysiological descriptors. Online supplemental appendix 6, lists the final candidate items used in the derivation of the classification criteria for GPA, MPA and EGPA.

### Expert review to derive a gold standard—defined final set of cases of AAV

Fifty-five independent experts reviewed vignettes derived from the case report forms for 2871 cases submitted with a diagnosis of either small-vessel vasculitis (90% of case report forms) or another type of vasculitis or a mimic of vasculitis (10% of case report forms). The characteristics of the expert reviewers are shown in online supplemental appendix 7. A flow chart showing the results of the expert review process is shown in online supplemental appendix 8. A total of 2072 cases (72%) passed the process and were designated as cases of vasculitis; these cases were used for the stage 5 analyses.

After expert review, 724 of 843 cases retained a reference diagnosis of GPA. There were 813 comparators randomly selected

**Table 1** Demographic and disease features of cases of GPA and comparators*

| | GPA (n=724) | Comparators (n=813)* | P value |
|---|---|---|---|
| Age, mean±SD years | 53.6±16.2 | 56.4±17.1 | 0.001 |
| Sex, no. (%) female | 340 (47.0) | 424 (52.2) | 0.048 |
| Maximum serum creatinine, mean | | | 0.077 |
| μmoles/L | 168.3 | 185.2 | |
| mg/dL | 1.9 | 2.1 | |
| cANCA positive, no. (%) | 531 (73.3) | 40 (4.9) | <0.001 |
| pANCA positive, no. (%) | 71 (9.8) | 342 (42.1) | <0.001 |
| Anti-PR3-ANCA positive, no. (%) | 595 (82.2) | 21 (2.6) | <0.001 |
| Anti-MPO-ANCA positive, no. (%) | 59 (8.1) | 399 (49.1) | <0.001 |
| Maximum eosinophil count ≥1×$10^9$/L, no. (%) | 196 (27) | 366 (45) | <0.001 |

*Diagnoses of comparators for the classification criteria for granulomatosis with polyangiitis (GPA) included microscopic polyangiitis (n=291), eosinophilic granulomatosis with polyangiitis (n=226), polyarteritis nodosa (n=51), non-ANCA-associated small-vessel vasculitis that could not be subtyped (n=51), Behçet's disease (n=50), IgA vasculitis (n=50), cryoglobulinaemic vasculitis (n=34), ANCA-associated vasculitis that could not be subtyped (n=25), primary central nervous system vasculitis (n=19) and antiglomerular basement membrane disease (n=16). ANCA, antineutrophil cytoplasmic antibody; anti-MPO-ANCA, anti-myeloperoxidase-ANCA; anti-PR3-ANCA, anti-proteinase 3-ANCA; cANCA, cytoplasmic antineutrophil cytoplasmic antibody; GPA, granulomatosis with polyangiitis; pANCA, perinuclear ANCA.

for analysis. Table 1 shows the demographic and disease features of the 1537 cases included in this analysis (724 patients with GPA and 813 comparators), of which 1230 (80%; 578 patients with GPA and 652 comparators) were in the development set, and 307 (20%; 146 patients with GPA and 161 comparators) were in the validation set.

### Derivation and validation of the final classification criteria for GPA

Lasso logistic regression analysis using all 91 items resulted in a model of 26 independent items (see online supplemental appendix 9B). The variables 'positive test for cytoplasmic ANCA (cANCA)' and 'positive test for anti-proteinase 3 (anti-PR3) antibody' and the variables 'positive test for perinuclear ANCA (pANCA)' and 'positive test for antimyeloperoxidase (anti-MPO) antibody' were strongly colinear and were combined within the model as 'positive test for cANCA or positive test for anti-PR3 antibody' and 'positive test for pANCA or positive test for anti-MPO antibody', respectively. Each item was scrutinised for inclusion based on statistical significance, clinical relevance and specificity to GPA, resulting in 10 final items. Weighting of an individual criterion was based on logistic regression fitted to the 10 selected items (see online supplemental appendix 10B).

### Model performance

Use of a cut-off of ≥5 for total risk score (see online supplemental appendix 11B, for different cut points) yielded a sensitivity of 92.5% (95% CI 86.9% to 96.2%) and a specificity of 93.8% (95% CI 88.9% to 97.0%) in the validation set. The area under the curve (AUC) for the model was 0.98 (95% CI 0.98 to 0.99) in the development set and 0.99 (95% CI 0.98 to 1.00) in the validation set (online supplemental appendix 12B). The final classification criteria for GPA are shown in figure 1 (for the slide presentation version, see online supplemental figure 1).

### Sensitivity analyses

The classification criteria for GPA were applied to 2511 patients randomly selected from the DCVAS database using the original physician-submitted diagnosis (n=483 GPA and 2028 comparators). Use of the same cut point of ≥5 points for the classification of GPA yielded a similar specificity of 94.6% but a lower sensitivity of 83.8%. This upheld the a priori hypothesis that specificity would remain unchanged but sensitivity would be reduced in a population with fewer clear-cut diagnoses of GPA (ie, cases that did not pass expert review).

When the 1990 ACR classification criteria for GPA were applied to the DCVAS data set, the criteria performed poorly due to low sensitivity (69.3%) and moderate specificity (75.8%), with an AUC of 0.73 (95% CI 0.70 to 0.75).

## DISCUSSION

Presented here are the final 2022 ACR/EULAR GPA classification criteria. A 5-stage approach has been used, underpinned by data from the multinational prospective DCVAS study and informed by expert review and consensus at each stage. The comparator group for developing and validating the criteria were other forms of AAV and other small- and medium-vessel vasculitides, the clinical entities where discrimination from GPA is difficult, but important. The new criteria for GPA have excellent sensitivity and specificity and incorporate ANCA testing and modern imaging techniques. The criteria were designed to have face and content validity for use in clinical trials and other research studies.

These criteria are validated and intended for the purpose of *classification* of vasculitis and are not appropriate for use in establishing a *diagnosis* of vasculitis. The aim of the classification criteria is to differentiate cases of GPA from similar types of vasculitis in research settings. Therefore, the criteria should only be applied when a diagnosis of small- or medium-vessel vasculitis has been made and all potential 'vasculitis mimics' have been excluded. The exclusion of mimics is a key aspect of many classification criteria, including those for Sjögren's syndrome[16] and rheumatoid arthritis.[14] The 1990 ACR classification criteria for vasculitis perform poorly when used for diagnosis (ie, when used to differentiate between cases of vasculitis vs mimics without vasculitis),[17] and it is expected that the 2022 criteria would also perform poorly if used inappropriately as diagnostic criteria in people in whom alternative diagnoses, such as infection or other non-vasculitis inflammatory diseases, are still being considered. The relatively low weight assigned to glomerulonephritis in these classification criteria highlights the distinction between classification and diagnostic criteria. While detection of kidney disease is important to diagnose GPA, glomerulonephritis is common among patients with either GPA or MPA and thus does not function as a strong classifier between these conditions.

These criteria differ from the previous 1990 ACR criteria in that they have been developed using cases presenting prospectively at the start of their disease process. This approach is different from the methods used to generate the 1990 ACR criteria, in which prevalent case records were used, potentially including items related to irreversible damage accrued over time. Inclusion of newly diagnosed cases in these criteria should improve their accuracy within the context of early intervention trials as well as refractory disease. The comparators used for these new criteria are also more appropriate and are closer mimics of GPA; for example, comparators with predominantly small-vessel vasculitis rather than predominantly giant cell arteritis were included. The new criteria perform better than previous criteria within this

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## CLASSIFICATION CRITERIA FOR GRANULOMATOSIS WITH POLYANGIITIS

### CONSIDERATIONS WHEN APPLYING THESE CRITERIA

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

### CLINICAL CRITERIA

| | |
|---|---|
| Nasal involvement: bloody discharge, ulcers, crusting, congestion, blockage, or septal defect / perforation | +3 |
| Cartilaginous involvement (inflammation of ear or nose cartilage, hoarse voice or stridor, endobronchial involvement, or saddle nose deformity) | +2 |
| Conductive or sensorineural hearing loss | +1 |

### LABORATORY, IMAGING, AND BIOPSY CRITERIA

| | |
|---|---|
| Positive test for cytoplasmic antineutrophil cytoplasmic antibodies (cANCA) or antiproteinase 3 (anti-PR3) antibodies | +5 |
| Pulmonary nodules, mass, or cavitation on chest imaging | +2 |
| Granuloma, extravascular granulomatous inflammation, or giant cells on biopsy | +2 |
| Inflammation, consolidation, or effusion of the nasal/paranasal sinuses, or mastoiditis on imaging | +1 |
| Pauci-immune glomerulonephritis on biopsy | +1 |
| Positive test for perinuclear antineutrophil cytoplasmic antibodies (pANCA) or antimyeloperoxidase (anti-MPO) antibodies | -1 |
| Blood eosinophil count ≥ 1 x$10^9$/liter | -4 |

**Sum the scores for 10 items, if present. A score of ≥ 5 is needed for classification of GRANULOMATOSIS WITH POLYANGIITIS.**

**Figure 1** 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for granulomatosis with polyangiitis.

data set.[11] ANCA is a major discriminator within these criteria, although patients can be classified as having GPA without having a positive test result for ANCA if they have a sufficient number of other features. These new criteria were validated in an independent data set and are weighted with threshold scores[14 16] to maximise predictive ability.

There are some study limitations to consider. Although this was the largest international study ever conducted in vasculitis, most patients were recruited from Europe, Asia and North America. The performance characteristics of the criteria should be further tested in African and South American populations, which may have different clinical presentations of vasculitis. These criteria were developed using data collected from adult patients with vasculitis. Although the clinical characteristics of GPA and the other vasculitides which these criteria were tested against are not known to differ substantially between adults and children, these criteria should be applied to children with some caution. The scope of the criteria is intentionally narrow and applies only to patients who have been diagnosed as having vasculitis. Diagnostic criteria are not specified. The criteria are intended to identify homogeneous populations of disease and, therefore, may not be appropriate for studies focused on the full spectrum of clinical heterogeneity in these conditions. To maximise relevance and face validity of the new criteria, study sites and expert reviewers were recruited from a broad range of countries and different medical specialties. Nonetheless, the majority of patients were recruited from academic rheumatology or nephrology units, which could have introduced referral bias.

A key strength of this study is the use of an independent expert review process to confirm cases of GPA and comparators to avoid the circularity of using predefined criteria to define the gold standard. Approximately one-quarter of cases were excluded via this process, due to either a lack of consensus on exact diagnosis or insufficient data available to make the diagnosis. A limitation of this approach, however, could be the exclusion of true, but less clearcut cases submitted by the original physicians. It is important that cases are classified accurately for inclusion in clinical trials; therefore, some loss of sensitivity

may be appropriate. Importantly, this study also demonstrated that applying the new criteria for GPA to the whole unselected DCVAS data set resulted in a reduction in sensitivity while maintaining specificity. Thus, the criteria should also be useful in a more generalised, 'real-world' population.

The 2022 ACR/EULAR classification criteria for GPA are the product of a rigorous methodological process that used an extensive data set generated by the work of a remarkable international group of collaborators. These criteria have been endorsed by the ACR and EULAR and are now ready for use to differentiate one type of vasculitis from another to define populations in research studies.

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# 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for microscopic polyangiitis

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## ABSTRACT

**Objective** To develop and validate classification criteria for microscopic polyangiitis (MPA).

**Methods** Patients with vasculitis or comparator diseases were recruited into an international cohort. The study proceeded in five phases: (1) identification of candidate items using consensus methodology, (2) prospective collection of candidate items present at the time of diagnosis, (3) data-driven reduction of the number of candidate items, (4) expert panel review of cases to define the reference diagnosis and (5) derivation of a points-based risk score for disease classification in a development set using least absolute shrinkage and selection operator logistic regression, with subsequent validation of performance characteristics in an independent set of cases and comparators.

**Results** The development set for MPA consisted of 149 cases of MPA and 408 comparators. The validation set consisted of an additional 142 cases of MPA and 414 comparators. From 91 candidate items, regression analysis identified 10 items for MPA, 6 of which were retained. The final criteria and their weights were as follows: perinuclear antineutrophil cytoplasmic antibody (ANCA) or anti-myeloperoxidase-ANCA positivity (+6), pauci-immune glomerulonephritis (+3), lung fibrosis or interstitial lung disease (+3), sino-nasal symptoms or signs (−3), cytoplasmic ANCA or anti-proteinase 3 ANCA positivity (−1) and eosinophil count ≥$1\times10^9$/L (−4). After excluding mimics of vasculitis, a patient with a diagnosis of small- or medium-vessel vasculitis could be classified as having MPA with a cumulative score of ≥5 points. When these criteria were tested in the validation data set, the sensitivity was 91% (95% CI 85% to 95%) and the specificity was 94% (95% CI 92% to 96%).

**Conclusion** The 2022 American College of Rheumatology/European Alliance of Associations for Rheumatology classification criteria for MPA are now validated for use in clinical research.



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## INTRODUCTION

The first description of 'periarteritis nodosa' was made by Kussmaul and Maier in 1866.[1] In 1948, Davson *et al* described 14 cases at autopsy that fitted the clinical description of periarteritis nodosa.[2] They divided the cases into two groups based on the histological findings in the kidneys. The clinical presentations of both groups were similar, but their pathological features differed: nine patients showed a distinctive pattern of necrotising glomerulonephritis with no arterial aneurysms, whereas the other five patients showed no glomerular lesions in the kidney but had widespread renal arterial aneurysms and renal infarcts. This is the first time that a clear distinction was made between the microscopic form of polyarteritis nodosa (now called microscopic polyangiitis (MPA)) and classic polyarteritis nodosa (PAN). The 1990 American College of Rheumatology (ACR) criteria for the classification of vasculitis did not make this distinction; instead both entities were included under the term 'polyarteritis nodosa'[3] or possibly 'granulomatosis with polyangiitis' (then called Wegener's granulomatosis).

The publication that resulted from the 1994 Chapel Hill Consensus Conference (CHCC) aimed to standardise the nomenclature and commented that 'different names are being used for the same disease and the same name is being used for different diseases'.[4] The distinction between MPA and PAN is recognised in the CHCC definitions. The main discriminating feature between MPA and PAN is the presence in MPA of pauci-immune vasculitis in arterioles, venules or capillaries. PAN is restricted to a medium-vessel disease, and MPA is a predominantly small-vessel vasculitis that can also involve medium-sized vessels.

The resulting inconsistency between disease definitions and existing classification criteria highlights an important need to update the classification criteria and to include MPA as its own entity. Additionally, over time there have been improvements in our understanding of the different forms of vasculitis, which have been informed in part by routine testing for antineutrophil cytoplasmic antibody (ANCA) in patients with vasculitis and increased utilisation of cross-sectional imaging, both of which have occurred since the 1990 ACR criteria were published. Indeed, most investigators regard MPA as part of the group of small-vessel vasculitides related to the presence of ANCA. This article outlines the development and validation of the new ACR/European Alliance of Associations for Rheumatology (EULAR)—endorsed classification criteria for MPA.

## METHODS

A detailed and complete description of the methods involved in the development and validation of the classification criteria for MPA is provided in online supplemental appendix 1. Briefly, an international

Steering Committee comprising clinician investigators with expertise in vasculitis, statisticians and data managers was established to oversee the overall Diagnostic and Classification Criteria in Vasculitis (DCVAS) project. The Steering Committee established a 5-stage plan using data-driven and consensus methodology to develop the criteria for each of six forms of vasculitis.

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

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

### Stage 2: DCVAS prospective observational study

A prospective, international multisite observational study was conducted (see collaborators for study investigators and sites). Consecutive patients representing the full spectrum of disease were recruited from academic and community practices. Patients were included if they were 18 years or older and had a diagnosis of vasculitis or a condition that mimics vasculitis. Patients with ANCA-associated vasculitis (AAV) could only be enrolled within 2 years of diagnosis. Only data present at diagnosis were recorded.

### Stage 3: refinement of candidate items specifically for AAV

The Steering Committee conducted a data-driven process to reduce the number of candidate items of relevance to cases and comparators for AAV. Items were selected for exclusion if they had a prevalence of <5% within the data set and/or they were not clinically relevant for classification criteria (eg, related to infection, malignancy or demographic characteristics). Low-frequency items of clinical importance could be combined, when appropriate.

### Stage 4: expert review to derive a gold standard—defined set of cases of AAV

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

### Stage 5: derivation and validation of the final classification criteria for MPA

The DCVAS AAV data set was randomly split into development (50%) and validation (50%) sets. Comparisons were performed between cases of MPA and a comparator group randomly selected from the DCVAS cohort in the following proportions: another type of AAV (including granulomatosis with polyangiitis (GPA) and eosinophilic granulomatosis with polyangiitis (EGPA)), 60%; another form of small-vessel vasculitis (eg, cryoglobulinaemic vasculitis) or medium-vessel vasculitis (eg, PAN), 40%. Least absolute shrinkage and selection operator (Lasso) logistic regression was used to identify items from the data set and create a parsimonious model including only the most important items. The final items in the model were formulated into a clinical risk-scoring tool with each factor assigned a weight based on its respective regression coefficient. A threshold that best balanced sensitivity and specificity was identified for classification.

In sensitivity analyses, the final classification criteria were applied to an unselected population of cases and comparators from the DCVAS data set based on the submitting physician diagnosis.

## RESULTS

### Generation of candidate classification items for the systemic vasculitides

The Steering Committee identified >1000 candidate items for the DCVAS case report form (see online supplemental appendix 2.

### DCVAS prospective observational study

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

### Refinement of candidate items specifically for AAV

Following a data-driven and expert consensus process, 91 items from the DCVAS case report form were retained for regression analysis, including 45 clinical (14 composite), 18 laboratory (2 composite), 12 imaging (all composite) and 16 biopsy (1 composite) items. Some clinical items were removed in favour of similar but more specific pathophysiological descriptors. For example, 'Hearing loss or reduction' was removed, and the composite item 'Conductive hearing loss/sensorineural hearing loss' was retained. See online supplemental appendix 6 for the final candidate items used in the derivation of the classification criteria for GPA, MPA and EGPA.

### Expert review to derive a gold standard—defined final set of cases of AAV

Fifty-five independent experts reviewed vignettes derived from the case report forms for 2871 cases submitted with a diagnosis of either small-vessel vasculitis (90% of case report forms) or another type of vasculitis or a mimic of vasculitis (10% of case report forms). The characteristics of the expert reviewers are shown in online supplemental appendix 7. A flow chart showing the results of the expert review process is shown in online supplemental appendix 8. A total of 2072 cases (72%) passed the process and were designated as cases of vasculitis; these cases were used for the stage 5 analyses.

After expert panel review by 55 investigators, 269 of 404 of the cases retained the submitting physician diagnosis of MPA and 22 additional cases were reclassified as having MPA by consensus of two expert reviewers. Compared with the 291 patients with a reference diagnosis of MPA, the 135 cases that were excluded had lower rates of perinuclear ANCA (pANCA) or anti-myeloperoxidase-ANCA (anti-MPO-ANCA) positivity (76% vs 98%; p<0.01), were less likely to have pauci-immune glomerulonephritis (16% vs 49%; p<0.01), were more likely to have maximum eosinophil counts $\geq 1\times10^9$/L (12% vs 6%; p=0.02), and were more likely to be cytoplasmic ANCA positive or proteinase 3-ANCA-positive (20% vs 4%; p<0.01). There were 822 comparators randomly selected for analysis. Table 1 shows the demographic and disease features of the 1113 cases included in this analysis (291 patients with MPA and 822 comparators), of which 557 (50%; 149 patients with MPA and 408 comparators) were in the development set and 556 (50%; 142 patients with MPA and 414 comparators) were in the validation set.

**Table 1** Demographic and disease features of cases of MPA and comparators*

| | MPA (n=291) | Comparators (n=822)* | P value |
|---|---|---|---|
| Age, mean±SD years | 65.5±13.2 | 52.0±16.9 | <0.001 |
| Sex, no. (%) female | 164 (56.4) | 394 (47.9) | 0.016 |
| Maximum serum creatinine, mean | | | <0.001 |
| μmoles/L | 126.4 | 185.2 | |
| mg/dL | 1.4 | 2.1 | |
| cANCA positive, no. (%) | 11 (3.8) | 257 (31.3) | <0.001 |
| pANCA positive, no. (%) | 236 (81.1) | 136 (16.5) | <0.001 |
| Anti-PR3-ANCA positive, no. (%) | 6 (2.1) | 265 (32.2) | <0.001 |
| Anti-MPO-ANCA positive, no. (%) | 279 (95.9) | 142 (17.3) | <0.001 |
| Maximum eosinophil count ≥1×$10^9$/L, no. (%) | 15 (5.2) | 244 (29.7) | <0.001 |

*Diagnoses of comparators for the classification criteria for microscopic polyangiitis (MPA) included granulomatosis with polyangiitis (n=300), eosinophilic granulomatosis with polyangiitis (n=226), polyarteritis nodosa (n=51), non-ANCA-associated small-vessel vasculitis that could not be subtyped (n=51), Behçet's disease (n=50), IgA vasculitis (n=50), cryoglobulinaemic vasculitis (n=34), ANCA-associated vasculitis that could not be subtyped (n=25), primary central nervous system vasculitis (n=19) and anti-glomerular basement membrane disease (n=16). ANCA, antineutrophil cytoplasmic antibody; anti-MPO-ANCA, anti-myeloperoxidase-ANCA; anti-PR3-ANCA, anti-proteinase 3-ANCA; cANCA, cytoplasmic antineutrophil cytoplasmic antibody; pANCA, perinuclear ANCA.

### Derivation and validation of the final classification criteria for MPA

Lasso regression of the previously selected 91 items yielded 10 independent items for MPA (see online supplemental appendix 9C. Each item was then adjudicated by the DCVAS Steering Committee for inclusion based on clinical relevance and specificity to MPA, resulting in six final items. Weighting of an individual criterion was based on logistic regression fitted to the six selected items (see online supplemental appendix 10C.

### Model performance

Use of a cut-off of ≥5 in total risk score (see online supplemental appendix 11C, for different cut points) yielded a sensitivity of 90.8% (95% CI 84.9% to 95.0%) and a specificity of 94.2% (95% CI 91.5% to 96.3%) in the validation set. The area under the curve for the model was 0.98 (95% CI 0.97 to 0.99) in the development set and 0.97 (95% CI 0.95 to 0.98) in the validation set for the final MPA classification criteria (online supplemental appendix 12C). The final classification criteria for MPA are shown in figure 1 (for the slide presentation version, see online supplemental figure 1).

### Sensitivity analysis

The classification criteria for MPA were applied to 2871 patients in the DCVAS database using the original physician-submitted diagnosis (n=404 cases of MPA and 2467 randomly selected comparators). Use of the same cut point of ≥5 points for the classification for MPA yielded a similar specificity of 92.5% but a lower sensitivity of 82.4%. This is consistent with the a priori hypothesis that specificity would remain unchanged but sensitivity would be reduced in a population with fewer clearcut diagnoses of MPA (ie, cases that did not pass expert panel review).

## DISCUSSION

Presented here are the 2022 ACR/EULAR MPA classification criteria. These are the first formal criteria for MPA. A 5-stage approach has been used, underpinned by data from the multinational prospective DCVAS study and informed by expert review and consensus at each stage. The comparator group for developing and validating the criteria were predominantly patients with other forms of AAV and other small- and medium-vessel vasculitides, the clinical entities where discrimination from MPA is difficult, but important. The new criteria for MPA have excellent sensitivity and specificity and incorporate ANCA testing and modern imaging techniques. The

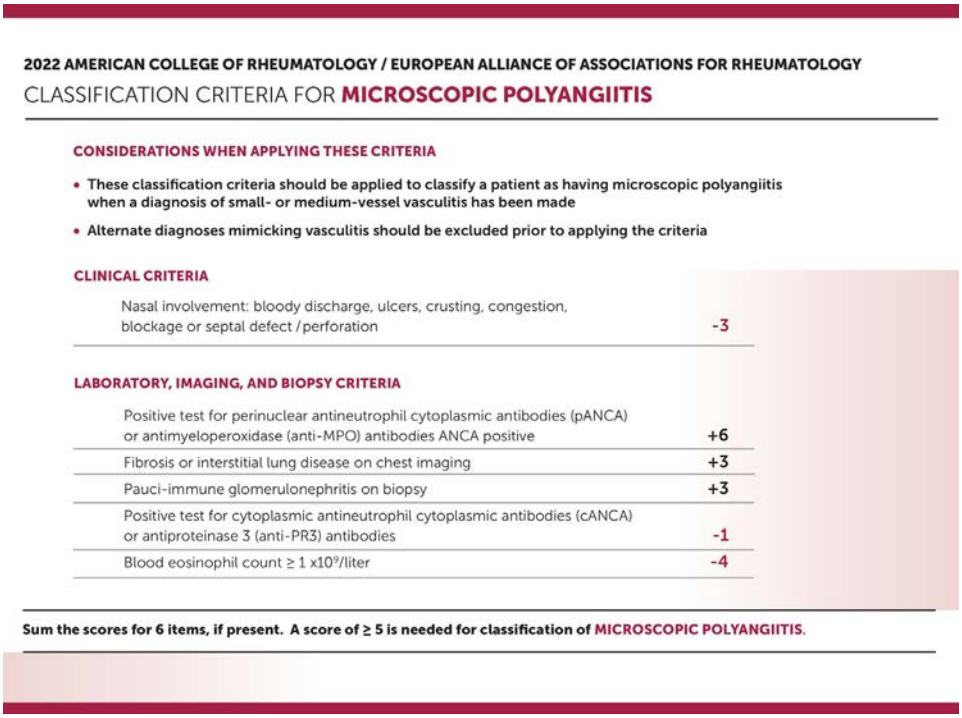


**Figure 1** 2022 American College of Rheumatology /European Alliance of Associations for Rheumatology classification criteria for microscopic polyangiitis.

criteria were designed to have face and content validity for use in clinical trials and other research studies.

These criteria are validated and intended for the purpose of *classification* of vasculitis and are not appropriate for use in establishing a *diagnosis* of vasculitis. The aim of the classification criteria is to differentiate cases of MPA from similar types of vasculitis in research settings. Therefore, the criteria should only be applied when a diagnosis of small- or medium-vessel vasculitis has been made and all potential 'vasculitis mimics' have been excluded. The exclusion of mimics is a key aspect of many classification criteria, including those for Sjögren's syndrome[5] and rheumatoid arthritis.[5] The 1990 ACR classification criteria for vasculitis perform poorly when used for diagnosis (ie, when used to differentiate between cases of vasculitis vs mimics without vasculitis),[6] and it is expected that the 2022 criteria would also perform poorly if used inappropriately as diagnostic criteria in people in whom alternative diagnoses, such as infection or other non-vasculitis inflammatory diseases, are still being considered. The relatively low weight assigned to glomerulonephritis in these classification criteria highlights the distinction between classification and diagnostic criteria. While detection of kidney disease is important to diagnose MPA, glomerulonephritis is common among patients with either GPA or MPA and thus does not function as a strong classifier between these conditions.

MPA was not recognised as a separate entity in the 1990 ACR classification criteria for vasculitis, although the disease was recognised as pathologically distinct from PAN over 40 years earlier. This omission of MPA caused difficulties in defining clear homogeneous populations for research; thus, over the last two decades, investigators have often relied on the disease definitions of the CHCC nomenclature for eligibility criteria when enrolling patients with MPA into clinical trials.[4 7–10] This approach resulted in heterogeneity between patients enrolled in therapeutic trials and epidemiological studies.[11] Due to inconsistent methods employed by researchers when applying the 1990 ACR criteria and the CHCC definitions in parallel, the European Medicines Agency (EMA) convened meetings to develop a consensus on how to use the two systems, leading to the publication of the EMA algorithm in 2007.[12] The algorithm works by first excluding EGPA and GPA, and then relying on the CHCC histological descriptions to discriminate between MPA and PAN. The new 2022 ACR/EULAR classification criteria for MPA and other vasculitides provide validated criteria that can replace the EMA interim solution and should harmonise future research studies.

A potential limitation of these new criteria is that, through the expert panel consensus methodology, only the most definite cases were included in the analyses. However, the purpose of these criteria is to enable homogeneous groupings so that individual diseases can be studied. Overall, the use of more definitive cases is consistent with the purpose of classification criteria. Additionally, positive testing for MPO-ANCA is weighted heavily in the criteria, and it is theoretically possible to classify a patient as having MPA on the basis of a positive test for MPO-ANCA only. However, the criteria are intended to only be applied to patients with an established diagnosis of small- or medium-vessel vasculitis; in this setting, the criteria sets should result in a reduction of the score away from a classification of MPA if the patient has features of another form of AAV. When criteria were tested in a much less clearly defined population using the submitting physician diagnosis as the gold standard, the sensitivity of the criteria fell substantially despite 91% of this group being pANCApositive or MPO-ANCA positive, which supports the contention that ANCA positivity is not overly dominant for the classification. Nonetheless, ANCA testing is obviously a key discriminator between the different forms of AAV and other small- and medium-vessel vasculitides.

There are some additional study limitations to consider. Although this was the largest international study ever conducted in vasculitis, most patients were recruited from Europe, Asia and North America. The performance characteristics of the criteria should be further tested in African and South American populations, which may have different clinical presentations of vasculitis. These criteria were developed using data collected from adult patients with vasculitis. Although the clinical characteristics of MPA and the other vasculitides which these criteria were tested against are not known to differ substantially between adults and children, these criteria should be applied to children with some caution. The scope of the criteria is intentionally narrow and applies only to patients who have been diagnosed as having vasculitis. Diagnostic criteria are not specified. The criteria are intended to identify homogenous populations of disease and, therefore, may not be appropriate for studies focused on the full spectrum of clinical heterogeneity in these conditions. To maximise relevance and face validity of the new criteria, study sites and expert reviewers were recruited from a broad range of countries and different medical specialties. Nonetheless, the majority of patients were recruited from academic rheumatology or nephrology units, which could have introduced referral bias.

The 2022 ACR/EULAR classification criteria for MPA are the product of a rigorous methodological process that used an extensive data set generated by the work of a remarkable international group of collaborators. These are the first classification criteria for this disease. The criteria can now be applied to patients who have been diagnosed as having a small- or medium-vessel vasculitis. These criteria have been endorsed by the ACR and EULAR and are now ready for use to differentiate one type of vasculitis from another to define populations in research studies.

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CLINICAL SCIENCE

# Multilevel factors predict medication adherence in rheumatoid arthritis: a 6-month cohort study

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## ABSTRACT

Non-adherence challenges efficacy and costs of healthcare. Knowledge of the underlying factors is essential to design effective intervention strategies.

**Objectives** To estimate the prevalence of treatment adherence in rheumatoid arthritis (RA) and to evaluate its predictors.

**Methods** A 6-month prospective cohort study of patients with RA selected by systematic stratified sampling (33% on first disease-modifying rheumatic drug (DMARD), 33% on second-line DMARD and 33% on biologics). The outcome measure was treatment adherence, defined by a score greater than 80% both in the Compliance Questionnaire in Rheumatology and the Reported Adherence to Medication scale, and was estimated with 95% CIs. Predictive factors included sociodemographic, psychological, clinical, drug-related, patient–doctor relationship related and logistic. Their effect on 6-month adherence was examined by multilevel logistic models adjusted for baseline covariates.

**Results** 180 patients were recruited (77% women, mean age 60.8). The prevalence of adherence was 59.1% (95% CI 48.1% to 71.8%). Patients on biologics showed higher adherence and perceived a higher medication need than the others; patients on second-line DMARDs had experienced more adverse events than the others. The variables explaining adherence in the final multivariate model were the type of treatment prescribed (second-line DMARDs OR=5.22, and biologics OR=3.76), agreement on treatment (OR=4.57), having received information on treatment adaptation (OR=1.42) and the physician perception of patient trust (OR=1.58). These effects were independent of disease activity.

**Conclusion** Treatment adherence in RA is far from complete. Psychological, communicational and logistic factors influence treatment adherence in RA to a greater extent than sociodemographic or clinical factors.

## Key messages

**What is already known about this subject?**

- Non-adherence is a common problem in chronic diseases such as rheumatoid arthritis that impacts on the costs and outcome of the disease.
- Most studies on adherence focus on patient-related determinants, neglecting other elements of the process, such as the attitude of the professionals, or system barriers.

**What does this study add?**

- The only way to measure the contribution of the various factors is to include all in the same study.
- This strategy helped confirm an interplay between patient-related and physician-related factors, highlighting the importance of communication and information as main determinants of adherence.

**How might this impact on clinical practice or future developments?**

- These findings support the need to train clinicians on trustworthy doctor–patient relationships, shared decision making and on providing information on practical aspects.

## INTRODUCTION

Non-adherence, defined as the extent to which a person's behaviour does not correspond with the agreed prescription,[1] is a common problem and has a significant impact on treatment efficacy and healthcare costs in patients with chronic diseases, such as rheumatoid arthritis (RA).[2–4]

The first problem in the study of adherence is the difficulty and variability of its definition and terminology. The term compliance, one of the first to be used and based on a purely clinical perspective, is defined as the 'degree to which the patient's behaviour coincides with medical recommendations' (not only treatments but also scheduled visits, health programmes, lifestyle modification, etc). However, the concept of adherence incorporates physician–patient collaboration in decision making, which includes the patient's 'active and voluntary participation in treatment adherence-related behaviour', accepted by mutual agreement, with a healthcare professional.[5]

The importance of patient involvement in decision making underlines the need to study the concept of adherence in chronic diseases. In RA, non-adherence can lead to treatment failure, delayed recovery, accelerated disease progression and the need for more aggressive treatment.[6–8] In addition, patients with RA often have associated comorbidity and thus are frequently polymedicated, which only worsens the situation of adherence.[9]

Knowledge of the factors underlying non-adherence is essential to design effective intervention strategies. Multiple barriers, defined as modifiable factors that limit or restrict adherence to a given regimen, can mediate treatment adherence. These barriers or factors can be grouped under (1) sociodemographic data; (2) patient characteristics,

including patients' beliefs and attitudes toward medication; (3) diseases; (4) treatments; (5) physician–patient relationship; and (6) relationship with the social and healthcare environments.[3 10–12]

Studies on adherence focus mainly on patient-related determinants, often neglecting other elements of the process, such as the attitude of the professionals, or system barriers. The combined contribution of all levels to adherence has seldom been quantified.[10] Our team conducted a systematic review[13] and several focus groups with the various stakeholders involved in the adherence process—that is, patients, nurses, physicians and pharmacists—with which we elaborated a list of major determinants of adherence. These factors affect patient and physician attitudes, and their components constitute the hypotheses for this study.

The main objectives of this longitudinal study were (1) to estimate the prevalence of medication adherence in patients with RA—overall and by whether the patient was on the first conventional disease-modifying rheumatic drug (csDMARD), second-line csDMARD (second csDMARD after the failure of the first one, for each patient), or biological disease-modifying rheumatic drug (bDMARD) or targeted synthetic disease-modifying rheumatic drug (tsDMARD) treatment; and (2) to study the association of adherence with factors related to the patient, the rheumatologist, the patient–doctor relationship, and the logistic factors, to better understand and quantify the contribution of the different levels to the burden of non-adherence.

## METHODS

### Design and population

This was a 6-month multicentre observational longitudinal prospective study. To obtain the necessary sample size and to facilitate the logistic of the study, 10 centres were selected at random from the list of tertiary or secondary care centres with rheumatology listed at the Spanish Society of Rheumatology. In general, the patients seen in hospital clinics are representative of all patients with the diagnosis under study, since the Spanish National Health System has universal access, and most rheumatology centres are connected to a hospital service. Although patients attending hospital clinics are somewhat more severe than those seen outside hospitals, the aforementioned conditions allow us to state that the probability of obtaining a representative sample of patients is very high.

Patients were then selected at each centre by systematic stratified sampling for 2 months, starting July 2019 (the last centre ended the study in November 2020). Every third patient of the day with RA was assigned to a stratum as defined by the current treatment: (1) first csDMARD, (2) second-line csDMARD and (3) bDMARD/tsDMARD. Strata had to be balanced at the centre level (33% per stratum). To participate in the study, patients were required to have a diagnosis of RA according to the European Alliance of Associations of Rheumatology and the American College of Rheumatology (EULAR-ACR) criteria,[14] and be treated with disease-modifying rheumatic drug (DMARDs), either bDMARDs, tsDMARDs or csDMARDs, irrespective of the activity or duration of their disease. All participants gave their written informed consent.

### Variables and measurements

The primary endpoint was adherence at 6 months of follow-up, evaluated by the Compliance Questionnaire Rheumatology (CQR)[15–17] and the Reported Adherence to Medication (RAM) scale.[18] The CQR is a rheumatology-specific instrument to measure patient compliance to drug regimens. It consists of 19 items and reflects statements about drug-taking behaviour. The instrument is well accepted by patients, has adequate psychometric properties and has been validated against electronic medication event monitoring systems. The total score can vary from 0 (complete non-compliance) to 100 (perfect compliance).[15] The RAM scale is a four-item composite self-report scale that assesses two aspects of adherence behaviour: active non-adherence (eg, tendency to deliberately alter the dose of mediation) and passive non-adherence (eg, the tendency to forget to take medication). The total score ranges from 4 to 20 but can be standardised to obtain a scale between 0 and 100.[18] Adherence was defined as a score over 80% on both scales.[15]

Explanatory factors included (1) sociodemographic; (2) psychological: perception of the need for treatment and concerns with the Beliefs about Medicines Questionnaire (BMQ),[19] adjustment to expectations, feeling privileged by the treatment prescribed (yes/no), anxious or depressed mood (taking medication for anxiety or depression), feeling of support (Visual Analogue Scale (VAS)); (3) clinical: previous DMARDs, previous adverse events to RA medication (yes/no), comorbidity, disease activity with the Disease Activity Score with 28 joint counts (DAS-28)[20] and impact with the Rheumatoid Arthritis Impact of Disease index (RAID)[21 22]; (4) drug-related: route and mode of administration, posology and type of drug; (5) patient–doctor relationship: trust in the physician (VAS), information received (VAS), visit times, accessibility (VAS), participation in shared decision making (yes/no); and (6) logistic factors: accessibility to the drug, costs and distance to point of treatment. In addition, summary variables on the number of RA independent comorbidities and treatments were created. The information was collected separately from the patient (blinded to the rheumatologist) and the responsible rheumatologist; some variables were collected from both sides and cross-checked. All variables were measured at baseline and the 6-month follow-up visit.

In addition, we collected variables at the centre level, such as access to a rheumatology nurse, or the possibility to choose dates for infusion or collection of medication at the hospital pharmacy. The rheumatology nurse has a key role to improve and/or increase self-management, self-efficacy and effective coping with the disease to promote patient independence. The main nursing care roles are vigilance of physical and psychological symptoms, drug toxicity and comorbidities; and providing information about treatment recommendations and ensuring continuity of care by acting as a contact person for the patient.

A secure and certified online platform was developed to facilitate data collection and monitoring. Each investigator from the participating centres entered their patient's clinical and examination data into the online platform. Questionnaires completed by patients were filled in on paper and returned to their respective physicians in a sealed envelope, who sent them to a central facility where a data manager entered the data. Data on drug administration (dates of administration and delivery, coverage periods, etc) were obtained from medical and pharmacy records. All variables were measured at the initial visit and a final visit at 6 months. The total number of visits per patient was two.

### Statistical analysis

The study sample was described by using summary statistics: mean, median, SD and IQR for continuous variables, and absolute and relative frequencies for categorical ones. Differences across the three treatment strata were refuted with parametric (analysis of variance) or non-parametric tests (Kruskal-Wallis, $\chi^2$), according to the distribution of the variables.

The prevalence of adherence as per the working definition was estimated with 95% CIs using a Poisson distribution.

The association between baseline factors and 6-month adherence by the working definition (score >80% on the CRQ and the RAM scale) was initially explored by bivariate logistic regression models and quantified by OR with 95% CI.

Since the data have a hierarchical structure (patients nested in physicians), the observations are not independent, which prevents the use of the common regression models and forces the construction of models that take into account the correlation structure between patients treated by the same physician. For this purpose, multilevel logistic regression analysis was used with two hierarchical levels: patient and physician. The predictors of adherence were estimated using multilevel logistic regression models with the same dependent variable as before. The inclusion of predictor variables in the models was guided by the results of the previous bivariate analysis and the conceptual model. Random intercept models were used considering random variability in average patient adherence due to the effects of the higher grouping level (physician). The best model was selected based on Akaike's information criterion and the Bayesian information criterion.

The minimum target sample size was 150 subjects, as we expected to include at least eight variables in a full model. To account for loss due to follow-up, the sample was increased by 10%, thus aiming at 165 patients. Missing data were not imputed.

## RESULTS

Participating centres had access to a rheumatology nurse. In most centres (62.5%), patients with hospital-administered medication could choose the time to pick up the drug, although in 50% they could not choose the time of drug administration. For infusions, the day hospital was shared with other specialities in 87.5% of the centres and had an average of 6.7±5.6 beds.

The 10 centres recruited 180 patients, 3 of whom were lost during follow-up (1 died and 2 had logistical problems due to the COVID-19 restrictions), yielding a retention rate of 98%. Table 1 shows the description of the sample, in total and by strata. The majority were women (76.7%) with a mean age of 60.8 years, brought up in Spain (90.3%), with primary or secondary education (79.7%), and living in a couple (76.7%). A fourth (25.6%) reported economic difficulties; 19.5% were active smokers; and the median score in the alcoholic habit questionnaire was 0.

The sample is characterised by low disease activity, with median joint counts of 0; mean erythrocyte sedimentation rate and C reactive protein (mg/dL) values of 18.3 and 0.40, respectively, and a patient global VAS in the last week of 4.0±2.9. The average DAS-28 was 2.32±1.06. The impact of RA, as defined by the RAID questionnaire, was 3.9. Regarding previous toxicity, 41.3% of the patients had presented previous adverse events that required a change in medication, and 16.7% were serious. The most frequent comorbidity was cardiovascular (47.8%), and the median number of concomitant treatments was 1. The percentage of patients taking steroids and non-steroidal anti-inflammatory drugs (NSAIDs) and steroids are 38.9% and 18.3%, respectively, without differences by treatment group. The time of evolution of the disease is 10.8±9.3 (longer for the bDMARD/tsDMARD).

In terms of psychosocial variables, as many as 19.5% of the patients stated they were depressed and, in general, rated their family support high (mean 8.6 in a 0–10 VAS). The scores about medication in the BMQ were high in both the needs (mean=20.6) and the concerns (mean=14.5) scales, and the majority of patients felt privileged to receive the prescribed medication (81.9%).

As to the patient–physician relationship and logistic variables, accessibility to the rheumatologist and trust in the professional were both rated very high (9.0 and 9.3, respectively, in 0–10 VAS). A vast majority of patients stated having agreed to their treatment with their physician (82.9%), and almost all patients considered the visit times to be adequate (94.8%). Also, the majority of patients found medication easy to use (92.8%) and did not fear it (only 16.3% expressed some concern) when they were asked these questions directly and not based on questionnaires like BMQ. The information received on different aspects of the treatment, such as efficacy, practical issues or adaptation to one's own needs was perceived as adequate (mean values around 8 on 0–10 VAS), although the perception of the adequacy of the information on toxicity was slightly lower (mean=6.8). In the opinion of the physicians, their patients had a high level of trust in them as professionals (mean=8.4) and in the treatment (mean=8.2), and they felt they had given adequate information on different aspects of the treatment (mean values above 8).

As per the stratified sampling, only 88 of the 180 studied patients received in-hospital treatment, which represents 49.2% of the sample. Most of them had the possibility of choosing the time to receive the treatment (88.4%) and have some kind of appointment reminder system (64.4%). On average, hospital treatment did not cause work-related problems (only 5.1% had problems). Hospital treatment had associated costs in 52.3% of the patients, with the centre being located less than 5 km from the patient's home in 47.3% of cases.

### Comparisons across treatment strata

Some baseline differences were observed across treatment groups (table 1). As expected, a history of adverse events requiring medication change was more frequent in the groups other than the first csDMARD. In terms of clinical factors, there was no variability across the groups, except for the RAID score, which was also lower in this first group in comparison to the other more experienced ones, although the DAS-28 showed no statistical differences.

Interestingly, while rheumatologists did not think they had explained better or worse depending on the groups, the patient perception of the adequacy of information was better in terms of practical aspects and adaptation to needs in the group with bDMARD/tsDMARD than in the others.

For psychological factors, patients from the bDMARD/tsDMARD had a perception of need higher than the other groups (p=0.021).

No other differences were found in the rest of the treatment-related, patient–doctor relationship-related or psychological factors across groups.

### Prevalence of adherence

Table 2 shows the prevalence data, overall and by treatment group, as well as the mean values of the scales that define this variable (CQR and RAM). The 6-month prevalence of adherence to DMARDs was estimated at 59.1% (95% CI 48.1% to 71.8%). By treatment group, the prevalence of adherence was 43.1% (95% CI 27.9% to 63.6%) for first csDMARD, 70.4% (95% CI 49.8% to 96.6%) for the second csDMARD and 64.4% (95% CI 45.6% to 88.4%) for bDMARD/tsDMARD.

Importantly, the period of adherence was calculated as the difference between the date of the last visit minus the first one, except in patients in whom a change in the treatment had been made after the first visit and the switching date was available (n=5). This period of measurement of adherence ranged from

**Table 1** Baseline factors, total and by treatment group

| Factors | Total (N=180) | First csDMARD (n=61) | Second-line csDMARD (n=57) | bDMARD/tsDMARD (n=62) | P value |
|---|---|---|---|---|---|
| Clinical factors | | | | | |
| Female sex | 138 (76.7) | 43 (70.5) | 45 (78.9) | 50 (80.6) | NS |
| Age (years) | 60.7±12.3 | 62.1±14.3 | 59.8±12.5 | 60.3±10.0 | NS |
| Swelling joints, $P_{50}$ ($P_{25}$–$P_{75}$) | 0 (0–1) | 0 (0–1) | 0 (0–2) | 0 (0–1) | NS |
| Painful joints, $P_{50}$ ($P_{25}$–$P_{75}$) | 1 (0–2) | 0 (0–2) | 1 (0–3) | 1 (0–3) | NS |
| Erythrocyte sedimentation rate (mm/hour) | 18.3±17.7 | 17.3±15.4 | 21.4±22.7 | 16.4±14.1 | NS |
| C reactive protein (mg/dL) | 0.4±0.7 | 0.5±1.0 | 0.3±0.5 | 0.4±0.7 | NS |
| Patient Global Assessment (0–10) | 4.0±2.9 | 3.2±2.9 | 4.3±2.9 | 4.4±2.9 | NS |
| Physician Global Assessment (0–10) | 2.5±2.5 | 2.2±2.3 | 2.7±2.7 | 2.5±2.6 | NS |
| DAS-28 | 2.32±1.06 | 2.10±0.80 | 2.57±1.19 | 2.32±1.13 | NS |
| RAID* | 3.9±2.7 | 3.1±2.7 | 4.3±2.7 | 4.1±2.6 | 0.04 |
| Number of comorbidities | 1.5±1.1 | 1.3±1.2 | 1.6±1.2 | 1.6±1.0 | NS |
| Time of evolution (years) | 10.8±9.3 | 8.8±9.5 | 9.4±8.2 | 13.9±9.3 | † |
| Treatment-related factors | | | | | |
| Treatment agreed | 146 (82.9) | 48 (81.4) | 45 (78.9) | 53 (88.3) | NS |
| Prior adverse events (patient-reported) | | | | | † |
| Yes, tolerable | 24 (13.4) | 10 (16.7) | 6 (10.5) | 8 (12.9) | |
| Yes, required medication change | 74 (41.3) | 8 (13.3) | 30 (52.6) | 36 (58.1) | |
| Prior serious adverse events (from eCR) | 30 (16.7) | 3 (4.9) | 16 (28.1) | 11 (17.7) | ‡ |
| Number of concomitant treatments | 1.6±1.3 | 1.4±1.4 | 1.7±1.2 | 1.7±1.1 | NS |
| Steroids | 70 (38.9) | 19 (31.1) | 24 (42.1) | 27 (43.5) | NS |
| NSAIDs | 33 (18.3) | 10 (16.4) | 9 (15.8) | 14 (22.6) | NS |
| Administration is felt easy | 167 (92.8) | 54 (88.5) | 52 (91.2) | 61 (98.4) | NS |
| Fear of medicine | 29 (16.3) | 11 (18.6) | 9 (15.8) | 9 (14.5) | NS |
| Patient–doctor relationship | | | | | |
| Accessibility to rheumatologist (0–10) | 9.0±1.5 | 9.0±1.6 | 8.8±1.3 | 9.3±1.4 | NS |
| Trust in the rheumatologist (0–10) | 9.3±1.3 | 9.2±1.5 | 9.0±1.4 | 9.6±0.9 | NS |
| Patient trust in doctor (physician, 0–10) | 8.4±1.1 | 8.5±0.9 | 8.2±1.2 | 8.4±1.2 | NS |
| Patient trust in treatment (physician, 0–10) | 8.2±1.3 | 8.5±1.1 | 8.0±1.4 | 8.2±1.4 | NS |
| Time of visit | | | | | NS |
| Very short | 4 (2.3) | 1 (1.7) | 1 (1.7) | 2 (3.3) | |
| Suitable | 165 (94.8) | 55 (96.5) | 54 (94.7) | 56 (93.3) | |
| Very long | 5 (2.9) | 1 (1.7) | 2 (3.5) | 2 (3.3) | |
| Information is felt consistent | 171 (96.6) | 59 (96.7) | 53 (96.6) | 59 (98.3) | NS |
| Adequacy of information (patient, 0–10) | | | | | |
| Efficacy | 7.9±2.2 | 7.6±2.1 | 7.7±2.4 | 8.6±2.1 | ‡ |
| Toxicity | 6.8±3.0 | 6.5±2.9 | 6.6±3.1 | 7.4±3.1 | NS |
| Practical aspects | 8.6±1.8 | 8.1±2.0 | 8.4±1.7 | 9.2±1.2 | † |
| Adaptation to needs | 8.0±2.0 | 7.5±2.3 | 7.8±2.0 | 8.8±1.5 | † |
| Adequacy of information (physician, 0–10) | | | | | |
| Efficacy | 8.5±1.1 | 8.5±1.0 | 8.4±1.3 | 8.6±1.0 | NS |
| Toxicity | 8.4±1.4 | 8.3±1.5 | 8.2±1.3 | 8.6±1.3 | NS |
| Practical aspects | 8.7±1.2 | 8.8±1.1 | 8.6±1.4 | 8.8±1.1 | NS |
| Adaptation to needs | 8.6±1.2 | 8.5±1.2 | 8.4±1.4 | 8.7±0.9 | NS |
| Access to health professionals if doubts | 164 (93.2) | 57 (95.0) | 50 (89.3) | 57 (95.0) | NS |
| Psychosocial factors | | | | | |
| BMQ score§ | | | | | |
| Need (0–25) | 20.6±3.9 | 19.7±3.9 | 20.6±3.9 | 21.5±3.8 | 0.02 |
| Concern/damage (0–25) | 14.5±4.3 | 15.4±4.8 | 14.0±5.6 | 14.2±4.3 | NS |
| Feel privileged with medication | 145 (81.9) | 46 (78.0) | 43 (76.8) | 56 (90.3) | NS |
| Anxiety/depression | 34 (17.8) | 10 (17.9) | 12 (21.4) | 12 (20.0) | NS |
| Family/social support (0–10) | 8.6±1.9 | 8.4±2.2 | 8.3±2.0 | 9.0±1.4 | NS |

Data are expressed as n (%) or as mean±SD unless otherwise noted.
*RAID values range from 0 to 10 with higher scores indicating worse status.
†<.001.
‡<.01.
§Higher scores indicate stronger beliefs; values range from 5 to 50 on each scale.
bDMARD, biological disease-modifying antirheumatic drug; BMQ, Beliefs about Medicines Questionnaire; csDMARD, conventional synthetic disease-modifying antirheumatic drug; eCR, electronic clinical records; NS, not significant; NSAID, non-steroidal anti-inflammatory drug; RAID, Rheumatoid Arthritis Impact of Disease Index; tsDMARD, targeted synthetic disease-modifying antirheumatic drug.

**Table 2** Adherence after 6 months

| Adherence variables | Total | First csDMARD | Second-line csDMARD | bDMARD/tsDMARD |
|---|---|---|---|---|
| Prevalence* (%) (95% CI) | 59.1 (48.1 to 71.8) | 43.1 (27.9 to 63.6) | 70.4 (49.8 to 96.6) | 64.4 (45.6 to 88.4) |
| CQR score, mean±SD | 86.0±9.4 | 83.6±9.3 | 87.2±9.2 | 86.0±9.4 |
| RAM score, mean±SD | 80.6±25.8 | 80.6±22.9 | 80.5±27.9 | 80.9±26.9 |

*Defined as a score above 80% in the CQR and RAM scales.
bDMARD, biological disease-modifying antirheumatic drug; CQR, Compliance Questionnaire Rheumatology; csDMARD, conventional synthetic disease-modifying antirheumatic drug; RAM, Reported Adherence to Medication; tsDMARD, targeted synthetic disease-modifying antirheumatic drug.

0.23 months (a patient with a treatment change in the last month) to 12.5 months (in a patient whose visit was scheduled on the first day of the COVID-19 lockdown), that is, a much greater variability than expected, since the design established a 6-month follow-up period.

### Predictors of adherence: bivariate analysis

In the bivariate analysis (table 3), the only clinical or treatment-related variables with which an association was found were the current treatment (OR=3.1 for second line csDMARDs and 2.4 for bDMARDs/tsDMARDs), in-hospital delivered treatment (OR 2.1) and having agreed on the treatment (OR=2.9). Taking NSAIDs also increased the probability of adherence (OR=2.4).

In the bivariate analysis, factors related to the patient–doctor relationship associated were the trust the patient had in his/her doctor (OR=1.7), patient-perceived adequacy of the information on treatment efficacy (OR=1.3) and on the possibility of adaptation to his/her needs (OR 1.4), the level of information adequacy as perceived by the doctor (all aspects with OR >1.2) and access to a health professional to consult doubts about treatment (OR=4.3).

Among the psychosocial factors, only feeling privileged by the medication was associated with adherence (OR=3.3).

In addition, the time to measure adherence had near to significance p value in the bivariate, and so it was decided to include in all models in the multivariate.

### Predictors of adherence: multivariate analysis

The results of the multilevel logistic regression are shown in table 4. The null model examines the random variability in the adherence due to the grouping level (physician) when none of the possible independent variables is taken into account; that is, heterogeneity or unobserved variability; in this model, the variance at the physician level was 0.15.

Model 1 examines the relationship between adherence and individual (patient) level variables. According to this model, the main predictor of treatment adherence was the agreement on treatment between patient and physician, with an OR of 4.32 (p=0.008). Other important factors for the patient were the prescription of hospital-administered treatment (OR=2.54, p=0.023) and receiving information on the possibility of adapting it to their needs (OR=1.44 p=0.023).

Model 2 studies the effects of the higher grouping level (physician) on adherence, after controlling for the effect of individual patient variables. The results showed that the physician variables that influence adherence are the type of treatment prescribed and the trust that patient has in his/her doctor, according to the physician's opinion. Consequently, and in comparison to first-line csDMARDs, adherence is higher in second-line csDMARDs (OR=4.24, p<0.006) and bDMARDs/tsDMARDs (OR=3.39, p=0.023). Finally, the information provided to the patient on the efficacy, assessed by the professional, increases significantly the probability that the patient will be adherent to treatment (OR=1.60, p=0.028).

Consequently, in the final and complete model, the variables that explain adherence are agreement on treatment between patient and physician (OR=4.29), receiving information on the possibility of adapting the treatment to the patient's needs (OR=1.54), the type of treatment prescribed (second-line csDMARDs and bDMARDs/tsDMARDs (OR=4.72 and OR=3.50, respectively), the information provided by the professional on treatment efficacy (OR=1.71) and the use of NSAIDs (OR=4.21). These effects are independent of baseline disease activity measured by the DAS-28.

## DISCUSSION

This study has shown that medication adherence in patients with RA in Spain is not 100% despite achieving good control of disease and that having patient–physician agreement on the treatment, the type of treatment prescribed—in favour of second-line csDMARDs and bDMARDs/tsDMARDs—and the patient feeling privileged by the medication received are consistent predictors of adherence.

A major difficulty in studying adherence is the variability of its definition and measurement. There is no single method to measure adherence, so it is generally recommended to use several simultaneously.[23] Most authors distinguish between adherence and persistence (time during which patients follow their prescription) and use the medication possession ratio (MPR)—only measurable in health systems with centralised pharmacy records linked to clinical records, or for drugs administered at the hospital—and survival time as measurement parameters for both concepts.[3] The majority of studies use questionnaires, among which the most commonly used are the Haynes-Sackett[24] and the Morisky-Green questionnaires.[25] In the case of rheumatic diseases, there is a specific questionnaire, the CQR, used in this study and validated in patients with inflammatory rheumatic diseases against a medication electronic monitoring system.[15–17] Therefore, a first cautionary message about any study of adherence is to first understand the definition and measure used; otherwise, comparisons will be difficult.[26] We used a double source definition based on complementary questionnaires, which makes our estimates of adherence stricter than using a single questionnaire or measure. Although we also included the MPR as a secondary measure of adherence, we have not shown the results here for the sake of clarity and because a large proportion of patients did not have a reliable measure due to differences in pharmacy procedures and information systems.

Roughly half of the patients could be considered good adherers in our study. These values are in line with the WHO estimate of adherence in chronic diseases, around 50%.[1] In studies on rheumatic diseases, the prevalence of adherence varies as much as from 9% to 94%, owing to differences in the definitions and methods used to measure adherence, but also

**Table 3** Predictors of adherence, bivariate analysis

| Factors | n | OR (95% CI) | P value |
|---|---|---|---|
| Sociodemographic factors | | | |
| Female sex | 171 | 0.90 (0.44 to 1.85) | 0.775 |
| Age (per year increase) | 171 | 0.98 (0.96 to 1.01) | 0.270 |
| Level of education | 165 | | |
| Primary | | 1 | |
| Secondary | | 1.66 (0.80 to 3.42) | 0.170 |
| College | | 1.37 (0.37 to 5.10) | 0.634 |
| University | | 0.86 (0.33 to 2.19) | 0.747 |
| Economic difficulties | 167 | 0.93 (0.45 to 1.90) | 0.840 |
| Living in a couple | 167 | 1.38 (0.67 to 2.84) | 0.381 |
| Smoking | 165 | | |
| Never smoker | | 1 | |
| Ex-smoker (>1 year) | | 0.65 (0.32 to 1.31) | 0.225 |
| Active smoker | | 0.75 (0.32 to 1.76) | 0.507 |
| Clinical factors | | | |
| Swelling joints | 171 | 0.96 (0.81 to 1.13) | 0.606 |
| Painful joints | 171 | 0.98 (0.89 to 1.08) | 0.686 |
| Erythrocyte sedimentation rate (mm/hour) | 149 | 1.02 (1.00 to 1.04) | 0.098 |
| C reactive protein (mg/dL) | 154 | 1.34 (0.79 to 2.29) | 0.274 |
| Patient Global Assessment (0–10) | 169 | 1.04 (0.94 to 1.15) | 0.451 |
| Physician Global Assessment (0–10) | 171 | 0.93 (0.82 to 1.04) | 0.214 |
| DAS-28 | 147 | 1.17 (0.85 to 1.60) | 0.329 |
| RAID | 167 | 0.99 (0.89 to 1.11) | 0.918 |
| Comorbidities (number) | 171 | 1.02 (0.79 to 1.33) | 0.852 |
| Time of evolution (years) | 156 | 1.00 (0.96 to .03) | 0.912 |
| Treatment-related factors | | | |
| Current treatment | 171 | | |
| First-line csDMARD | | 1 | |
| Second-line csDMARD | | 3.13 (1.43 to 6.85) | 0.004 |
| bDMARD/tsDMARD | | 2.39 (1.13 to 5.03) | 0.022 |
| Glucocorticoids | 171 | 1.11 (0.59 to 2.08) | 0.754 |
| NSAIDs | 171 | 2.41 (1.01 to 5.75) | 0.046 |
| In-hospital treatment | 170 | 2.14 (1.14 to 4.01) | 0.017 |
| Treatment agreed | 167 | 2.88 (1.26 to 6.58) | 0.012 |
| Prior adverse events (patient-reported) | 170 | | |
| No | | 1 | |
| Yes, but tolerable | | 1.07 (0.42 to 2.67) | 0.891 |
| Yes, with changes | | 1.89 (0.96 to 3.71) | 0.065 |
| Prior serious adverse events (from eCR) | 171 | 1.21 (0.52 to 2.84) | 0.654 |
| Concomitant treatments | 171 | 1.03 (0.81 to 1.31) | 0.796 |
| Administration is felt easy. | 171 | 0.70 (0.20 to 2.44) | 0.580 |
| Fear of medicine | 169 | 0.81 (0.35 to 1.87) | 0.627 |
| Patient–doctor relationship | | | |
| Accessibility to rheumatologist (0–10) | 167 | 1.12 (0.91 to 1.38) | 0.278 |
| Trust in the rheumatologist (0–10) | 165 | 1.14 (0.90 to 1.44) | 0.289 |
| Patient trust in doctor (physician, 0–10) | 171 | 1.71 (1.25 to 2.33) | 0.001 |
| Patient trust in treatment (physician, 0–10) | 169 | 1.22 (0.97 to 1.54) | 0.095 |
| Time of visit | 165 | | |
| Very short | | 1 | |
| Suitable | | 1.48 (0.20 to 10.7) | 0.701 |
| Very long | | 4.0 (0.211 to 75.6) | 0.355 |
| Information is felt consistent. | 168 | 0.73 (0.13 to 4.09) | 0.718 |
| Adequacy of information (patient, 0–10) | | | |
| Efficacy | 170 | 1.25 (1.08 to 1.45) | 0.002 |
| Toxicity | 168 | 1.06 (0.95 to 1.17) | 0.281 |
| Practical aspects | 169 | 1.14 (0.96 to 1.36) | 0.137 |
| Adaptation to needs | 166 | 1.35 (1.14 to 1.59) | 0.001 |
| Adequacy of information (physician, 0–10) | | | |
| Efficacy | 171 | 1.72 (1.26 to 2.35) | 0.001 |
| Toxicity | 171 | 1.44 (1.10 to 1.87) | 0.007 |
| Practical aspects | 170 | 1.40 (1.07 to 1.84) | 0.014 |
| Adaptation to needs | 171 | 1.34 (1.02 to 1.75) | 0.033 |
| Access to health professionals if there are doubts | 167 | 4.27 (1.09 to 16.7) | 0.037 |
| Psychosocial factors | | | |
| BMQ score | | | |
| Need (0–25) | 170 | 1.03 (0.95 to 1.11) | 0.487 |
| Concern/damage (0–25) | 170 | 0.97 (0.91 to 1.03) | 0.318 |
| Feeling privileged by the medication | 168 | 3.26 (1.45 to 7.37) | 0.004 |
| Anxiety/depression | 163 | 0.73 (0.33 to 1.63) | 0.447 |
| Family/social support (0–10) | 165 | 1.09 (0.93 to 1.28) | 0.284 |
| Time to measure adherence (months) | 171 | | 0.064 |
| <6 | | 1 | |
| ≥6 | | 1.98 (0.96 to 4.07) | |

bDMARD, biological disease-modifying antirheumatic drug; BMQ, Beliefs about Medicines Questionnaire; csDMARD, conventional synthetic disease-modifying antirheumatic drug; DMARD, disease-modifying anti-rheumatic drug (c, conventional; eCR, electronic clinical records; NSAID, non-steroidal anti-inflammatory drug; RAID, Rheumatoid Arthritis Impact of Disease Index.

on the interventions and populations studied.[13 27–33] The average disease activity is, however, very low in our study, which would be counterintuitive, given an adherence prevalence of only 50%. A plausible explanation is that the working definition in our study was very stringent compared with other studies.

As our study also confirms, adherence is greater among bDMARD/tsDMARD users than among first-line csDMARD users[6 8 13 30 32 33]; however, although one could think this is due to the control of medication by the hospital—both bDMARDs and tsDMARD are delivered at the hospital pharmacy or are administered in-hospital[12]—users of second-line DMARDs also showed better adherence in comparison to first line. This could be in relation to the use of previous treatment that failed, making the patient feel privileged of having alternatives, an explanation that is also supported by our results.

A systematic review quantified over 700 factors involved in adherence.[31] This illustrates the complexity of the problem

**Table 4** Multilevel analysis: predictors of adherence from patient and physician

| Fixed effects | Model 1 (patient) | Model 2 (physician) |
|---|---|---|
| Individual level (patient) | | |
| Treatment agreement | 4.32 (1.48 to 12.6) (0.008) | 4.29 (1.41 to 13.0) (0.010) |
| Information: adaptation | 1.44 (1.05 to 1.98) (0.023) | 1.54 (1.09 to 2.17) (0.015) |
| Information: practical aspects | 0.74 (0.53 to 1.03) (0.071) | 0.66 (0.45 to 0.96) (0.030) |
| In-hospital treatment | 2.54 (1.08 to 6.01) (0.033) | |
| Time to measure adherence | | |
| <6 months | 1 | 1 |
| ≥6 months | 3.91 (1.34 to 11.4) (0.012) | 3.85 (1.22 to 12.2) (0.022) |
| DAS-28 | 1.20 (0.80 to 1.78) (0.375) | 1.17 (0.77 to 1.78) (0.471) |
| Grouping level (physician) | | |
| Treatment | | |
| First-line csDMARDS | | 1 |
| Second-line csDMARDS | | 4.72 (1.61 to 13.9) (0.005) |
| bDMARDs/tsDMARDS | | 3.50 (1.14 to 10.8) (0.029) |
| Information: efficacy | | 1.71 (1.10 to 2.64) (0.016) |
| NSAIDs | | 4.21 (1.25 to 14.2) (0.021) |
| Constant | 0.060 | 0.001 |
| Random effects | | |
| $\sigma^2$ (variance) | 0.32 | 0.16 |

Cells include ORs with 95% CIs and p values unless otherwise noted.
bDMARD, biological disease-modifying antirheumatic drug; csDMARD, conventional synthetic disease-modifying antirheumatic drug; NSAID, non-steroidal anti-inflammatory drug; tsDMARD, targeted synthetic disease-modifying antirheumatic drug.

and the challenge in designing one-fit-all intervention strategies.[34 35] Many studies focusing on predictors, however, have not approached the problem at multiple levels at a time, like ours. According to the WHO multidimensional framework, there are five dimensions of factors influencing medication adherence: social and economic factors, health system-related factors, therapy-related factors, illness-related factors and patient-related factors.[1] In our study, we tried to include factors from all dimensions and to measure the contribution of the different levels. For this, we used models that allowed us to quantify the magnitude of the variation in therapeutic adherence that depends on patient-related factors and the variance corresponding to the higher aggrupation level (physician).

Regarding socioeconomic factors, we could not see any association with gender, age, level of education or having economic difficulties. This could reflect the health system in Spain, with universal coverage of visits, admissions and medications, highly accessible in all the territories of Spain. In countries with other types of health systems, with no universal coverage or high payment for health services, economic factors may impact directly adherence and are a source of long-term concern, even for health.[36] Another socioeconomic aspect could be work-related problems due to scheduled visits or due to the medication having been administered in-hospital. We did not detect an association as well, although it could be because only a third, or less, of the patients, had to go to the hospital pharmacy to collect the medication.

Health system-related factors, such as information about the frequency of follow-up, patient–provider communication, perceived quality of healthcare delivery, level of treatment information and a good relationship with the treating physician, all influence adherence.[29 37 38] Patients likely increase trust in the treatment efficacy if they feel they can rely on and trust the treating physician. Our results confirm these data as the privilege by the medication received, the agreement with the doctor, the good access to health professionals and the received information about different aspects of treatment increase, all of which increase treatment adherence.

Different factors related to therapy, such as type of medication used, the complexity of the treatment regimen, side effects and duration of medication, have been associated with adherence.[27 30] As already mentioned, our results support an increased adherence in second-line csDMARDs and bDMARDs/tsDMARDs, but other treatment-related factors, such as the number of medications or the ease of use, might not contribute as much to the collective adherence in the specific case of RA as the other highlighted factors. The results also show a significant effect of NSAIDs on adherence. These drugs control pain very well in the acute phase, producing an immediate response that improves the patient's clinical situation, which could reinforce treatment maintenance and increase adherence.

Patient-related factors, such as type of disease, duration, disease activity, functional disability, depressive symptoms and other comorbidities, have been studied with inconsistent results.[10] Except for the RAID in the bivariate analysis, no other clinical variables of the patient predict who will be adherent in our study. Only psychological variables, that is, the belief in the need for treatment and feeling privileged by the treatment prescribed, showed an association. Both can be modified by educating the patient in his/her disease and the treatment.

Special mention is the strong association of adherence with having agreed on the treatment. The shared decision between patient and physician about treatment is the first principle of the treat-to-target strategy.[39] Communication with the patient to clarify and agree on the treatment goal and the means to attain it is of utmost importance.[40] On the other hand, shared decision making is a right and a principle of adherence.[34] If a patient has not agreed on a specific treatment, we cannot say that the patient is not adherent, as adherence is, by definition, a volunteer decision based on an agreed prescription.[1 34] We tend to blame the patient for not being adherent, something understandable as it is a behaviour; however, there are many barriers that we can modify to help the patient. Instead of focusing on developing reminders, or assessing adherence, we should focus on training physicians on communication skills, making sure they approach the shared decision-making process efficiently and provide the practical information the patient demands.[34] Thus, our results support the concept that adherence is not just an individual characteristic but rather a complex and dynamic experience in which each part—patient, healthcare physician and the community—plays a specific role.[27 34]

Our study is not without limitations. In longitudinal studies, obtaining reliable and unbiased estimates depends, to a large extent, on complete follow-up. Because the intended follow-up in the study design was 6 months, we expected low attrition. There are two considerations: first, only three patients were lost to follow-up, representing a retention rate of 98%; second, there was significant variability in the follow-up time and adherence measurement period. We tried to control for the possible effect of these differences by introducing this variable in the multivariate models. Although we included the results of this variable in the tables, we should not draw any conclusions about its association with adherence. Also, the sample is very homogeneous, with all centres having access to a nurse in rheumatology and with a large majority of patients with low disease activity. Although with the sampling design we tried to reach a representative sample, some may find this with limited external validity. Consistently, however, studies on RA in Spain show very good control of the disease.[41 42] This control, in principle, would facilitate adherence. However, despite the use of a very stringent definition of adherence, 41% of the sample was non-adherent to treatment. Therefore, we believe that the prevalence of adherence is representative of the RA population in our country.

Finally, the hypothesis of the study was that adherence is influenced by psychological, communicational and logistic factors to a greater extent than by the sociodemographic and clinical characteristics of the patients. Our results confirm the hypothesis, since the factors that determine treatment adherence, besides the line of treatment, are those derived from the doctor–patient relationship, that is, agreement on the treatment, and receiving information on practical aspects, independently of disease activity. Our task now is to focus on improving these aspects.

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**Contributors** LC and AB conceived the study, which was designed by LC, guarantor, and MJGY; the ADHIERA study group reviewed and approved the protocol, recruited the patients and collected the data; MJGY and LC analysed the

data. All authors contributed to drafting the manuscript and approved the final version.

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CLINICAL SCIENCE

# Safety of baricitinib for the treatment of rheumatoid arthritis over a median of 4.6 and up to 9.3 years of treatment: final results from long-term extension study and integrated database

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## ABSTRACT

**Objective** To report long-term safety from the completed extension trial of baricitinib, an oral selective Janus kinase inhibitor, in patients with active rheumatoid arthritis (RA).

**Methods** Treatment-emergent adverse events are summarised from an integrated database (9 phase III/II/Ib and 1 long-term extension) of patients who received any baricitinib dose (All-bari-RA). Standardised incidence ratio (SIR) for malignancy (excluding non-melanoma skin cancer (NMSC)) and standardised mortality ratio (SMR) were estimated. Additional analysis was done in a subset of patients who had ever taken 2 mg or 4 mg baricitinib.

**Results** 3770 patients received baricitinib (14 744 patient-years of exposure (PYE)). All-bari-RA incidence rates (IRs) per 100 patient-years at risk were 2.6, 3.0 and 0.5 for serious infections, herpes zoster and major adverse cardiovascular events (MACE), respectively. In patients aged ≥50 with ≥1 cardiovascular risk factor, the IR for MACE was 0.77 (95% CI 0.56 to 1.04). The IR for malignancy (excluding NMSC) during the first 48 weeks was 0.6 and remained stable thereafter (IR 1.0). The SIR for malignancies excluding NMSC was 1.07 (95% CI 0.90 to 1.26) and the SMR was 0.74 (95% CI 0.59 to 0.92). All-bari-RA IRs for deep vein thrombosis (DVT)/pulmonary embolism (PE), DVT and PE were 0.5 (95% CI 0.38 to 0.61), 0.4 (95% CI 0.26 to 0.45) and 0.3 (95% CI 0.18 to 0.35), respectively. No clear dose differences were noted for exposure-adjusted IRs (per 100 PYE) for deaths, serious infections, DVT/PE and MACE.

**Conclusions** In this integrated analysis including long-term data of baricitinib from 3770 patients (median 4.6 years, up to 9.3 years) with active RA, baricitinib maintained a similar safety profile to earlier analyses. No new safety signals were identified.

**Trial registration number** NCT01185353, NCT00902486, NCT01469013, NCT01710358, NCT02265705, NCT01721044, NCT01721057, NCT01711359 and NCT01885078.

## Key messages

**What is already known about this subject?**

- The efficacy and safety of baricitinib, an oral, reversible and selective Janus kinase (JAK)1/JAK2 inhibitor in rheumatoid arthritis (RA), have been reported from previous phase II and III randomised controlled trials and open-label, long-term extension studies.
- The efficacy of baricitinib has been demonstrated in populations that cover the clinical disease continuum.
- Previous integrated analyses of the long-term safety of baricitinib in patients with RA included placebo-controlled and dose–response assessments.
- Adverse events were stable over time and no new safety risks were observed.
- The safety profile of JAK inhibitors in clinical trials includes an increased risk of herpes zoster and associations with increased cardiovascular events, venous thromboembolic events (VTE) and malignancies.
- As disease-modifying antirheumatic drugs, JAK inhibiotors are used chronically in patients with RA, and it is important to continuously monitor and assess the evolving long-term safety profile.

## INTRODUCTION

Baricitinib, an oral, reversible and selective Janus kinase (JAK)1/JAK2 inhibitor,[1] is indicated for the treatment of rheumatoid arthritis (RA). In clinical trials, once-daily baricitinib at 2 mg and 4 mg doses have shown significant clinical efficacy with acceptable safety.[2–5] The most commonly reported serious adverse events (SAEs) during the placebo-controlled period were infections.[2–5] Integrated long-term safety of baricitinib in 3770 patients (10 127 patient-years of exposure (PYE)) during the RA clinical development programme has been previously reported.[6 7] As baricitinib, like other disease-modifying antirheumatic drugs (DMARDs), is used chronically in patients with RA, it is important to continuously monitor and assess the evolving long-term safety profile. These long-term data are most relevant to assess the incidence and risk of uncommon adverse events of special interest (AESI), including malignancies and major adverse cardiovascular events (MACE). Since the previous analysis, the long-term extension (LTE) study has concluded, and we present the final update of integrated data of up to 9.3 years of treatment, representing an additional 4617 PYE.[6]

## Key messages

**What does this study add?**

- This final report of the long-term safety of baricitinib describes the highest level of patient exposure use of up to 9 years and over 14 000 patient-years of exposure, across the spectrum of the RA population, from integrated data of randomised clinical trials and the completed long-term extension study.
- The safety profile of baricitinib remained consistent with previous reports.
- Rates of safety events of special interest, including deaths, malignancies, major adverse cardiovascular event (MACE) and deep vein thrombosis/pulmonary embolism, remained stable through exposures up to 9.3 years and were generally similar between the 2 mg and 4 mg groups.
- The potential risk of MACE, VTE and malignancy events with JAK inhibitors warrants further characterisation, including registries.

**How might this impact on clinical practice or future developments?**

- This study is the largest integrated safety analysis of baricitinib.
- The results suggest that baricitinib has a consistent safety profile as demonstrated in previous reports and is in line with other JAK inhibitors and biologic disease-modifying antirheumatic drugs.
- Because RA is a chronic inflammatory disease that requires long-term treatment, this study gives assurances that baricitinib can be used for prolonged periods of time.
- Continued follow-up and further research, including long-term population-based studies, are needed to fully understand the risk of outcomes, including malignancies, MACE and VTE, and the comparative real-world risk of baricitinib and therapies in RA.

## MATERIALS AND METHODS

### Study design and patients

Pooled data of patients ≥18 years old with moderate-to-severe active RA from nine randomised clinical trials (five phase III, three phase II, one phase Ib) and one completed LTE trial (online supplemental table 1) were analysed.[2–5 8–10] Exclusion criteria included current or recent (<30 days prior to study entry) clinically serious infection requiring antimicrobial treatment and selected laboratory abnormalities (eg, hepatic/renal function tests, selected haematology and markers of infection). Baricitinib doses ranged from 1 mg to 15 mg daily, with 2 mg and 4 mg daily doses in the phase III and LTE trials. All patients provided written informed consent.

Patients completing phase III trials and phase II trial (NCT01185353) were eligible for the LTE. Patients randomised to baricitinib 2 mg and not rescued in the originating study continued on baricitinib 2 mg in the LTE; all other patients received baricitinib 4 mg at LTE entry. Patients receiving 4 mg for at least 15 months without rescue and achieving sustained low disease activity (Clinical Disease Activity Index (CDAI) score ≤10) or remission (CDAI score ≤2.8)[11] were blindly rerandomised to 4 mg or tapered down to 2 mg.

### Patient and public involvement

This research was done without patient and public involvement.

### Analysis sets

The All-bari-RA analysis set includes data of all patients who received ≥1 dose of baricitinib using all available data after the first dose without censoring for rescue or dose change. This analysis set is uncontrolled and provides reliable estimates for adverse event incidence within the baricitinib programme, which is particularly relevant for less common event types and for evaluating the incidence after long-term exposure. An exploratory analysis of AESI and death was done in a subset of data from All-bari-RA that included patients who had ever taken baricitinib at 2 mg or 4 mg. As a postmarketing study found an increased risk of MACE and malignancies excluding non-melanoma skin cancer (NMSC) with tofacitinib versus tumour necrosis factor inhibitors (TNFi) in patients ≥50 years with cardiovascular risk factors, the incidence rate (IR) of MACE was analysed in a similar subpopulation.

Safety evaluations included treatment-emergent adverse events (TEAEs), adverse events leading to temporary interruption or permanent discontinuation of study drug, SAEs, deaths, and AESI, including serious and opportunistic infections, malignancies, MACE, deep vein thrombosis (DVT)/pulmonary embolism (PE), and gastrointestinal perforations. SAEs were any event meeting the International Conference on Harmonisation E2A seriousness criteria.[12] Cardiovascular adverse events from the five phase III studies and LTE, identified by investigators or according to a predefined list of event terms, were adjudicated for MACE by an independent, external Clinical Endpoint Committee that remained blinded to treatment assignments. Venous thromboembolic events were not externally adjudicated in the baricitinib RA programme. Gastrointestinal perforations were based on events identified from a medical review of the gastrointestinal perforations Standardised Medical Dictionary for Regulatory Activities Queries and were considered definite or probable perforations after internal medical review.

### Statistical analysis

Baseline characteristics were descriptively analysed. For adverse events (except AESI), the exposure-adjusted incidence rate (EAIR) was calculated as the number of patients with an event per 100 PYE, including observation time during the follow-up period. For AESI, the IR was calculated as the number of patients with an event per 100 patient-years at risk (PYR), including follow-up time censored at event onset date. Poisson distribution was used to calculate 95% CI. The EAIR for death, serious infections, MACE and DVT/PE was calculated for groups of patients receiving baricitinib 2 mg or 4 mg within All-bari-RA, with the EAIR based on the dose at the time of the event, given that patients in this subset could contribute events to both treatment groups depending on their drug dose at the time of the event. The IR for MACE was evaluated in subgroups of patients aged ≥50 years and presenting with cardiovascular risk factors (current smoker, hypertension, high-density lipoprotein (HDL) cholesterol <40 mg/dL, diabetes or arteriosclerotic cardiovascular disease). This IR was calculated as 100 times the number of patients experiencing MACE divided by PYR (exposure time up to the event for patients with MACE and exposure time up to the end of the period for patients without MACE) in years in the subgroup of the specific factor. To account for ageing of the cohort, standardised incidence ratio (SIR) was calculated as the ratio of observed to expected number of malignancies (excluding NMSC) using age-specific malignancy data from the Surveillance, Epidemiology, and End Results 17 (SEER17), 2013–2017 US population cancer rates.[13] Standardised mortality

ratio (SMR) was estimated using 2019 population mortality calculated as compared with the general US population with the same age and sex distribution.[14]

## RESULTS

### Patients

Patient demographics and disease activity are presented in online supplemental table 2. In this final analysis of All-bari-RA, 3770 patients received ≥1 dose of baricitinib for a total of 14 744 PYE, an additional 4617 PYE from our previous report.[6] The majority of PYE (80.5%) were baricitinib 4 mg, while 18.1% of PYE were baricitinib 2 mg; 78.5% of patients had ≥1 year and 47.1% had ≥5 years of baricitinib treatment. The median exposure was 4.6 years and the maximum exposure was 9.3 years (table 1).

### Adverse events including SAEs

In All-bari-RA, the EAIRs (per 100 PYE) for any TEAE and SAE were 22.6 and 7.4, respectively. The most common TEAEs were nasopharyngitis, upper respiratory tract infections, bronchitis, urinary tract infections and herpes zoster (online supplemental table 3). Interruptions and discontinuations were most frequently due to infections. There were 85 deaths, and the IR (patient-years=15 114, IR=0.56, 95% CI 0.45 to 0.70) increased over time. After controlling for age and sex, the baricitinib SMR was <1 (SMR 0.74, 95% CI 0.59 to 0.92; online supplemental figure 1). Of the 85 deaths, categories (system organ class based on Medical Dictionary for Regulatory Activities Terminology V.23.1) for causes that included >2 deaths were cardiovascular-related (n=19, 22.4%), infections (n=19, 22.4%), neoplasms (n=19, 22.4%), respiratory-related (n=13, 15.3%, including 4 due to PE, 2 of which included comorbid cancer and 1 who had comorbid diverticulitis with sepsis), general disorders (n=6, 7.1%), nervous system-related (n=5, 5.9%) and vascular disorders (n=3, 3.5%). The EAIRs for death were similar in the 2 mg and 4 mg subsets of All-bari-RA (table 2).

### Adverse events of special interest

#### Infections

Infections were the most common TEAE. The IR for serious infections (2.6, 95% CI 2.33 to 2.86) remained stable from the previous report[6] and did not increase with prolonged exposure (figure 1); the IRs for serious infection in patients <65 years and ≥65 years were 2.1 (95% CI 1.85 to 2.37) and 5.5 (95% CI 4.53 to 6.60), respectively. The EAIRs for serious infections were 2.13 (95% CI 1.61 to 2.76) for the 2 mg subset and 2.62 (95% CI 2.34 to 2.93) for the 4 mg subset of All-bari-RA (table 2). The most common serious infections were pneumonia (n=84, EAIR 0.6), herpes zoster (n=44, EAIR 0.3), urinary tract infection (n=25, EAIR 0.2) and cellulitis (n=23, EAIR 0.2). Multivariable risk factor analysis for serious infections in patients treated with baricitinib was previously reported.[15]

The IR for herpes zoster (3.0, 95% CI 2.70 to 3.28) remained essentially unchanged from our previous report[6] and did not increase with prolonged exposure (figure 1). The IR for herpes zoster was highest in Asia (IR 5.2, 95% CI 4.42 to 6.01). Majority of the herpes zoster cases were mild (39.8%) or moderate (54.5%) in severity and occurred mostly in patients who were older (75.1% in patients ≥50 years), without prior episodes (96.0%) or without prior vaccination (96.1%), and 91% of the patients recovered. There were 15 complicated cases of herpes zoster (ocular/ophthalmic, n=10 (2 were SAEs); herpes zoster meningitis, n=1 (SAE); palsy, n=4), and 42 cases of multidermatomal herpes zoster of which 18 were disseminated.

The IR for tuberculosis in All-bari-RA (0.1, 95% CI 0.08 to 0.20) (table 1) did not increase with prolonged exposure.[6] No cases were reported with 2 mg, and the events occurred almost exclusively in endemic countries (Argentina, China, India,

**Table 1** Safety summary among patients with RA treated with at least one dose of baricitinib (All-bari-RA analysis set)

| | All-bari-RA (N=3770) |
|---|---|
| Exposure | |
| Total patient-years of exposure to baricitinib | 14 744.4 |
| Total patient-years (including follow-up period) | 15 114.1 |
| Number of patients with ≥52 weeks, n (%) | 2961 (78.5) |
| Number of patients with ≥104 weeks, n (%) | 2519 (66.8) |
| Number of patients with ≥208 weeks, n (%) | 2093 (55.5) |
| Number of patients with ≥260 weeks, n (%) | 1775 (47.1) |
| Median duration, days | 1682.5 |
| Longest exposure, days | 3405 |
| ≥1 AE, n (EAIR) | |
| Any TEAE | 3421 (22.6) |
| SAE | 1117 (7.4) |
| Temporary study drug interruption due to AE | 1282 (8.5)* |
| Permanent discontinuation of the study drug due to AE | 704 (4.7) |
| Death, n (IR) | 85 (0.56) |
| Infections, n (IR) | |
| Treatment-emergent infections† | 2590 (17.1) |
| Serious infection | 372 (2.6) |
| Herpes zoster | 422 (3.0) |
| Infection leading to death† | 19 (0.1) |
| TB† | 19 (0.1) |
| Opportunistic infection excluding TB | 69 (0.5) |
| Malignancy, n (IR) | |
| Malignancy excluding NMSC | 139 (0.9) |
| Lymphoma | 9 (0.06) |
| NMSC | 50 (0.3) |
| Adverse CV events of special interest, n (IR) | |
| MACE‡ | 73 (0.5) |
| MI | 24 (0.2) |
| CV death | 20 (0.1) |
| Stroke | 38 (0.3) |
| DVT/PE | 73 (0.5) |
| DVT§ | 52 (0.4) |
| PE | 39 (0.3) |
| GI disorder, n (IR) | |
| GI perforations | 9 (0.06) |

*Some studies did not collect temporary interruption of study drug.
†Used EAIR per 100 PY (patient exposure not censored at the event).
‡Potential CV adverse events from the phase III and LTE trials, identified by investigators or according to a predefined list of event terms, were adjudicated by an independent, external Clinical Endpoint Committee that remained blinded to treatment assignments.
§DVT includes distal events below the knee.
AE, adverse events; bari, baricitinib; CV, cardiovascular; DVT, deep vein thrombosis; EAIR, exposure-adjusted incidence rate; GI, gastrointestinal; IR, incidence rate; LTE, long-term extension; MACE, major adverse cardiovascular events; MI, myocardial infarction; N, number of patients in the analysis set; n, number of patients in the specified category; NMSC, non-melanoma skin cancer; PE, pulmonary embolism; PY, patient-years; RA, rheumatoid arthritis; SAE, serious adverse event; TB, tuberculosis; TEAE, treatment-emergent adverse event.

**Table 2** Exposure-adjusted incidence rates of adverse events of special interest in the 2 mg and 4 mg subsets of the All-bari-RA analysis set

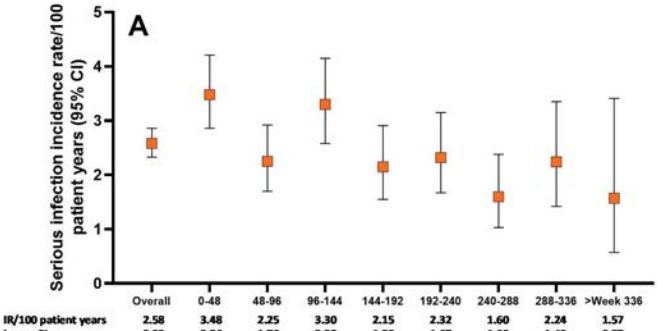
| | Ever on 2 mg (N=1077) (PYE=2678) EAIR (95% CI) | Ever on 4 mg (N=3401) (PYE=11 872) EAIR (95% CI) | All-bari-RA (N=3770) (PYE=14 744) IR (95% CI) |
|---|---|---|---|
| Death | 0.56 (0.31 to 0.92) | 0.57 (0.44 to 0.73) | 0.56 (0.45 to 0.70) |
| Serious infections | 2.13 (1.61 to 2.76) | 2.62 (2.34 to 2.93) | 2.58 (2.33 to 2.86) |
| Thromboembolic events | | | |
| DVT/PE | 0.49 (0.26 to 0.83) | 0.51 (0.39 to 0.66) | 0.49 (0.38 to 0.61) |
| DVT | 0.41 (0.21 to 0.73) | 0.35 (0.25 to 0.48) | 0.35 (0.26 to 0.45) |
| PE | 0.26 (0.11 to 0.54) | 0.27 (0.18 to 0.38) | 0.26 (0.18 to 0.35) |
| MACE* | 0.42 (0.21 to 0.74) | 0.54 (0.41 to 0.69) | 0.51 (0.40 to 0.64) |

*Positively adjudicated events of myocardial infarction, stroke and cardiovascular deaths.
bari, baricitinib; DVT, deep vein thrombosis; EAIR, exposure-adjusted incidence rate; IR, incidence rate; MACE, major adverse cardiovascular events; N, number of patients in the analysis set; PE, pulmonary embolism; PYE, patient-years of exposure; RA, rheumatoid arthritis.

Lithuania, Mexico, Russia, South Africa, South Korea and Taiwan), with one report in the USA.

## Malignancies

In All-bari-RA, the IR for malignancy (excluding NMSC) during the first 48 weeks was 0.6 (95% CI 0.34 to 0.91) and remained stable thereafter at approximately 1.0 (overall IR 0.9, 95% CI 0.77 to 1.09) (figure 2A). The most commonly reported types of malignancy were respiratory and mediastinal (n=26, EAIR=0.17), breast (n=23, EAIR=0.15) and gastrointestinal (n=19, EAIR=0.13) (table 3). The number of malignancy events, excluding NMSC, in each 5-year age category was compared with the expected number of malignancies based on SEER17 data (online supplemental figure 2). The resulting overall age-adjusted SIR was 1.07 (95% CI 0.90 to 1.26), suggesting similar incidence of malignancies as in the general US population. In All-bari-RA, the IR for NMSC was 0.3 (95% CI 0.25 to 0.44) and did not increase over time (figure 2B). The IR for lymphoma was 0.06 (95% CI 0.03 to 0.11), with diffuse large B cell lymphoma remaining the most common subtype.



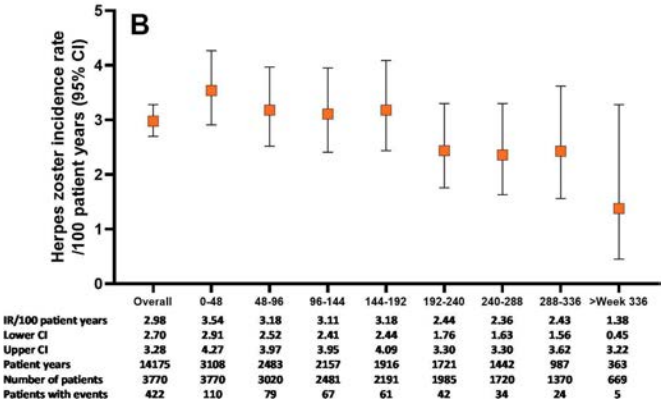
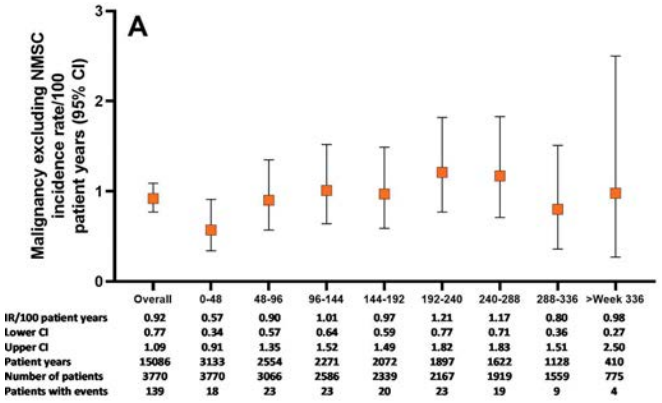




**Figure 1** Serious infections and herpes zoster over time for the All-bari-RA analysis set. Cumulative incidence rate of serious infections (A) and herpes zoster (B) by time period for the All-bari-RA analysis set. Data are presented by IR per 100 PY at risk. The number of total patients and patients with events, as well as the total PY per time period, are also provided. bari, baricitinib; IR, incidence rate; PY, patient-years; RA, rheumatoid arthritis.



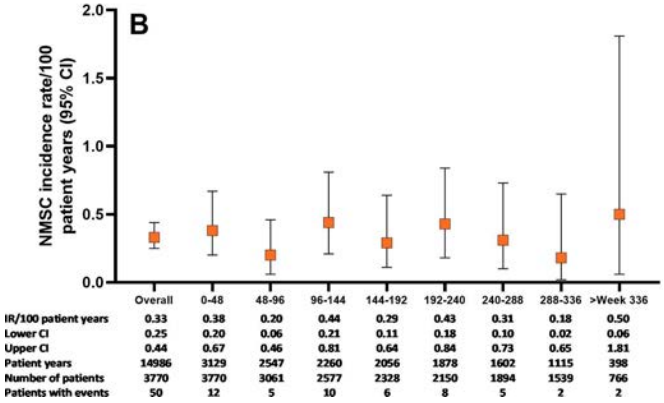




**Figure 2** Malignancy-related events over time for the All-bari-RA analysis set. Cumulative incidence rate of (A) malignancy (excluding NMSC) and (B) NMSC by time period for the All-bari-RA analysis set. Data are presented by IR per 100 PY at risk. The number of total patients and patients with events, as well as the total PY per time period, are also provided in both panels. bari, baricitinib; IR, incidence rate; NMSC, non-melanoma skin cancer; PY, patient-years; RA, rheumatoid arthritis.

## Cardiovascular events

In All-bari-RA, the IR for positively adjudicated MACE was 0.5 (95% CI 0.40 to 0.64) and remained stable with longer baricitinib exposure (figure 3). The IRs for stroke, myocardial infarction and cardiovascular-related death were 0.3 (95% CI 0.19 to 0.36), 0.2 (95% CI 0.11 to 0.25) and 0.1 (95% CI 0.08 to 0.21), respectively. In the overall population, 54.8% of patients had ≥1 cardiovascular risk factor, with an IR for MACE of 0.70 (95% CI 0.53 to 0.92) in this group (table 4). In patients aged ≥50 with ≥1 cardiovascular risk factors (n=1325), 44 patients (3.3%) had

**Table 3** Exposure-adjusted incidence rates of malignancies excluding NMSC by high-level term

| High-level (group) term | n | EAIR (95% CI) |
|---|---|---|
| Respiratory and mediastinal neoplasms malignant and unspecified | 26 | 0.17 (0.11 to 0.25) |
| Breast neoplasms malignant and unspecified (including nipple) | 23 | 0.15 (0.10 to 0.23) |
| Gastrointestinal neoplasms malignant and unspecified | 19 | 0.13 (0.08 to 0.20) |
| Reproductive neoplasms female malignant and unspecified | 16 | 0.11 (0.06 to 0.17) |
| Reproductive neoplasms male malignant and unspecified (all reported cases were prostatic neoplasms) | 10 | 0.07 (0.03 to 0.12) |
| Skin neoplasms malignant and unspecified (other than NMSC) | 10 | 0.07 (0.03 to 0.12) |
| Renal and urinary tract neoplasms malignant and unspecified | 9 | 0.06 (0.03 to 0.11) |
| Lymphomas non-Hodgkin's B cell | 6 | 0.04 (0.01 to 0.09) |
| Endocrine neoplasms malignant and unspecified | 4 | 0.03 (0.01 to 0.07) |
| Metastases | 3 | 0.02 (0.00 to 0.06) |
| Others* | 15 | 0.10 (0.06 to 0.16) |

*Others are all high-level group terms with 2 cases or fewer, including haematopoietic neoplasms (excluding leukaemias and lymphomas); hepatobiliary neoplasms malignant and unspecified; leukaemias; lymphomas non-Hodgkin's T cell; lymphomas non-Hodgkin's unspecified histology; miscellaneous and site unspecified neoplasms malignant and unspecified; neoplasm-related morbidities; nervous system neoplasms malignant and unspecified not elsewhere classified (NEC); not coded; ocular neoplasms; and soft tissue neoplasms malignant and unspecified.
EAIR, exposure-adjusted incidence rate; n, number of subjects in the specified category; NMSC, non-melanoma skin cancer.

MACE (IR 0.77, 95% CI 0.56 to 1.04). The EAIRs were similar in the 2 mg (0.42, 95% CI 0.21 to 0.74) and 4 mg (0.54, 95% CI 0.41 to 0.69) subsets of All-bari-RA (table 2).

### Venous thromboembolic events

In All-bari-RA, the overall IRs for DVT/PE, DVT and PE were 0.49 (95% CI 0.38 to 0.61), 0.35 (95% CI 0.26 to 0.45) and 0.26 (95% CI 0.18 to 0.35) (table 1) and remained stable over time (figure 4). The EAIRs for DVT/PE were similar in the 2 mg (0.49, 95% CI 0.26 to 0.83) and 4 mg (0.51, 95% CI 0.39 to 0.66) subsets of All-bari-RA (table 2).

### Diverticulitis and lower gastrointestinal perforation

There were 23 treatment-emergent events of diverticulitis (EAIR 0.15). Diverticulitis occurred in patients with risk factors including pre-existing diverticulosis, older age, overweight and obesity, and chronic corticosteroid or non-steroidal anti-inflammatory drug (NSAID) treatment. Since the prior reported analysis, five additional cases of gastrointestinal perforations have been reported in All-bari-RA, bringing the total to nine (IR 0.06, 95% CI 0.03 to 0.11) (table 1). There were seven (IR 0.05) lower gastrointestinal perforations.

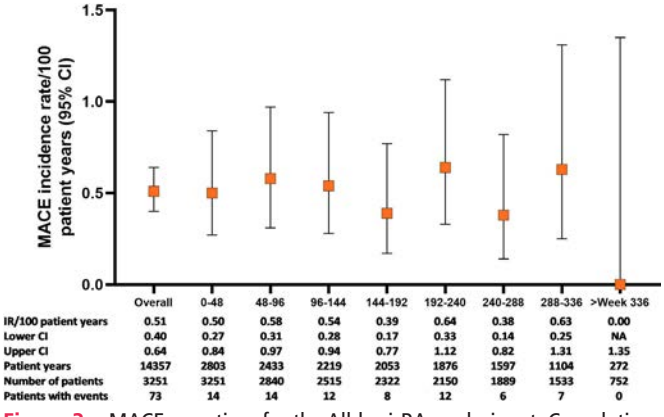



**Figure 3** MACE over time for the All-bari-RA analysis set. Cumulative incidence rate of MACE (calculated for the five phase III studies and the LTE) by time period for the All-bari-RA analysis set. Data are presented by IR per 100 PY at risk. The number of total patients and patients with events, as well as the total PY per time period, are also provided. bari, baricitinib; IR, incidence rate; LTE, long-term extension; MACE, major adverse cardiovascular event; PY, patient-years; RA, rheumatoid arthritis.

### Laboratory

In All-bari-RA treatment-emergent shifts of selected laboratory parameters are presented in online supplemental table 4. Changes in selected haematological parameters following once-daily baricitinib dose were previously disclosed.[16] The IR for laboratory-related treatment-emergent events included anaemia (1.74, 95% CI 1.53 to 1.97), neutropaenia (0.4, 95% CI 0.31 to 0.52), lymphopaenia (1.04, 95% CI 0.89 to 1.22) and thrombocytosis (0.3, 95% CI 0.24 to 0.43).

## DISCUSSION

We report an updated assessment of safety from an integrated database of baricitinib in patients with RA through 9.3 years of treatment for a total of 14 744 years of patient exposure, in which baricitinib maintained a safety profile similar to that previously reported.[6] The incidence of death, SAEs (including infections), MACE and malignancy in the baricitinib analysis is similar to those observed for other therapeutic trials of JAK inhibitors[17–20] and biologic DMARDs.[21] Few patients (EAIR 4.7) discontinued due to adverse events. In the baricitinib 2 mg and 4 mg subsets of All-bari-RA, the incidence of AESI was generally similar between the two dosing groups.

Although the incidence of deaths appears to be increasing over time, the overall IR for death (0.56) and the EAIR per dose (2 mg, 0.56; 4 mg, 0.57) are lower than the reported IR of 1.5–2.4/100 patient-years in epidemiological studies of RA.[22 23] The risk of mortality in patients treated with baricitinib was not increased compared with the general population after controlling for age and sex, with the SMR for baricitinib <1. Causes of death for baricitinib-treated patients are in line with the percentages of total deaths in the US general population,[24] as well as those reported in clinical trials of other RA therapies.[17 20 25 26]

Due to disease and therapeutic interventions, patients with RA are at an elevated risk of infection. The EAIR of treatment-emergent infections for patients in the current analysis decreased to 17.1 from previously reported EAIRs of 23.7–26.9.[6 15] Similarly, EAIRs for TEAEs that led to temporary or permanent discontinuations from study drug have continued to decrease with prolonged exposure. The overall incidence of serious

**Table 4** Patient demographics and cardiovascular risk factors in patients with and without MACE

| | Patients with MACE (N=73) | Patients without MACE (N=3178) | IR (95% CI) | All-bari-RA (N=3251)* |
|---|---|---|---|---|
| Patients with ≥1 cardiovascular risk factor, n (%)† | 55 (75.3) | 1725 (54.3) | 0.70 (0.53 to 0.92) | 1780 (54.8) |
| Age, mean (SD) | 58.9 (10.1) | 52.2 (12.2) | | 52.3 (12.2) |
| <50 years, n (%) | 13 (17.8) | 1214 (38.2) | 0.23 (0.12 to 0.40) | 1227 (37.7) |
| ≥50 years, n (%) | 60 (82.2) | 1964 (61.8) | 0.68 (0.52 to 0.88) | 2024 (62.3) |
| Sex | | | | |
| Male | 29 (39.7) | 659 (20.7) | 0.93 (0.62 to 1.33) | 688 (21.2) |
| Female | 44 (60.3) | 2519 (79.3) | 0.39 (0.28 to 0.53) | 2563 (78.8) |
| BMI category, n (%) | | | | |
| Underweight (<18.5 kg/m$^2$) | 2 (2.8) | 138 (4.3) | 0.35 (0.04. 1.27) | 140 (4.3) |
| Normal or underweight (≥18.5 and <25 kg/m$^2$) | 16 (22.2) | 1156 (36.4) | 0.31 (0.18 to 0.50) | 1172 (36.1) |
| Overweight (≥25 and <30 kg/m$^2$) | 27 (37.5) | 951 (30.0) | 0.62 (0.41 to 0.90) | 978 (30.1) |
| Obese (≥30 kg/m$^2$) | 27 (37.5) | 930 (29.3) | 0.65 (0.43 to 0.94) | 957 (29.5) |
| Current cigarette smoker, n (%) | 22 (30.1) | 581 (18.3) | 0.81 (0.51 to 1.23) | 603 (18.5) |
| Arteriosclerotic cardiovascular disease, n (%)‡ | 6 (8.2) | 68 (2.1) | 2.01 (0.74 to 4.37) | 74 (2.3) |
| Cardiac disorder (SOC), n (%) | 21 (28.8) | 292 (9.2) | 1.60 (0.99 to 2.45) | 313 (9.6) |
| Hypertension, n (%) | 43 (58.9) | 1126 (35.4) | 0.86 (0.62 to 1.15) | 1169 (36.0) |
| Diabetes, n (%) | 14 (19.2) | 283 (8.9) | 1.17 (0.64 to 1.97) | 297 (9.1) |
| Hypercholesterolaemia§, n (%) | 45 (61.6) | 1482 (46.6) | 0.68 (0.49 to 0.91) | 1527 (47.0) |
| Treatment-emergent thrombocytosis, n (%) | 4 (5.5) | 154 (4.8) | 0.57 (0.16 to 1.47) | 162 (5.0) |
| Baseline corticosteroid use, n (%) | 46 (61.6) | 1650 (51.9) | 0.60 (0.44 to 0.80) | 1695 (52.1) |
| HDL cholesterol <40 mg/dL, n (%) | 9 (12.3) | 280 (8.8) | 0.72 (0.33 to 1.37) | 289 (8.9) |

*All-bari-RA for MACE is only from phase II and III studies where MACE was adjudicated.
†The five possible cardiovascular risk factors included in this analysis were current smoker, hypertension, HDL cholesterol <40 mg/dL, diabetes mellitus and ASCVD.
‡Arteriosclerotic cardiovascular disease (ASCVD) is defined at baseline by medical history of myocardial infarction, coronary artery bypass, stroke, transient ischaemic attack or peripheral vascular disease.
§Hypercholesterolaemia was defined by (1) baseline total cholesterol ≥200 mg/dL or LDL ≥130 mg/dL; or (2) preferred terms of 'blood cholesterol abnormal, blood cholesterol increased, LDL abnormal, LDL increased, very LDL abnormal, very LDL increased, LDL/HDL ratio increased, total cholesterol/HDL ratio increased, total cholesterol/HDL ratio abnormal, lipids abnormal'; and high-level terms of 'elevated cholesterol, hyperlipidaemias NEC'.
bari, baricitinib; BMI, body mass index; HDL, high-density lipoprotein; HDL, high-density lipoprotein; IR, incidence rate; LDL, low-density lipoprotein; MACE, major adverse cardiovascular event; n, number of patients in the specified category; N, number of patients in the analysis set; RA, rheumatoid arthritis; SOC, system organ class.

infections has remained stable over time. EAIRs of serious infections in the baricitinib 2 mg group could be numerically lower than 4 mg, related to lower disease activity and lower corticosteroid and methotrexate (MTX) use at the start of dose. Infections leading to death were rare in this patient population. The incidence of herpes zoster remained stable and is similar to that of other JAK inhibitors, including tofacitinib[27] and upadacitinib.[28] In our study, the rates of herpes zoster were highest in Asia and

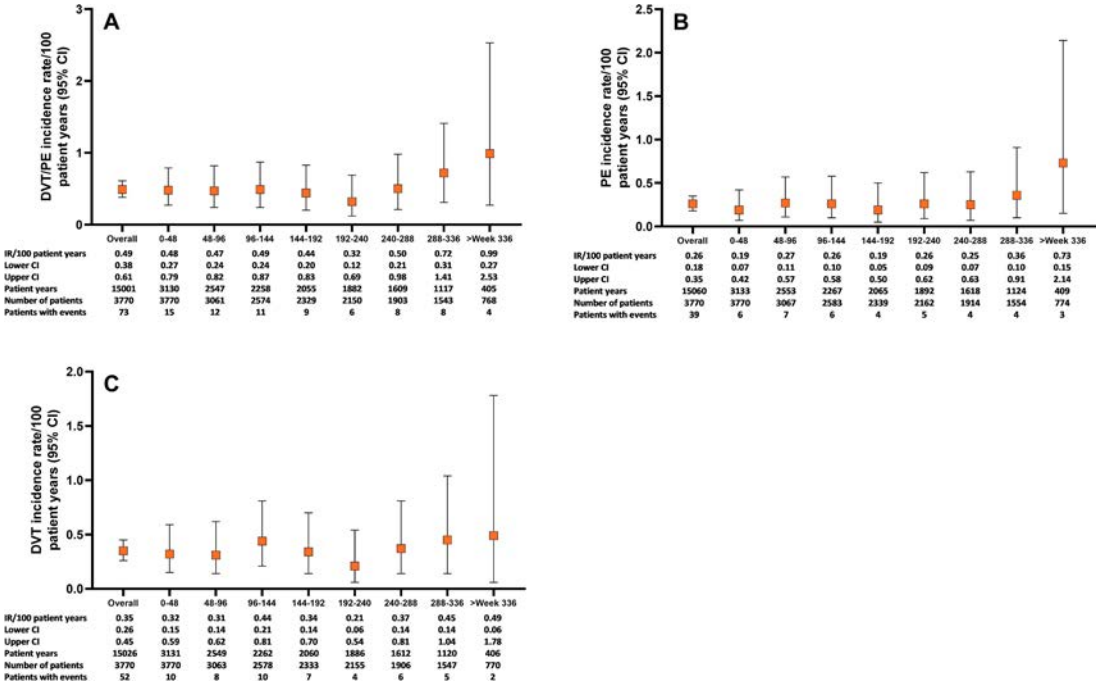



**Figure 4** Thromboembolic events over time for the All-bari-RA analysis set. Cumulative incidence rate of (A) DVT/PE, (B) PE and (C) DVT by time period for the All-bari-RA analysis set. Data are presented by IR per 100 PY at risk. The number of total patients and patients with events, as well as the total PY per time period, are also provided in both panels. bari, baricitinib; DVT, deep vein thrombosis; IR, incidence rate; PE, pulmonary embolism; PY, patient-years; RA, rheumatoid arthritis.

driven by higher rates in Japan, Taiwan and South Korea, as shown in a previous report of herpes zoster in patients treated with baricitinib.[29]

There were an additional 54 cases of malignancy (excluding NMSC) since our previous report, with a similar IR (0.9 in the current analysis vs 0.8).[6] Patients with RA are predisposed to an increased risk of malignancy, especially lymphoma, lung cancer and NMSC.[30] The incidence of lymphoma remained unchanged at 0.06 from previous reports of baricitinib[6] and similar to rates reported for other RA therapies, including an IR of 0.1 for adalimumab[21] and 0.096 in patients using TNFi.[31]

The effects of JAK inhibitors on the risk of malignancies remain unclear and need further research. Data reported from a recent systematic review and meta-analysis concluded that there was no increased risk of malignancies in patients who combined a JAK inhibitor with MTX compared with those treated with MTX alone.[32] It must be noted that 79% of the patients included in our analysis had concomitant use of MTX. Furthermore, evidence from observational studies reported no increased risk of malignancy between other JAK inhibitors (tofacitinib) compared with conventional synthetic DMARDs or biologic DMARDs, such as TNFi.[33 34] However, preliminary findings from a prospective, randomised, postmarketing safety study of tofacitinib (A3921133, NCT02092467), comparing outcomes between treatments in patients with RA who were aged ≥50 years and had ≥1 additional cardiovascular risk factor, showed an increased rate of malignancies for tofacitinib (IR/100 patient-years, 1.13) relative to TNFi (IR/100 patient-years, 0.77),[35] with the observed IR for tofacitinib remaining within reported boundaries in patients treated with biologic DMARDs (IR 0.8–2.3).[36] Further data on this study are, however, needed to appropriately contextualise these findings. In this report, the observed number of malignancies for the baricitinib population was similar to the expected events for the US population sample, resulting in an SIR of 1.07. Although the present long-term data on baricitinib do not show an increased risk of malignancy, lung cancer or lymphoma with longer exposure to baricitinib, long-term direct comparative data are not yet available from the ongoing randomised trial of baricitinib versus TNFi.

Patients with RA are at an increased risk of DVT and PE (IR 0.3–0.8/100 patient-years)[16] compared with the general population.[37 38] In this analysis, the IR of DVT/PE in patients treated with baricitinib was consistent with previously reported data[6 39] and comparable with other JAK inhibitors.[16 17 40 41] In the subset of patients receiving baricitinib 2 mg or 4 mg, the EAIRs were similar between dose groups and comparable with those previously reported.[6] While recent meta-analyses of randomised controlled trials of JAK inhibitors (including tofacitinib, baricitinib and upadacitinib) in patients with RA have shown no increased risk of venous thromboembolic events during the placebo-controlled periods, longer-term data are needed to fully characterise the risk of these events.[42 43]

The incidence of MACE in the current study (0.5) remained low and stable from previous reports.[6 37] The IRs showed no increase with longer exposure to baricitinib despite the ageing of the study population and were observed at similar rates to TNFi (0.62/100 patient-years)[44] and other JAK inhibitors (0.4/100 patient-years; 0.6–1.0/100 patient-years).[18 19 42] The EAIR of MACE was similar between baricitinib 2 mg (0.42) and 4 mg (0.54). Of the patients, 55% had at least one of five cardiovascular risk factors at baseline used in the analysis (current smoker, hypertension, diabetes, history of atherosclerotic disorder or HDL cholesterol <40 mg/dL), and as expected the IR for MACE was higher in this at-risk subpopulation (0.70), remaining similar to rates reported for TNFi in the preliminary data of the tofacitinib postmarketing study (IR/100 patient-years, 0.98 for tofacitinib compared with 0.73 for TNFi).[35 40] It should be noted that the higher IR observed with tofacitinib in a study (A3921133) remains within the wide boundaries (IR 0.2–2.4) reported for MACE in epidemiological studies within the general RA population.[44–47] It has been hypothesised that TNFi could provide a protective effect against MACE.[48 49]

The EAIR for diverticulitis in the current study (0.15) is consistent with published data among patients with RA reported at 0.2[50–52] and consistent with IR for diverticulitis of 0.27 among a general population of similar mean age.[53] Important risk factors for diverticulitis in the general population include age, obesity, diet, smoking and medication use, in particular opioids, corticosteroids and NSAIDs.[54 55] Diverticulitis in our study occurred in patients with risk factors. The IR for gastrointestinal perforations (0.06) remains low in the context of reports from tofacitinib, tocilizumab and other biologic DMARDs in real-world data[56] and upadacitinib (0.08/100 patient-years).[19]

Moderate decreases in haemoglobin and neutrophils and increases in transaminase and creatinine phosphokinase observed with baricitinib were consistent with laboratory changes previously reported and observed with other JAK inhibitors.[19 20]

As previously reported, there are limitations to this analysis, including lack of control group in the LTE and possible modifications of background therapy by clinicians in the study extension; however, these factors more closely resemble real-world treatment plans. Additionally, during the LTE, because dose changes were allowed whether for tapering from baricitinib 4 mg to 2 mg or rescue to baricitinib 4 mg, the ability to assess the effects of dose on outcomes is restricted. Despite this limitation, the subset analysis provides a view of dose time of event for death, serious infections, MACE and DVT/PE. The study is also limited by survival bias; patients with adverse events and/or lack of efficacy that led to discontinuation from their originating study were not included in the LTD, therefore yeilding a more robust cohort for analysis at the end of 9 years. All data in this analysis are from randomised controlled trials with specific inclusion criteria and protocols, which may limit the applicability of these data to clinical practice. Caution should also be taken when interpreting the results for patients with the shortest and longest baricitinib exposure due to differences in patient numbers, which are fewer in later months. Safety in the baricitinib placebo-controlled analysis set is not included in the current study as there are no new data from the short placebo-controlled period that was previously reported.[6]

In summary, this report describes the highest level of patient exposure to baricitinib across the spectrum of the RA population, including the LTE study, RA-BEYOND, which is now completed. The study included 3770 patients and over 14 000 PYE with rigorous safety monitoring throughout the clinical trials and robust mortality data. Baricitinib maintained a safety profile similar to that previously reported, with rates of safety events of special interest (including deaths, malignancies, MACE and DVT/PE) remaining stable through exposures up to 9.3 years.

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CLINICAL SCIENCE

# Fatigue in patients with early rheumatoid arthritis undergoing treat-to-target therapy: predictors and response to treatment

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## ABSTRACT

**Objectives** Fatigue is a frequent symptom in rheumatoid arthritis (RA) and has high impact on quality of life. We explored associations between disease activity and fatigue in patients with early RA during the initial 24 months of modern treat-to-target therapy and predictors of fatigue after 24 months of follow-up.

**Methods** Data were obtained from the treat-to-target, tight control Aiming for Remission in Rheumatoid Arthritis: a Randomised Trial Examining the Benefit of Ultrasound in a Clinical Tight Control Regime (ARCTIC) trial. Fatigue was measured on a visual analogue scale (VAS) from 0 to 100 mm and defined as clinically relevant if VAS was ≥20 mm. Baseline predictors of fatigue at 24 months were analysed by multivariable logistic regression.

**Results** 205 patients with fatigue data at baseline and 24 months were included. Median (25th, 75th percentiles) symptom duration was 5.4 months (2.8, 10.4), fatigue VAS 37.0 mm (13.0, 62.0) and mean Disease Activity Score (DAS) 3.4 (SD 1.1) at baseline. Prevalence of fatigue declined from 69% at baseline to 38% at 24 months. Fewer swollen joints (OR 0.92, 95% CI 0.87 to 0.98, p=0.006), lower power Doppler ultrasound score (OR 0.95, 95% CI 0.90 to 0.99, p=0.027) and higher patient global assessment (PGA) (OR 1.03, 95% CI 1.01 to 1.04, p<0.001) increased the risk of clinically relevant fatigue at 24 months. Not achieving remission at 6 months was associated with a higher risk of reporting fatigue at 24 months.

**Conclusions** Fatigue in patients with early RA was prevalent at disease onset, with a rapid and sustained reduction during treatment. Low objective disease activity and high PGA at baseline were predictors of clinically relevant fatigue at 24 months.

## Key messages

**What is already known about this subject?**

► Fatigue is common in patients with rheumatoid arthritis (RA). Knowledge about the causes of fatigue is limited, and associations between fatigue and disease activity are uncertain.

**What does this study add?**

► This study shows that there is an association between disease activity and fatigue, and that achieving early disease remission decreases the risk of fatigue. Baseline predictors of fatigue at 24 months are lower number of swollen joints, lower ultrasound power Doppler score and higher patient global assessment.

**How might this impact on clinical practice or future developments?**

► These results support the effect of early treat-to-target treatment on fatigue in a majority of patients with early RA and provide baseline predictors of fatigue at 24 months. A non-pharmacological approach to fatigue might be favourable in the presence of these predictors.

## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by joint inflammation with subsequent joint destruction and loss of function.[1] Up to 70% of patients with RA experience fatigue,[2] and patients have ranked fatigue as one of the most important disease-related outcomes in RA.[3 4] There is no generally accepted definition of RA-related fatigue, but the symptoms have been described 'as an overwhelming, debilitating, and sustained sense of exhaustion that decreases the ability to function and carry out daily activities'.[5]

Fatigue is considered a multidimensional phenomenon involving disease processes, personal and social aspects,[2] and with implications for the patient's quality of life as well as increased societal costs related to reduced work productivity and frequent physician consultations.[6] Physical function, age, gender, mental health, pain, sleep disturbances and inflammation have been found to be associated with fatigue. However, none of these variables show a consistently strong relationship with fatigue across studies in systematic reviews, and the impact of inflammatory disease activity on fatigue has not been established.[6–9]

The goal in modern RA treatment is sustained remission and augmentation of long-term health-related quality of life through control of symptoms, prevention of structural damage, and participation in social and work-related activities.[10 11] In addition to the introduction of biological disease-modifying antirheumatic drugs (DMARDs), more aggressive treatment with higher doses of methotrexate, earlier initiation of DMARDs and tight control



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strategies have led to a large proportion of patients with early RA reaching sustained remission.[11] However, fatigue in patients with early RA treated according to modern treatment strategies is still not well understood.

The objectives of this study were, first, to explore the longitudinal prevalence of fatigue in patients with early RA followed up in a treat-to-target strategy trial and to investigate the relationship between fatigue and disease activity. Second, we aimed to identify baseline predictors of unresolved fatigue after 24 months of follow-up and, finally, to assess the impact of early treatment response and remission on fatigue.

## MATERIALS AND METHODS

### Study design

Data were obtained from the Aiming for Remission in Rheumatoid Arthritis: a Randomised Trial Examining the Benefit of Ultrasound in a Clinical Tight Control Regime (ARCTIC) trial.[12] Participants were randomised 1:1 to a treat-to-target strategy with or without applying musculoskeletal ultrasonography in clinical examinations and treatment decisions. All patients were treated according to a predefined algorithm that started with a combination of methotrexate (15 mg/week escalated to 20–25 mg/week) and prednisolone (15 mg tapered to 0 mg) during the initial 7 weeks. The treatment target was remission defined as Disease Activity Score (DAS) in 44 joints of <1.6 and no swollen joints, with an additional target of no power Doppler signal in any examined joint in the ultrasound arm. Each patient was scheduled for 13 visits during the 2-year follow-up.[12]

### Participants

Patients (18–75 years) who fulfilled the American College of Rheumatology (ACR)/European Alliance of Associations for Rheumatology (EULAR) classification criteria for RA[13] were recruited from 11 Norwegian rheumatology centres between 2010 and 2013. All patients provided written consent, and all had symptom duration of less than 24 months, no prior DMARD use and indication for DMARD treatment at inclusion.[12]

### Participant involvement

Two participants recruited from the ARCTIC trial were involved in the planning and interpretation of the analyses presented in this article.

### Fatigue

Fatigue was measured at all visits on a Visual Analogue Scale (VAS) ranging from 0 mm (fatigue is not a problem) to 100 mm (fatigue is a major problem).[14–16] The question was articulated, 'Have you had problems with fatigue during the last week'? Fatigue is recommended as a core outcome measure in clinical trials by the Outcome Measures in Rheumatology, ACR and the EULAR.[17 18]

As there are no standardised cut-offs for clinically relevant fatigue, we dichotomised fatigue in accordance with previous studies: <20 mm (no fatigue) and ≥20 mm (clinically relevant fatigue).[19–21] For the predictor analyses, the outcome was fatigue of ≥20 mm at 24 months. Additionally, we divided the fatigue VAS in <20, 20–39 and ≥40 mm corresponding to low or no fatigue, clinically relevant fatigue and high level of fatigue, respectively.[22–24] Fatigue was also assessed on a continuous scale. The percentage of participants who achieved a minimal clinically important improvement in fatigue VAS of ≥10 mm was assessed at 24 months.[25]

### Clinical assessments

Disease activity was measured at all visits by DAS (range 0–10),[26] which incorporates assessment of tender joints (Ritchie Articular Index range 0–78), number of swollen joints (0–44), the patient global assessment (PGA) of disease activity on a VAS (VAS 0–100 mm) and erythrocyte sedimentation rate (ESR, mm/hour). DAS of <1.6 corresponds to remission; DAS of <2.4 corresponds to low disease activity; DAS of >2.4–3.7 corresponds to moderate disease activity; and DAS of >3.7 corresponds to high disease activity[27]. In addition, the Boolean-based ACR/EULAR remission,[28] Simplified Disease Activity Index remission[29] and Clinical Disease Activity Index remission[30] were assessed. Treatment response was evaluated by EULAR good/moderate response.[31]

The clinical evaluation also included C reactive protein (CRP, mg/L) and ultrasound examination of 32 joints at baseline and yearly in all patients (0–3 semiquantitative scoring of grey scale and power Doppler using an atlas for reference).[32 33]

### Patient-reported outcomes and demographic measures

Sleep difficulty was assessed by a component of the Rheumatoid Arthritis Impact of Disease (RAID) on a Numerical Rating Scale (range 0–10, higher scores representing poorer outcome). Mental health was assessed by the 36-Item Short Form Survey Mental Component Summary Score consisting of the components mental health, vitality, role–emotional and social functioning (range 0–100, with lower scores indicating poorer outcome).[34] Physical function was assessed by Patient-Reported Outcome Information System (PROMIS) on a range of 20–100, translated to a T score with a mean of 50 and an SD of 10, where lower scores implied poorer outcome.[35] Patient-reported outcomes were acquired electronically at 0, 3, 6, 12, 16 and 24 months during study visits.[12] In addition, PGA was measured at all 13 visits. Baseline characteristics included age, gender, anti-CCP positivity, rheumatoid factor, body mass index (BMI) of ≥25 kg/m$^2$ and a comorbidity score measured by the Self-administered Comorbidity Questionnaire (score 0–45).[36] Education was dichotomised below/above 12 years.

### Statistics

Data from the two study groups in ARCTIC were pooled for the current analyses as there were no statistically significant differences in primary or secondary endpoints between the two study groups.[12] Patients with complete fatigue data at baseline and 24 months were included. Missing fatigue data as well as other continuous variables between baseline and 24 months were imputed with last observation carried forward. Categorical variables missing at 24 months were imputed with worst outcome, and missing data before 24 months with last observation carried forward.[12] Continuous variables are described in means (SD), or medians (25th, 75th percentiles) as appropriate. Categorical variables are presented as frequencies (%).

We explored changes in median fatigue from baseline to 24 months and calculated the proportions of patients in the three fatigue categories at baseline and at 6 and 24 months. The correspondence between changes in fatigue VAS and changes in DAS according to categories was assessed.

Potential baseline predictors of fatigue of ≥20 mm at 24 months of follow-up were explored by univariable logistic regression, and a p value of less than 0.10 was required for the variable to be included in the subsequent multivariable analysis. Continuous variables were tested for linearity. A multivariable prediction model was built using backward stepwise selection

requiring a p value of <0.05 to keep the covariate in the model. All models were adjusted for age and gender. Variables excluded from the final model were re-entered one by one and kept in the model if they changed the coefficient of any other variable by more than 20%. Potential interactions between variables in the final model were analysed by including interaction terms one by one. Joint pain VAS, PROMIS physical function and RAID sleep were excluded from analyses due to high correlation with PGA (r=0.83, r=−0.67 and r=0.58, p<0.001, respectively) at baseline, but separate analyses of a model including these variables were also performed. DAS was not included as a composite measure, but the components were included separately. As CRP and ESR are closely related, we included CRP in the analyses in favour of ESR. ORs were calculated to explore the associations between early treatment response or remission and fatigue after 24 months of treatment. Robustness analyses were performed using only complete case data, and sensitivity analyses were performed for patients with fatigue of ≥20 mm at all time points from baseline to 24 months.

All analyses were performed using Stata/IC V.14.0 and 16.0.

## RESULTS

### Baseline characteristics

Of the 230 patients analysed for the primary outcome in the ARCTIC trial, 205 had complete fatigue data at baseline and 24 months and were included in the current analyses. There were no statistically significant differences in baseline variables between the subset of 205 and the full set of 230 patients. Less than 5/205 (2.4%) of the observations for any variables were imputed at any time point during the 24 months of follow-up. Baseline demographics and disease characteristics are presented in table 1. Fatigue was highly prevalent at baseline with 142/205 (69%) reporting fatigue of ≥20 mm, and median fatigue was 37.0 mm (25th, 75th percentiles 13.0, 62.0). Mean DAS of 3.4 (SD 1.1) corresponded to moderate disease activity level at baseline.

**Table 1** Baseline demographics and disease characteristics

| Variable | Patients n=205 |
|---|---|
| Age (years) | 52.2 (13.4) |
| Female gender | 126 (61.5) |
| Education >12 years | 123 (60) |
| Symptom duration (months) | 5.4 (2.8, 10.4) |
| Anti-CCP positive | 169 (82.4) |
| RF positive | 142 (69.3) |
| Comorbidity score (SCQ) | 4.0 (2.0, 7.0) |
| Depression | 11 (5.4) |
| Fibromyalgia | 1 (0.5) |
| BMI ≥25 | 111 (54.7) |
| DAS | 3.4 (1.1) |
| Swollen joint count* | 9.0 (4.0, 14.0) |
| Tender joint count, RAI† | 6.0 (4.0, 12.0) |
| ESR | 19.0 (11.0, 31.0) |
| C reactive protein | 7.0 (3.0, 18.0) |
| Patient global assessment (VAS) score | 48.8 (24.4) |
| Physician global assessment (VAS) score | 39.2 (20.0) |
| Ultrasound power Doppler score (0–96) | 7.0 (3.0, 14.0) |
| van der Heijde Modified Sharp Score (0–480)‡ | 4.5 (1.5, 9.0) |
| Fatigue VAS | 37.0 (13.0, 62.0) |
| Joint pain VAS | 42.6 (23.4) |
| PROMIS physical function score | 39.5 (8.6) |
| SF-36 MCS score | 49.4 (10.6) |
| Sleep (RAID) | 3.8 (3.0) |

Values are presented as mean (SD), n (%) or median (25th, 75th percentiles). SD: 95%.
DAS: 44 joints (0–10), <1.6 (remission), ≥1.6–2.4 (low disease activity), >2.4–3.7 (moderate disease activity), >3.7 (high disease activity). ESR (mm/hr): 1-140. PROMIS: 20–100. MCS score: 0–100, RAID: 0–10. SCQ: 0–45. VAS (mm) score: 0-100.
*Assessment of 44 joints (0–44).
†RAI score: 0–78.
‡Including erosion score and joint space narrowing score.
BMI, body mass index; CCP, cyclic citrullinated peptide; DAS, Disease Activity Score; ESR, erythrocyte sedimentation rate; MCS, mental component summary; PROMIS, Patient-Reported Outcome Information System; RA, rheumatoid arthritis; RAI, Ritchie Articular Index; RAID, Rheumatoid Arthritis Impact of Disease; RF, rheumatoid factor; SCQ, Self-administered Comorbidity Questionnaire; SF-36, 36-Item Short Form Survey; VAS, Visual Analogue Scale.

### Changes in fatigue from baseline to 24 months

There was a rapid and sustained reduction in fatigue with the largest reduction observed within the first 3 months (figure 1 and online supplemental figure 1). Median fatigue was 37 mm (25th, 75th percentiles 13.0, 62.0) at baseline and 9 mm (25th, 75th percentiles 2.0, 34.0) at 24 months, and mean fatigue was 39 mm (SD 27.9) and 21 mm (SD 24.5) at baseline and 24 months, respectively.

At baseline, 142/205 (69%) reported a fatigue score of ≥20 mm, compared with 77/205 (38%) at 24 months (p<0.001) (figure 2). A total of 57% of the patients had a minimal clinically important improvement in fatigue (≥10 mm) at 24 months, and the proportion of patients reaching minimal clinically important improvement according to baseline fatigue of <20, 20–40 and >40 mm were 11%, 64% and 84%, respectively, displaying that a majority of patients with moderate and high fatigue achieved an improvement corresponding to minimal clinically important improvement.

### Changes in fatigue and disease activity

There was a parallel reduction in fatigue and disease activity as 80% of the patients had moderate or high disease activity according to DAS (>2.4) at baseline and 9% at 24 months, while 69% of patients reported clinically relevant fatigue (≥20 mm) at baseline and 38% at 24 months (figure 2). At baseline, 95/205 (46%) had a fatigue score of 40 mm or higher, and this proportion was reduced to 39/205 (19%) at 24 months (figure 2). There was a corresponding increase in patients with low or no fatigue from baseline to 24 months; 63/205 (31%) of the patients scored <20 mm on the fatigue scale at baseline, compared with 128/205 (62%) at 24 months. In concurrence, 10/205 (5%) were in DAS remission at baseline vs 156/205 (76%) at 24 months (figure 2).

The proportion of patients with fatigue was highest among the patients with moderate to high disease activity both at baseline with 122/165 (74%) and 24 months with 14/18 (78%).

A fraction of the patients in DAS remission reported fatigue: 4/10 (40%) at baseline and 45/156 (29%) at 24 months, while some patients with moderate or high DAS (>2.4) did not report fatigue: 43/165 (26%) at baseline and 4/18 (22%) at 24 months (figure 2).

### Baseline predictors of fatigue

Sleep disturbances, Mental Component Summary Score, physical function and PGA at baseline were predictors of fatigue at 24 months, in addition to low number of swollen joints and low ultrasound power Doppler score (table 2). In the multivariable

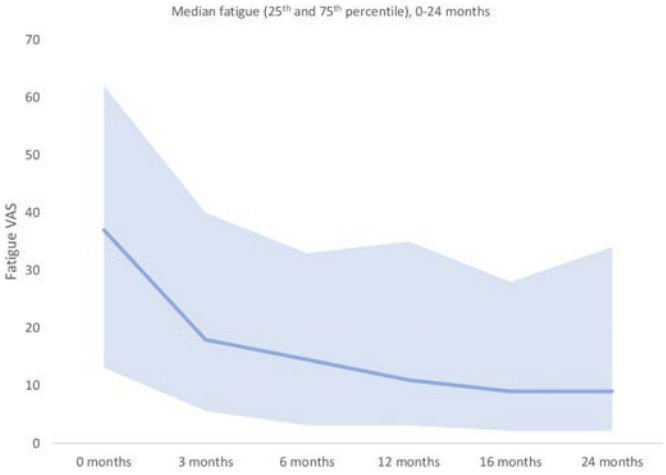


**Figure 1** Change in median fatigue VAS (0–100 mm) over 24 months. Percentiles (25th and 75th) illustrated by the shaded area. VAS, Visual Analogue Scale.

analyses, number of swollen joints (OR=0.92, 95% CI 0.87 to 0.98, p value of 0.006), ultrasound power Doppler score (OR=0.95, 95% CI 0.90 to 0.99, p value of 0.027) and PGA (OR per mm=1.03, 95% CI 1.01 to 1.04, p<0.001) were significant predictors of reporting fatigue at 24 months, when controlling for the other factors in the model, as described in table 2.

Similar results were observed in robustness analyses in complete case data and sensitivity analyses for patients with sustained, clinically relevant fatigue from baseline to 24 months (data not shown).

Moreover, neither BMI, depression as a comorbidity (online supplemental table 1), methotrexate dosage (online supplemental table 2) nor the extent of adverse events (online supplemental table 3) were associated with fatigue in our data.

### Associations between fatigue and early treatment response or remission at 6 months

There was no significantly decreased risk of reporting fatigue of ≥20 mm at 24 months for patients who achieved EULAR good/moderate response at 3 months (OR=0.62, 95% CI 0.28, 1.34, p value of 0.277) (table 3). However, there was a significantly decreased risk of reporting fatigue of ≥20 mm at 24 months for patients who achieved remission at 6 months by all listed remission criteria (table 3).

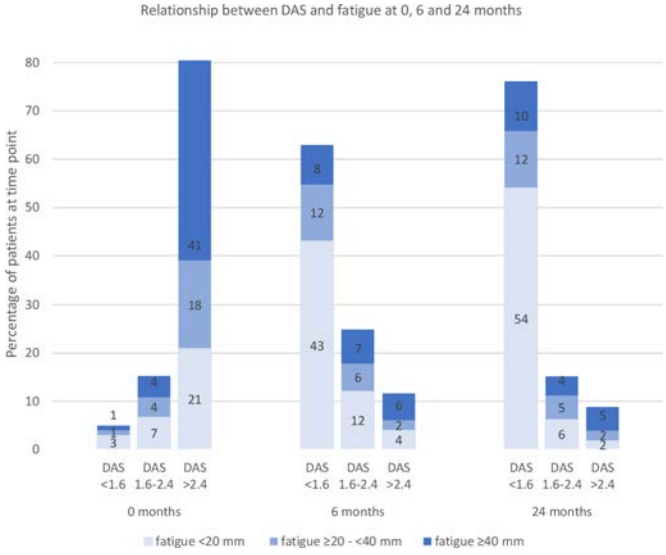


**Figure 2** Percentage of patients in fatigue categories <20, ≥20–<40 and ≥40 mm according to DAS categories <1.6, 1.6–2.4 and >2.4 at 0, 6 and 24 months. DAS, Disease Activity Score.

## DISCUSSION

In this inception cohort of patients with early RA, clinically relevant fatigue of ≥20 mm was highly prevalent at treatment onset. There was an overall rapid and sustained reduction in fatigue corresponding to the reduction in disease activity, and the majority of patients were in remission or low disease activity with no clinically relevant fatigue at 24 months. Prediction analyses demonstrated that few swollen joints, low power Doppler ultrasound score and high PGA at baseline increased the risk of reporting fatigue at 24 months. In addition, not reaching remission at 6 months increased the risk of reporting fatigue at 24 months.

Differences in fatigue measures, cut-offs and study designs create some challenges in the comparison of fatigue across studies. We saw a lower baseline level of fatigue in the present analyses than in comparable studies by Rat *et al*,[20] Scott *et al*[37] and Gossec *et al*,[38] where patients with early RA reported mean fatigue level of 47.8 (SD 28.2) and mean fatigue of >50 and >60 mm, respectively. Furthermore, the reduction in fatigue during the 24 months of follow-up was greater in the ARCTIC cohort than in a longitudinal register study by Druce *et al*,[21] where mean fatigue VAS was above 50 mm at baseline and at 1 and 4 years of follow-up, and smaller reductions in fatigue were observed in studies on patients with early RA as well as in patients with established RA.[19 23 24] Mean fatigue at 24 months in the ARCTIC cohort was similar to the level of fatigue (mean 20.5 mm (SD 0.02)) in Norwegian healthy controls reported by Slatkowsky-Christensen *et al*, supporting that the overall level of fatigue in the ARCTIC cohort at 24 months was at the same level as a normal population.[39] In agreement with previous research, our findings suggest that fatigue is prevalent in patients with early RA in about two of three patients, and that the prevalence is similar to what has been observed in established RA.[19 20 24 40]

We found that the improvement in fatigue over time corresponded to the reduction in disease activity, indicating a treatment response. There was a higher proportion of patients with high fatigue among patients with the highest disease activity at all assessed time points, which implies a positive relationship between the two factors. At the same time, our analyses showed that some patients in DAS remission reported fatigue and that some patients with high disease activity experienced low or no fatigue. Proinflammatory cytokines involved in the inflammatory responses in RA have been suggested to trigger fatigue,[6 41 42] and some trials have indicated an association between disease activity and fatigue.[24 43–45] However, other studies show that in some cases, fatigue persists even though inflammation and disease activity are low.[22 46 47]

The predictor analyses support that fatigue is a multidimensional phenomenon.[2] The multivariable analyses indicated that low inflammatory disease activity represented by few swollen joints and low ultrasound power Doppler score, and high scores of PGA at baseline were associated with a higher risk of fatigue at 24 months of follow-up. It could seem contradictory that we observed a positive association between fatigue and disease activity as well as between early remission and unresolved fatigue at 24 months, and at the same time found little inflammation at baseline to predict fatigue at 24 months. One explanation could be that there were two subsets of fatigue: patients where high

**Table 2** Baseline predictors of clinically relevant fatigue (≥20 mm) at 24 months

| | Fatigue ≥20 mm at 24 months, n=77/205 | | | |
|---|---|---|---|---|
| | Univariable analysis | | Multivariable analysis | |
| Variables | OR (95% CI) | P value | OR (95% CI) | P value |
| Female gender | 1.52 (0.84 to 2.74) | 0.167 | 1.30 (0.67 to 2.53) | 0.441 |
| Age | 0.99 (0.98 to 1.02) | 0.844 | 1.01 (0.99 to 1.03) | 0.465 |
| Education >12 years | 0.63 (0.35 to 1.12) | 0.112 | | |
| Anti-CCP positivity | 1.46 (0.67 to 3.16) | 0.341 | | |
| BMI ≥25 | 0.91 (0.52 to 1.61) | 0.794 | | |
| Swollen joint count* | 0.92 (0.88 to 0.97) | **0.001**‡ | 0.92 (0.87 to 0.98) | **0.006** |
| Tender joint count (RAI) | 1.01 (0.96 to 1.05) | 0.737 | | |
| Patient global assessment (VAS) score | 1.01 (1.00 to 1.03) | **0.016**‡ | 1.03 (1.01 to 1.04) | **<0.001** |
| C reactive protein | 0.99 (0.97 to 1.00) | 0.146 | | |
| Ultrasound power Doppler score† | 0.93 (0.88 to 0.99) | **0.022**‡ | 0.95 (0.90 to 0.99) | **0.027** |
| PROMIS physical function | 0.96 (0.93 to 0.99) | **0.021**‡ | | |
| SF-36 MCS | 0.96 (0.94 to 0.99) | **0.007**‡ | | |
| RAID sleep | 1.16 (1.05 to 1.27) | **0.003**‡ | | |

P values <0.05 in bold. MCS score: 0–100. PROMIS score: 20–80. RAI score: 0–78. RAID: NRS score: 0–10. VAS (mm): 0–100.
*Assessment of 44 joints (0–44)
†Range 0–96
‡Variables with univariable p values <0.10
BMI, body mass index; CCP, cyclic citrullinated peptide; 95% CI, 95% confidence interval; MCS, Mental Component Summary; NRS, Numerical Rating Scale; OR, odds ratio; PROMIS, Patient-Reported Outcome Information System; RAI, Ritchie Articular Index; RAID, Rheumatoid Arthritis Impact of Disease; SF-36, 36-Item Short Form Survey; VAS, Visual Analogue Scale.

inflammatory disease activity was the cause of fatigue and for whom early, intensive treatment improved fatigue, and patients with fatigue at baseline for whom fatigue could have been triggered by different factors that were not affected by DMARD treatment.[23 48] These factors might be captured by PGA, and further research is warranted on the relationship between fatigue and PGA. In the 38% of the patients who reported clinically relevant fatigue at 24 months, the source of fatigue might not have been adequately addressed. Associations between comorbidities such as depression and fibromyalgia and fatigue have been documented in prior studies[8 49] but were not confirmed in our data. Non-pharmacological interventions to relieve fatigue are tailored physical activity, behavioural modification, treatment of pain or depression, and improving sleep.[8]

Our results indicate that achievement of treatment target at 6 months reduced the risk of fatigue at 24 months, and similar results were observed by Scott *et al*, who found that fatigue was significantly lower in patients with RA in an intensive treat-to-target strategy compared with patients who received standard treatment.[24 37]

This study has limitations. There is a lack of standardised fatigue measures and definitions in RA, and we used global, unidimensional fatigue VAS in the present analyses. Fatigue VAS does not yield detailed information; however, fatigue VAS is validated as more sensitive to change than some multidimensional measures and has high reliability, construct, content and face validity.[14–16 50] Furthermore, it is one of the most frequently applied measures of fatigue in RA.[16] The lack of standardised cut-offs for fatigue VAS generates uncertainties regarding the prevalence and severity of fatigue, including the extent of residual fatigue, and complicates comparison of results across studies.

A strength of this study is the longitudinal, prospective and multivariable analyses which have been recommended.[7] In addition, data from the ARCTIC trial provided a unique opportunity to explore fatigue in patients with early RA followed by modern treat-to-target strategies, and to our knowledge, this is the first

**Table 3** ORs of fatigue ≥20 mm at 24 months according to EULAR response at 3 months, DAS remission, ACR/EULAR Boolean remission, SDAI remission and CDAI remission at 6 months

| | | Fatigue ≥20 mm at 24 months | |
|---|---|---|---|
| | Classification n/N (%) | OR (95% CI) | P value |
| Response at 3 months | | | |
| EULAR good/moderate response | 170/199 (85) | 0.62 (0.28 to 1.34) | 0.227 |
| Remission at 6 months | | | |
| DAS 44 remission | 124/197 (63) | 0.31 (0.17 to 0.57) | **<0.001** |
| ACR/EULAR Boolean remission | 78/197 (40) | 0.30 (0.16 to 0.58) | **0.002** |
| SDAI remission | 90/197 (46) | 0.23 (0.12 to 0.43) | **<0.001** |
| CDAI remission | 92/197 (47) | 0.19 (0.10 to 0.36) | **<0.001** |

EULAR good/moderate response defined as DAS ≤2.4 and a decrease by >1.2, DAS ≤2.4 and a decrease by >0.6 and ≤1.2, or a DAS >2.4 and ≤3.7 and decreases by >1.2 and >0.6 and ≤1.2, or DAS >3 and a decrease by >1.2. DAS (44 joints, ESR) remission defined as DAS <1.6. ACR/EULAR Boolean remission criteria defined as swollen joints ≤1, tender joints ≤1, CRP ≤10 and PGA ≤10. SDAI: defined as SDAI ≤3.3. CDAI: remission defined as CDAI ≤2.8. P values <0.05 in bold.
ACR, American College of Rheumatology; CDAI, Clinical Disease Activity Index; DAS, Disease Activity Score; ESR, erythrocyte sedimentation rate; EULAR, European Alliance of Associations for Rheumatology; SDAI, Simplified Disease Activity Index.

study that have explored associations in patients with early RA between fatigue and ultrasound, biochemical and clinical assessments, in addition to a comprehensive assessment of patient-reported outcome measures.

In conclusion, this study showed that the majority of patients with early RA treated according to current EULAR treatment recommendations experienced a rapid and sustained reduction of fatigue. However, patients who did not reach remission at 6 months were at risk of experiencing fatigue at the 2-year follow-up, which could be of importance to clinicians in identifying patients at risk of long-term fatigue. In addition, there was a higher risk of fatigue in patients with RA with low objective disease activity measures and high patient reported global assessment of disease at baseline, and a non-pharmacological approach to fatigue in these patients might be considered.

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**Contributors** All authors were involved in drafting the article or revising it critically for important intellectual content, approved the final manuscript to be submitted and agreed to be accountable for all aspects of the work. Conception and design of the study: KH, NPS, SL, EAH and A-BA. Acquisition of data: NPS, HBH, EM, TU, EAH and A-BA. Analysis and interpretation of data: KH, NPS, SL, JS, LBN, EM, HBH, TU, TKK, EAH and A-BA.

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**Patient consent for publication** Not required.

**Ethics approval** The Aiming for Remission in Rheumatoid Arthritis: a Randomised Trial Examining the Benefit of Ultrasound in a Clinical Tight Control Regime trial was approved by an independent ethics committee (the Regional Committee for Medical and Health Research Ethics South-East; reference number 2010/744) and appropriate institutional review boards, and followed the guidelines outlined in 'Guidelines for Good Clinical Practice' developed by the International Conference of Harmonization.

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CLINICAL SCIENCE

# Efficacy and safety of risankizumab for active psoriatic arthritis: 24-week results from the randomised, double-blind, phase 3 KEEPsAKE 2 trial

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## ABSTRACT

**Objectives** Risankizumab is an interleukin-23 inhibitor under study for the treatment of patients with psoriatic arthritis (PsA). The phase 3 KEEPsAKE 2 trial investigated the efficacy and safety of risankizumab versus placebo in patients with active PsA who had previous inadequate response or intolerance to ≤2 biological therapies (Bio-IR) and/or ≥1 conventional synthetic disease-modifying antirheumatic drug (csDMARD-IR). Results through week 24 are reported here.

**Methods** Adults with PsA who were Bio-IR and/or csDMARD-IR were randomised to receive subcutaneously administered risankizumab 150 mg or placebo at weeks 0, 4 and 16 during a 24-week, double-blind treatment period. The primary endpoint was the proportion of patients who achieved ≥20% improvement in American College of Rheumatology score (ACR20) at week 24. Secondary endpoints assessed key domains of PsA and patient-reported outcomes.

**Results** A total of 444 patients (median age 53 years, range 23–84 years) were randomised to risankizumab (n=224) or placebo (n=220); 206 patients (46.5%) were Bio-IR. At week 24, a significantly greater proportion of patients receiving risankizumab achieved the primary endpoint of ACR20 (51.3% vs 26.5%, p<0.001) and all secondary endpoints (p<0.05) compared with placebo. Serious adverse events were reported for 4.0% and 5.5% of risankizumab-treated and placebo-treated patients, respectively; serious infections were reported for 0.9% and 2.3%, respectively.

**Conclusion** Treatment with risankizumab resulted in significant improvements versus placebo in key disease outcomes and was well tolerated in patients with PsA who were Bio-IR and/or csDMARD-IR.

**Trial registration number** NCT03671148.

## Key messages

**What is already known about this subject?**

- Many patients with psoriatic arthritis (PsA) do not achieve an adequate response or are intolerant to conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) or biological agents, highlighting a need for additional effective treatments.

**What does this study add?**

- This study demonstrates the efficacy of the interleukin-23 inhibitor, risankizumab, across multiple domains of PsA, including patient-reported outcomes assessing disease burden in patients who had previous inadequate responses to csDMARDs or biological agents.
- Risankizumab was well tolerated based on low rates of serious adverse events (AEs), severe AEs, serious and opportunistic infections, and discontinuation of treatment due to AEs by <1% of patients receiving risankizumab.

**How might this impact on clinical practice or future developments?**

- Results from the phase 3 KEEPsAKE 2 trial demonstrate that risankizumab is effective and well tolerated to treat active PsA.
- Risankizumab may provide an additional treatment option for patients with PsA who have had an inadequate response or are intolerant to currently approved therapies.

## INTRODUCTION

Psoriatic arthritis (PsA) is a progressive, chronic, inflammatory condition that affects approximately 30% of patients with psoriasis.[1 2] Symptoms of PsA involve the synovium, tendons, entheses and bone in axial or peripheral joints, and progression is characterised by joint degeneration, leading to disability and increased risk of mortality.[3–5] Comorbid conditions such as cardiovascular disease, metabolic syndrome, obesity, diabetes and mood disorders are common among patients with PsA, contributing to functional impairment and decreased quality of life.[3 6] PsA is also associated with considerable individual, societal and economic burdens, including reduced employment and increased healthcare costs compared with the general population.[7]

The aim of PsA treatment is to reduce symptoms, structural damage and inflammation, while restoring overall function, with a goal of remission (REM) and/or reduced disease activity and increased long-term, health-related quality of life.[8 9] Initial recommended treatment for PsA is non-steroidal anti-inflammatory drugs (NSAIDs), which may be combined with local corticosteroid injections. Second-line treatment includes use of conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) such as methotrexate, followed by



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therapy using antitumour necrosis factor medications, and/or other biological agents.[8 9]

Although biological agents are effective in treating PsA,[10] approximately 25%–40% of patients do not achieve at least 20% improvement in American College of Rheumatology score (ACR20), and clinical REM and minimal disease activity (MDA) are often short-lived.[11–19] Lack of efficacy frequently leads to treatment switching or discontinuation, which may negatively affect patients' clinical outcomes and increase treatment costs,[20–24] revealing a need for well-tolerated treatments with sustained efficacy.

Risankizumab is a humanised IgG1 monoclonal antibody that specifically inhibits interleukin (IL)-23 by binding to its p19 subunit.[25 26] IL-23 is a key component driving the release of IL-17 from Th17 cells, and overexpression of IL-23 has been reported in affected skin in psoriasis and in the synovial tissue of patients with PsA.[26–28] KEEPsAKE 2 is an ongoing clinical trial that is evaluating the efficacy and safety of risankizumab to treat PsA in patients with a history of inadequate response or intolerance to csDMARD and/or biological therapies. The results of the initial 24-week double-blind period of the KEEPsAKE 2 trial are reported here.

## METHODS

### Study design and treatment

This was a phase 3, global, multicentre study assessing the efficacy and safety of risankizumab 150 mg vs placebo to treat PsA in patients with inadequate response or intolerance to biological agents (Bio-IR) and/or inadequate response or intolerance to conventional synthetic disease-modifying antirheumatic drugs (csDMARD-IRs). During a screening period of approximately 35 days, patients were stratified by current csDMARD use (0 vs ≥1), number of prior biological therapies (0 vs ≥1) and extent of psoriasis (≥3% vs <3% body surface area affected by psoriasis), then randomised using an interactive response technology system in a 1:1 ratio to receive double-blind treatment with risankizumab 150 mg or matched placebo for 24 weeks, administered subcutaneously at weeks 0, 4 and 16. Patients then received open-label risankizumab every 12 weeks through week 208. The current report presents results for the 24-week double-blind period only, which was from 7 March 2019 to 22 June 2020. Study modifications for the COVID-19 pandemic included out-of-window study visits, phone calls and/or at-home visits for patients unable to attend on-site visits due to travel restrictions, quarantine or COVID-19 infection. The study drug was not administered to patients with suspected or confirmed COVID-19 infection; study drug administration and study visits could be resumed after patients recovered from infection.

### Patient and public involvement

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

### Patient eligibility

Eligible patients were adults (aged 18 years or older) with a clinical diagnosis of active PsA defined as ≥5 tender joints and ≥5 swollen joints, meeting the Classification Criteria for Psoriatic Arthritis, with symptoms of ≥6 months before screening, and active plaque psoriasis with ≥1 psoriatic plaque of ≥2 cm in diameter or nail changes consistent with psoriasis at screening. Patients were also required to be Bio-IR and/or csDMARD-IR, as described further.

### Prior or concomitant medications

Stable treatment with ≤2 concomitant csDMARDs at study entry was permitted if treatment was started ≥12 weeks before baseline at protocol-approved doses. In addition, patients could remain taking stable doses of concomitant NSAIDs, oral corticosteroids (equivalent to prednisone ≤10 mg/day) and other analgesics if they were started ≥1 week before baseline. Patients with a demonstrated lack of efficacy after ≥12 weeks or those who experienced intolerance or had a contraindication to methotrexate, sulfasalazine, leflunomide, apremilast, bucillamine, iguratimod or ciclosporin A were defined as csDMARD-IR.

Patients previously treated with biologic agents, except for IL-23, IL-12/23 or IL-17 antagonists, were also eligible for enrolment. The discontinuation of biological agents was required for prespecified durations before the first study treatment (≥4 weeks for etanercept; ≥8 weeks for adalimumab, infliximab, certolizumab, golimumab and abatacept; ≥1 year (or ≥6 months with normalisation of B cells) for rituximab; or ≥5 times the mean terminal elimination half-life for any other permitted biological agent). Patients with a demonstrated lack of efficacy after ≥12 weeks of treatment, or intolerance to one or two eligible biological agents, were defined as Bio-IR.

### Assessments

#### Efficacy

The primary endpoint was the proportion of patients who achieved ACR20 at week 24. Ranked secondary endpoints assessed at week 24, except where noted, were change from baseline in Health Assessment Questionnaire–Disability Index (HAQ-DI), proportion of patients who achieved ≥90% reduction in Psoriasis Area and Severity Index (PASI 90), proportion of patients who achieved ACR20 at week 16, proportion of patients who achieved MDA, change from baseline in 36-Item Short Form Health Survey Physical Component Summary (SF-36 PCS) score and change from baseline in Functional Assessment of Chronic Illness Therapy–Fatigue Questionnaire (FACIT-Fatigue) score.

Additional non-ranked secondary endpoints included the proportion of patients who achieved ACR50, ACR70, resolution of enthesitis (Leeds Enthesitis Index=0) and resolution of dactylitis (Leeds Dactylitis Index=0) at week 24. Post hoc analyses included the proportions of patients who achieved very low disease activity (VLDA), Disease Activity in Psoriatic Arthritis (DAPSA) REM (defined as DAPSA score ≤4), low disease activity (LDA) +REM (defined as DAPSA score ≤14), ≥50% and ≥85% reductions in DAPSA, HAQ-DI score ≤0.5, ≥10% and ≥30%, and ≥50% reductions in pain (as measured on a visual analogue scale (VAS)), and minimally clinically important difference (MCID) for PtGA (defined as a reduction of 10 mm or more from baseline as measured on a VAS).

#### Safety

Safety assessments were based on monitoring of treatment-emergent adverse events (TEAEs), which were defined as adverse events (AEs) with onset after the first dose of study drug and were summarised based on the Medical Dictionary for Regulatory Activities V.23.1. Findings from physical examinations, vital sign measurements and clinical laboratory tests (haematology and chemistry) were also assessed. Unblinded safety data were reviewed periodically by an external independent data monitoring committee through the week 24 interim analysis.

## Statistics

### Sample size determination

It was estimated that 210 patients per treatment group would have a 90% power to detect a mean difference of 0.24 for the changes from baseline in HAQ-DI between risankizumab and placebo, assuming a common SD of 0.72. This sample size would also ensure that analyses would have at least a 90% power to detect a 20% treatment difference in ACR20 at week 24, with an assumed placebo response rate of 35%, using a two-sided test at a significance level of 0.05 and accounting for a 10% dropout rate.

### Efficacy and safety analyses

Efficacy and safety analyses were conducted based on the full analysis set, defined as all randomised patients who received one or more doses of the study drug. Patient demographic and medical characteristics were summarised using categorical variables or continuous variables as appropriate.

For the efficacy analyses, the Cochran-Mantel-Haenszel test adjusted for the stratification factors was used for categorical variables, and a mixed-effect model repeat-measurement method was used for continuous variables, each with a two-sided $\alpha$ of 0.05. Due to the smaller number of patients with enthesitis and dactylitis at baseline, it was prespecified that data for the analyses of resolution of enthesitis and dactylitis were to be pooled from the companion study, KEEPsAKE 1 (NCT03675308), and KEEPsAKE 2 to increase sample size. Pooled data for these endpoints were analysed under the multiplicity control of KEEPsAKE 1 and are reported separately.[29] A multiple testing procedure was used to control the type I error rate by comparing risankizumab versus placebo in a fixed hypothesis testing procedure that began with the primary endpoint, proceeded through the ranked secondary endpoints in sequence, and continued until an endpoint did not achieve statistical significance. For categorical efficacy endpoints, missing data were handled by non-responder imputation incorporating multiple imputation to handle missing data due to COVID-19 (NRI-C). Missing data unrelated to COVID-19 were handled by non-responder imputation, and missing data due to COVID-19 (infection or logistical restrictions) were handled by multiple imputation. In addition, patients were considered non-responders after the initiation of rescue therapy or concomitant medications for PsA that could have meaningfully impacted efficacy assessments. For continuous efficacy endpoints, observations after the initiation of rescue therapy or concomitant medications for PsA that could have meaningfully impacted efficacy assessments were considered as missing and were excluded from the model. Safety results are summarised as the number and proportion of patients for whom TEAEs were reported within each treatment group.

# RESULTS

## Patients

A total of 444 patients at 99 sites in 23 countries were randomised to receive risankizumab (n=224) or placebo (n=220); of these patients, 215 (96.0%) and 199 (90.5%), respectively, completed the week 24 study visit (figure 1). One patient was randomised but never received the study drug and was excluded from the efficacy analyses; therefore, 443 patients were included in the full analysis set. Reasons for study discontinuation are summarised in figure 1. No patients discontinued from the study because of COVID-19 infection during the double-blind period; however, one patient discontinued because of COVID-19-related logistical restrictions. Less than 2.5% of patients had missing efficacy data for any parameter in either treatment group because of COVID-19 (online supplemental table 1).


**Figure 1** Patient disposition. *One patient was randomised but never received study drug and was therefore excluded from the efficacy analyses, resulting in 219 patients included in the PBO group in the full analysis set. PBO, placebo; RZB, risankizumab.

Demographics and baseline disease characteristics were generally balanced between treatment groups (table 1). The median age (range) was 53 (23–84) years and 55.1% were female. A total of 46.5% patients were Bio-IR. Demographics and baseline disease characteristics for the Bio-IR and csDMARD-IR subgroups are presented in online supplemental table 2. Baseline enthesitis and dactylitis were present for slightly greater proportions of patients in the placebo group compared with the risankizumab group.

## Efficacy assessments

The primary endpoint and all ranked secondary endpoints were met (table 2). For the primary endpoint, 51.3% of patients treated with risankizumab and 26.5% treated with placebo achieved ACR20 at week 24 ($p<0.001$). Changes from baseline in each ACR component at week 24 are summarised in online supplemental table 3. Higher ACR20 response rates were observed for patients treated with risankizumab versus placebo, regardless of whether patients received concomitant csDMARDs (50.4% vs 33.9%) or risankizumab as monotherapy (53.0% vs 16.0%), and among the csDMARD-IR (56.3% vs 36.6%) and Bio-IR (45.7% vs 14.9%) patient populations (online supplemental table 4). For the full patient population, similar results favouring risankizumab were also observed for ACR50 (26.3% vs 9.3%, nominal $p<0.001$) and ACR70 (12.0% vs 5.9%, nominal $p<0.05$) (table 2).

At week 4 (after a single dose), a greater proportion of patients treated with risankizumab than placebo achieved ACR20 (nominal $p=0.016$), and the improvement was sustained for all subsequent time points (figure 2A), including a significant difference for the secondary endpoint of ACR20 at week 16 (48.3% vs 25.3%, $p<0.001$; table 2). Similar patterns occurred with ACR50 (figure 2B) and ACR70 (figure 2C) results.

At week 24, a greater proportion of patients treated with risankizumab versus placebo experienced resolution of enthesitis (42.9% vs 30.4%, nominal $p<0.01$) and dactylitis (72.5% vs 42.1%, nominal $p<0.001$; table 2). These results are consistent with the pooled results from KEEPsAKE 1 and 2 previously reported.[29] Additionally, a significantly greater proportion of patients treated with risankizumab versus placebo achieved PASI

**Table 1** Demographics and baseline characteristics

| Characteristics | RZB 150 mg N=224 | PBO N=219 |
|---|---|---|
| Female, n (%) | 124 (55.4) | 120 (54.8) |
| Age (years), median (range) | 53 (23–84) | 52 (24 to 83) |
| Race, n (%) | | |
| White | 218 (97.3) | 210 (95.9) |
| Black or African–American | 2 (0.9) | 3 (1.4) |
| Asian | 2 (0.9) | 3 (1.4) |
| Other | 2 (0.9) | 3 (1.4) |
| Not Hispanic/Latino, n (%) | 182 (81.3) | 176 (80.4) |
| BMI (kg/m$^2$), mean (SD) | 31.5 (8.0) | 31.2 (6.8) |
| PsA duration (years), mean (SD) | 8.2 (8.2) | 8.2 (8.3) |
| Swollen joint count,* mean (SD) | 13.0 (8.7) | 13.6 (9.0) |
| Tender joint count,† mean (SD) | 22.8 (14.9) | 22.3 (13.8) |
| Patient's assessment of pain,‡ mean (SD) | 55.0 (23.5) | 57.0 (23.1) |
| PtGA of disease activity,‡ mean (SD) | 56.2 (21.8) | 56.2 (23.0) |
| PGA of disease activity,‡ mean (SD) | 63.0 (17.0) | 60.7 (16.4) |
| HAQ-DI, mean (SD) | 1.10 (0.62) | 1.13 (0.63) |
| hsCRP (mg/L),§ mean (SD) | 7.5 (10.9) | 8.2 (17.1) |
| Presence of psoriasis affecting ≥3% BSA, n (%) | 123 (54.9) | 119 (54.3) |
| BSA (%),¶ mean (SD) | 12.5 (15.4) | 11.7 (14.9) |
| PASI,¶ mean (SD) | 7.7 (6.7) | 8.4 (9.9) |
| MDA, n (%) | 5 (2.2) | 5 (2.3) |
| Presence of enthesitis,** n (%) | 147 (65.6) | 158 (72.1) |
| LEI,†† mean (SD) | 3.0 (1.5) | 3.0 (1.6) |
| Presence of dactylitis,‡‡ n (%) | 40 (17.9) | 57 (26.3) |
| LDI,§§ mean (SD) | 78.9 (98.4) | 109.8 (155.3) |
| SF-36 PCS score, mean (SD) | 35.6 (8.8) | 35.2 (9.1) |
| FACIT-Fatigue score, mean (SD) | 28.2 (11.5) | 27.7 (12.7) |
| Prior csDMARDs, n (%) | | |
| 0 | 12 (5.4) | 11 (5.0) |
| 1 | 88 (39.3) | 81 (37.0) |
| 2 | 60 (26.8) | 60 (27.4) |
| ≥3 | 64 (28.6) | 67 (30.6) |
| Any prior biologic, n (%) | 105 (46.9) | 101 (46.1) |
| Prior failed biologics, n (%) | | |
| 0 | 137 (61.2) | 132 (60.3) |
| 1 | 72 (32.1) | 64 (29.2) |
| ≥2 | 15 (6.7) | 23 (10.5) |
| Prior TNF antagonist, n (%) | 103 (46.0) | 100 (45.7) |
| Concomitant medication at baseline, n (%) | | |
| MTX¶¶ | 110 (49.1) | 99 (45.2) |
| csDMARD other than MTX*** | 31 (13.8) | 30 (13.7) |
| MTX and another csDMARD | 8 (3.6) | 10 (4.6) |
| Oral corticosteroids | 28 (12.5) | 22 (10.0) |
| NSAIDs | 141 (62.9) | 145 (66.2) |

*Based on 66 joints.
†Based on 68 joints.
‡Scored as millimetres on a 100 mm horizontal visual analogue scale.
§Reference range: 0–10 mg/dL.
¶Among patients with ≥3% BSA affected by psoriasis (RZB, n=23; PBO, n=119).
**LEI >0.
††Among patients with LEI >0 (RZB, n=147; PBO, n=158).
‡‡LDI >0.
§§Among patients with LDI>0 (RZB, n=40; PBO, n=57).
¶¶As monotherapy or in combination with another csDMARD.
***Sulfasalazine, leflunomide or apremilast, without MTX.
BMI, body mass index; BSA, body surface area; csDMARD, conventional synthetic disease-modifying antirheumatic drug; FACIT-Fatigue, Functional Assessment of Chronic Illness Therapy–Fatigue; HAQ-DI, Health Assessment Questionnaire–Disability Index; hsCRP, high-sensitivity C reactive protein; LDI, Leeds Dactylitis Index; LEI, Leeds Enthesitis Index; MDA, minimal disease activity; MTX, methotrexate; NSAID, non-steroidal anti-inflammatory drug; PASI, Psoriasis Area and Severity Index; PBO, placebo; SF-36 PCS, 36-Item Short Form Health Survey Physical Component Summary; PGA, physician's global assessment; PsA, psoriatic arthritis; PtGA, patient's global assessment; RZB, risankizumab; TNF, tumour necrosis factor.

90 at week 24 (55.0% vs 10.2%, p<0.001); a difference was observed as early as week 4 (figure 2D).

In the analysis of patient-reported outcomes, the change from baseline in HAQ-DI score was significantly greater in the risankizumab group compared with the placebo group (−0.22 vs −0.05, p<0.001; table 2). In a prespecified analysis of patients with HAQ-DI ≥0.35 at baseline, a greater proportion of patients treated with risankizumab achieved a clinically meaningful improvement in HAQ-DI (≥0.35 from baseline[30]) at week 24 versus placebo (39.9% vs 23.6%, nominal p<0.001). Significantly greater changes from baseline were also observed for patients treated with risankizumab versus placebo for both SF-36 PCS score (5.9 vs 2.0, p<0.001) and FACIT-Fatigue score (4.9 vs 2.6, p<0.01). The proportion of patients achieving MDA was significantly greater for risankizumab versus placebo (25.6% vs 11.4%, p<0.001; table 2). Additional outcomes on VLDA, DAPSA REM and LDA+REM; percentage reductions in DAPSA and pain; HAQ-DI score ≤0.5; and MCID for PtGA are reported in online supplemental table 5.

## Safety

TEAEs were reported for 124 (55.4%) and 120 (54.8%) patients in the risankizumab and placebo groups, respectively (table 3). Most events reported in the risankizumab group were mild or moderate. The most frequently reported TEAE was upper respiratory tract infection (risankizumab, n=17 (7.6%); placebo, n=12 (5.5%); table 4); no other event was reported for ≥5% of patients in either treatment group. Frequencies of serious and severe TEAEs were similar between treatment groups, and, except for severe psoriatic arthropathy (risankizumab, n=1 (0.4%); placebo, n=2 (0.9%)), no severe TEAE was reported for more than one patient in either group. TEAEs leading to discontinuation of treatment were more frequent in the placebo group (n=5, 2.3%) than in the risankizumab group (n=2, 0.9%). No deaths occurred during the 24-week double-blind period.

Frequencies of AEs of safety interest were low and comparable between treatment groups (table 3). However, injection site reactions were more frequently reported in the risankizumab group (n=3, 1.3%) than the placebo group (n=1, 0.5%). None of the injection site reactions occurring in the risankizumab group were serious or resulted in patient discontinuation, and no anaphylactic reactions were reported. One (0.4%) patient in the risankizumab group with a history of hypertension experienced a non-fatal stroke adjudicated as a major adverse cardiac event. Serious infections were reported for two (0.9%) patients in the risankizumab group and for five (2.3%) patients in the placebo group. There were no reports of active tuberculosis or other opportunistic infection in either treatment group, and only one case of herpes zoster was reported for a patient receiving placebo. There was one reported AE of uveitis in a patient treated with risankizumab and no reported AEs of inflammatory bowel disease.

Mean changes in haematology and clinical chemistry values were small, not clinically meaningful, and comparable between the risankizumab and placebo groups. There were no grade 3 transaminase elevations (as judged by Common Terminology Criteria for Adverse Events V.4.03) reported in either treatment group. Shifts in transaminase levels from baseline are reported in online supplemental table 6.

## DISCUSSION

In this phase 3 study, treatment with the IL-23 p19 inhibitor, risankizumab, led to significant improvements in key efficacy measures for patients with active PsA who were csDMARD-IR or

**Table 2** Primary and secondary efficacy endpoints

| | RZB 150 mg N=224 | PBO N=219 | Difference (95% CI) | P value |
|---|---|---|---|---|
| Primary endpoint | | | | |
| ACR20 at week 24, n (%) | 115 (51.3) | 58 (26.5) | 24.5 (15.9, 33.0) | <0.001* |
| Ranked secondary endpoints | | | | |
| Change in HAQ-DI at week 24, mean (95% CI) | −0.22 (−0.28 to −0.15) | −0.05 (−0.12 to 0.02) | −0.16 (−0.26 to 0.07) | <0.001* |
| PASI 90 at week 24,† n (%) | 68 (55.0) | 12 (10.2) | 44.3 (33.9 to 54.6) | <0.001* |
| ACR20 at week 16, n (%) | 108 (48.3) | 55 (25.3) | 22.6 (13.9 to 31.2) | <0.001* |
| MDA at week 24, n (%) | 57 (25.6) | 25 (11.4) | 14.0 (7.0 to 21.0) | <0.001* |
| Change in SF-36 PCS score at week 24, mean (95% CI) | 5.9 (4.9 to 6.9) | 2.0 (0.9 to 3.1) | 3.9 (2.4 to 5.3) | <0.001* |
| Change in FACIT-Fatigue score at week 24, mean (95% CI) | 4.9 (3.7 to 6.0) | 2.6 (1.4 to 3.9) | 2.2 (0.6 to 3.9) | <0.01* |
| Non-ranked secondary endpoints | | | | |
| ACR50 at week 24, n (%) | 59 (26.3) | 20 (9.3) | 16.6 (9.7 to 23.6) | <0.001 |
| ACR70 at week 24, n (%) | 27 (12.0) | 13 (5.9) | 6.0 (0.8 to 11.3) | <0.05 |
| Resolution of enthesitis at week 24,‡ n (%) | 63 (42.9) | 48 (30.4) | 13.8 (3.5 to 24.2) | <0.01 |
| Resolution of dactylitis at week 24,§ n (%) | 29 (72.5) | 24 (42.1) | 38.8 (22.9 to 54.8) | <0.001 |

All changes are LS mean changes from baseline.
Results for binary endpoints are based on non-responder imputation incorporating multiple imputation if there are missing data due to COVID-19 or non-responder imputation if there are no missing data due to COVID-19. Results for continuous endpoints are based on mixed models for repeated measures.
ACR20/50/70, ≥20/50/70% improvement in American College of Rheumatology score; BSA, body surface area; FACIT-Fatigue, Functional Assessment of Chronic Illness Therapy–Fatigue Questionnaire; HAQ-DI, Health Assessment Questionnaire–Disability Index; LDI, Leeds Dactylitis Index; LEI, Leeds Enthesitis Index; LS, least square; MDA, minimal disease activity; PASI 90, ≥90% reduction in Psoriasis Area and Severity Index; PBO, placebo; RZB, risankizumab; SF-36 PCS, 36-Item Short Form Health Survey Physical Component Summary.
*Statistically significant under overall type I error control.
†Among patients with ≥3% BSA affected by psoriasis at baseline (RZB, n=123; PBO, n=119).
‡Defined as LEI=0 among patients with LEI >0 at baseline (RZB, n=147; PBO, n=158).
§Defined as LDI=0 among patients with LDI>0 at baseline (RZB, n=40; PBO, n=57).

Bio-IR. A significantly greater proportion of patients treated with risankizumab versus placebo achieved ACR20 at 24 weeks and all secondary endpoints, including assessments of disease activity in joints and skin, and patient-reported outcomes. Overall, the safety profile was consistent with that described in the UltIMMa-1, UltIMMa-2,[31] IMMvent,[32] IMMhance,[33] and IMMerge[34] studies of risankizumab in patients with moderate-to-severe plaque psoriasis, and no new safety signals were observed. Of note, serious infections were reported for <1% of patients treated with risankizumab through week 24, and there were no cases of opportunistic infection, including herpes zoster, systemic candidiasis, or active tuberculosis. There was one major adverse cardiac event reported among patients receiving risankizumab (a non-fatal stroke in a patient with a history of hypertension

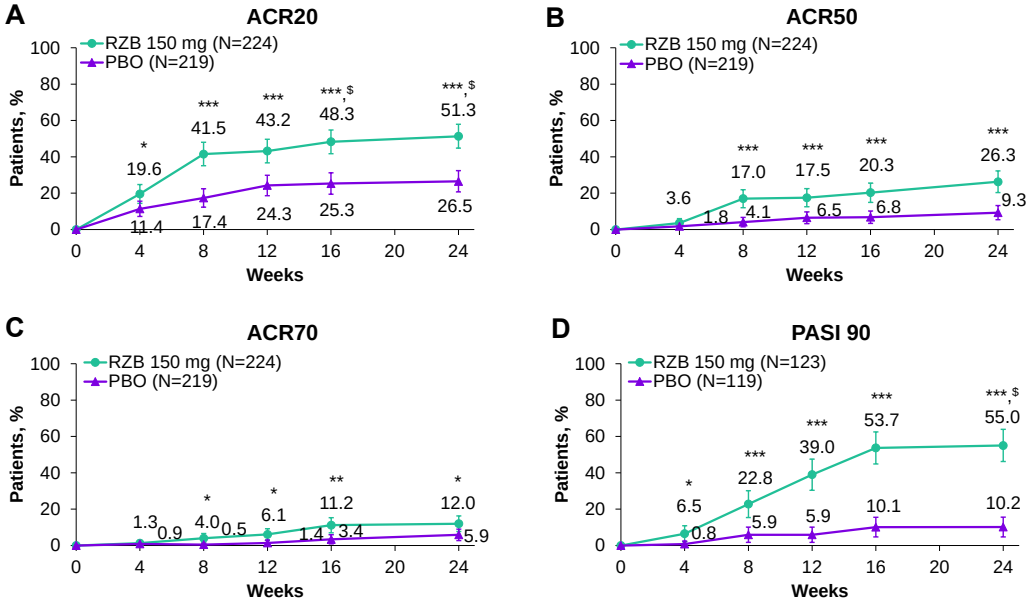


**Figure 2** ACR and PASI response rates over time. (A) ACR20, (B) ACR50, (C) ACR70 and (D) PASI 90 response rates for RZB 150 mg and PBO over the 24-week, double-blind treatment period. PASI 90 results are among patients with ≥3% body surface area affected by psoriasis at baseline. *P≤0.05, **P≤0.01, ***P≤0.001. $Statistically significant under overall type I error control. ACR20/50/70, ≥20/50/70% improvement in American College of Rheumatology criteria score; PASI 90, ≥90% reduction in Psoriasis Area and Severity Index; PBO, placebo; RZB, risankizumab.

**Table 3** Safety summary

| Patients, n (%) | RZB 150 mg N=224 | PBO N=219 |
|---|---|---|
| TEAE | 124 (55.4) | 120 (54.8) |
| COVID-19-related TEAE | 1 (0.4) | 0 |
| Serious AE | 9 (4.0) | 12 (5.5) |
| Severe TEAE | 6 (2.7) | 7 (3.2) |
| TEAE leading to discontinuation of study drug | 2 (0.9) | 5 (2.3) |
| Death | 0 | 0 |
| Serious infections* | 2 (0.9) | 5 (2.3) |
| Active tuberculosis | 0 | 0 |
| Herpes zoster | 0 | 1 (0.5) |
| Any other opportunistic infections | 0 | 0 |
| Malignancy† | 1 (0.4) | 1 (0.5) |
| Anaphylactic reactions | 0 | 0 |
| Injection site reactions | 3 (1.3)‡ | 1 (0.5) |
| MACE | 1 (0.4) | 0 |

*Serious infections reported in the RZB group were abscess and cellulitis (one patient) and gastroenteritis (one patient); in the placebo group, serious infections were erysipelas, gastroenteritis, postoperative abscess, upper respiratory tract infection and urinary tract infection (each reported for one patient).
†Both were non-melanoma skin cancer.
‡All were non-serious and did not result in discontinuation of the study drug.
AE, adverse event; MACE, major adverse cardiovascular event; PBO, placebo; RZB, risankizumab; TEAE, treatment-emergent adverse event.

that did not result in discontinuation of risankizumab). Additionally, only two patients (0.9%) in the risankizumab group discontinued the study due to AEs as the primary reason, which is comparable to the rate in the placebo group (three patients; 1.4%). Together, these results show that risankizumab 150 mg is effective and well-tolerated in patients with PsA.

Multiple agents that target IL-23 or its downstream pathway component, IL-17, are approved to treat PsA.[10] Data from the KEEPsAKE 1[29] and KEEPsAKE 2 trials provide further evidence that specifically targeting the p19 subunit of IL-23 is an effective therapeutic strategy to treat PsA. Notably, similar efficacy was observed with or without background csDMARDs or methotrexate for patients treated with risankizumab (ACR20 response rates, 48% for csDMARDs other than methotrexate and 51% for any methotrexate vs 53% for no csDMARD; online supplemental table 4). However, patients receiving placebo had higher ACR20 response rates when treated with concomitant csDMARDs or methotrexate than did those without any csDMARD or methotrexate use (27% for csDMARDs other than methotrexate and 36% for any methotrexate vs 16% for no csDMARD). The KEEPsAKE 2 study results also support the effectiveness of this mechanism of action for patients with a history of inadequate response to other biological therapies (ie, patients who are generally considered to be more treatment refractory as evidenced by higher rates of treatment discontinuation and switching).[21 22 35] As a larger proportion of patients who had failed prior biologics achieved ACR20 at week 24 when treated with risankizumab (45.7%) versus placebo (14.9%), risankizumab may provide an additional effective treatment option for these patients. Among patients who were not Bio-IR, 56.3% versus 36.6% in the risankizumab versus placebo groups achieved ACR20 at week 24. Overall, ACR20 response rates among patients receiving risankizumab were similar among patients who were Bio-IR and those who were csDMARD-IR (45.7% and 56.3%, respectively).

**Table 4** Frequently reported TEAEs

| Patients, n (%) | RZB 150 mg N=224 | PBO N=219 |
|---|---|---|
| TEAEs reported in ≥2% of patients in either group | | |
| Upper respiratory tract infection | 17 (7.6) | 12 (5.5) |
| Hypertension | 10 (4.5) | 6 (2.7) |
| Nasopharyngitis | 9 (4.0) | 8 (3.7) |
| Arthralgia | 7 (3.1) | 7 (3.2) |
| Nausea | 6 (2.7) | 4 (1.8) |
| Psoriatic arthropathy | 6 (2.7) | 9 (4.1) |
| Bronchitis | 5 (2.2) | 4 (1.8) |
| Diarrhoea | 5 (2.2) | 5 (2.3) |
| Headache | 5 (2.2) | 8 (3.7) |

PBO, placebo; RZB, risankizumab; TEAE, treatment-emergent adverse event.

Greater improvement across key domains of PsA, including psoriasis, enthesitis (among the 69% of the study population who had LEI>0 at baseline) or dactylitis (among the 22% of the study population who had LDI>0 at baseline) was observed in patients treated with risankizumab versus placebo. Importantly, risankizumab treatment significantly improved physical function, fatigue and health-related quality of life as assessed by HAQ-DI, FACIT-Fatigue and SF-36 scores, respectively. Together, results from this study demonstrate the efficacy of risankizumab across key domains of PsA for not only musculoskeletal manifestations but also patient-reported outcomes.

This study enrolled a relatively large, representative population of patients with PsA and assessed a broad range of meaningful endpoints. However, there were some limitations. First, the study was performed during the COVID-19 pandemic, which introduced health-related and logistical challenges. These were addressed by implementing specific mechanisms to handle missing data resulting from the pandemic. Overall, less than 2.5% of patients had missing efficacy data because of COVID-19, and this did not affect the overall study results. In addition, no safety concerns attributed to COVID-19 were observed. Though the study is currently limited by the relatively brief assessment period of 24 weeks, the open-label portion of this study, which remains ongoing at the time of this report, will provide safety and efficacy data for risankizumab in this patient population over a 4-year period.

In conclusion, results from the 24-week, double-blind portion of this phase 3 clinical trial in patients with active PsA reveal that risankizumab was well tolerated and effective in treating patients who have experienced previous intolerance and/or inadequate response to csDMARDs or prior biological therapies. Overall, treatment with risankizumab demonstrated efficacy in key clinical PsA domains, providing additional evidence that targeting the p19 unit of IL-23 is a rational therapeutic approach to treat PsA.

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**Data availability statement** AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymised, individual and trial-level data (analysis data sets), as well as other information (eg, protocols and clinical study reports), as long as the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications. Clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research and will be provided following review and approval of a research proposal and statistical analysis plan and execution of a data sharing agreement. Data requests can be submitted at any time and the data will be accessible for 12 months, with possible extensions considered. For more information on the process, or to submit a request, visit the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.



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CLINICAL SCIENCE

# Efficacy and safety of guselkumab in patients with active psoriatic arthritis who are inadequate responders to tumour necrosis factor inhibitors: results through one year of a phase IIIb, randomised, controlled study (COSMOS)

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## ABSTRACT

**Objective** To evaluate efficacy and safety of guselkumab, an anti-interleukin-23p19-subunit antibody, in patients with psoriatic arthritis (PsA) with prior inadequate response (IR) to tumour necrosis factor inhibitors (TNFi).

**Methods** Adults with active PsA (≥3 swollen and ≥3 tender joints) who discontinued ≤2 TNFi due to IR (lack of efficacy or intolerance) were randomised (2:1) to subcutaneous guselkumab 100 mg or placebo at week 0, week 4, then every 8 weeks (Q8W) through week 44. Patients receiving placebo crossed over to guselkumab at week 24. The primary (ACR20) and key secondary (change in HAQ-DI, ACR50, change in SF-36 PCS and PASI100) endpoints, at week 24, underwent fixed-sequence testing (two-sided $\alpha$=0.05). Adverse events (AEs) were assessed through week 56.

**Results** Among 285 participants (female (52%), one (88%) or two (12%) prior TNFi), 88% of 189 guselkumab and 86% of 96 placebo→guselkumab patients completed study agent through week 44. A statistically significantly higher proportion of patients receiving guselkumab (44.4%) than placebo (19.8%) achieved ACR20 (%difference (95% CI): 24.6 (14.1 to 35.2); multiplicity-adjusted p<0.001) at week 24. Guselkumab was superior to placebo for each key secondary endpoint (multiplicity-adjusted p<0.01). ACR20 response (non-responder imputation) in the guselkumab group was 58% at week 48; >80% of week 24 responders maintained response at week 48. Through week 24, serious AEs/serious infections occurred in 3.7%/0.5% of 189 guselkumab-randomised and 3.1%/0% of 96 placebo-randomised patients; the guselkumab safety profile was similar through week 56, with no deaths or opportunistic infections.

**Conclusion** Guselkumab significantly improved joint and skin manifestations and physical function in patients with TNFi-IR PsA. A favourable benefit–risk profile was demonstrated through 1 year.

**Trial registration number** NCT03796858.

## Key messages

What is already known about this subject?

- Patients with psoriatic arthritis (PsA) with an inadequate response or intolerance to tumour necrosis factor inhibitors (TNFi) often have lower response rates to additional TNFi, and current treatment guidelines generally support only one switch within the TNFi class before selecting an alternate mechanism of action.
- Guselkumab, a fully human interleukin (IL)–23 p19-subunit inhibitor, is efficacious in improving the signs and symptoms of active PsA both in TNFi-naïve and TNFi-experienced patients.

What does this study add?

- In the phase III, randomised, placebo-controlled COSMOS study in adults with active PsA, guselkumab-treated patients had significantly higher response rates and greater mean improvements in assessments of the signs and symptoms of PsA at week 24 when compared with placebo; response rates and mean improvements were maintained or improved through 1 year in the guselkumab group.
- The COSMOS safety results were consistent with the known safety profile of guselkumab in biologic-naïve patients with PsA.

How might this impact on clinical practice or future developments?

- The efficacy and safety results of COSMOS suggest that guselkumab may be an appropriate therapy for patients with PsA with lack or efficacy from or intolerance to TNFi.

## INTRODUCTION

Psoriatic arthritis (PsA) is a heterogeneous, chronic, inflammatory disease, with distinct classes of therapy now increasingly recommended based on the disease domains predominantly involved, such as enthesitis and dactylitis, in the individual patient.[1 2] Current treatment guidelines[3] recommend the use of a biologic disease-modifying antirheumatic drug (bDMARD) when conventional synthetic DMARDs (csDMARDs) have proven ineffective. The introduction of tumour necrosis factor inhibitors (TNFi) into the rheumatologist's armamentarium has substantially improved the ability

to achieve lower states of PsA activity[3]; however, up to 40% of patients receiving their first TNFi do not achieve response (assessed by ≥20% improvement in American College of Rheumatology criteria (ACR20)) with 6 months of treatment.[3] An analysis of patients with PsA in the DANBIO registry who switched biologics after initiating TNFi therapy found decreased ACR20 response rates with the second and third TNFi (47%, 22% and 18%, respectively).[4] In addition, real-world registry data have demonstrated diminished drug persistence with each successive TNFi.[4–6]

Alternate mechanisms of action may prove more beneficial in patients who experience a lack of response to TNFi,[7] highlighting the need for treatments targeting alternate disease pathways. Accordingly, several bDMARDs with alternative mechanisms of action are now approved for PsA,[8–10] including those targeting interleukin (IL)−17A, p40 (IL-12/23), and p19 (IL-23).

Guselkumab, a high-affinity, human monoclonal antibody targeting the IL-23p19-subunit, demonstrated efficacy and safety across two phase III PsA studies (DISCOVER-1 (TNFi-experienced and biologic-naïve), DISCOVER-2 (biologic-naïve only)).[8 9] Approximately 31% of the 381 patients in DISCOVER-1 were previously exposed to 1–2 TNFi, and of those, 37% had discontinued TNFi therapy due to inadequate efficacy. The objective of the phase IIIb COSMOS study was to further assess the efficacy and safety of guselkumab through 1 year in patients with PsA with an inadequate response (IR; inadequate efficacy or intolerance) to TNFi.

## PATIENTS AND METHODS

### Patients

Eligible adults had a diagnosis of PsA according to the ClASsification criteria for Psoriatic ARthritis (CASPAR) at screening and had active disease (≥3 swollen; ≥3 tender joints) and active (≥1 psoriatic plaque of ≥2 cm) or documented history of plaque psoriasis or current nail psoriasis, and who had also demonstrated lack of benefit or intolerance to 1–2 TNFi. Patients could continue stable baseline use of methotrexate (MTX), sulfasalazine, hydroxychloroquine or leflunomide; oral corticosteroids; and non-steroidal anti-inflammatory drugs (NSAIDs)/other analgesics. Targeted synthetic DMARDs were prohibited before and during study participation. Patients with active tuberculosis (TB) were excluded; those with latent TB received appropriate prophylaxis.

### Study design

This phase IIIb, randomised, double-blind study (COSMOS) was conducted at 84 European sites from March 2019 to November 2020 (see online supplemental methods). The study comprised a 6-week screening period and placebo-controlled (weeks 0–24) and active-treatment (weeks 24–48; final study intervention at week 44) periods. The primary endpoint assessment was at week 24, with final efficacy and safety assessments at week 48 and week 56, respectively.

At week 0, participants were randomised (2:1) to receive subcutaneous injections of either guselkumab 100 mg (week 0, week 4, then every 8 weeks (Q8W) through week 44) or placebo (weeks 0, 4, 12, 20, followed by guselkumab 100 mg at weeks 24, 28, 36, 44). Randomisation was stratified by baseline csDMARD use (yes/no) and number of prior TNFi (1 or 2). Study personnel, including independent joint assessors and the study team, were blinded throughout the study. Participants with <5% improvement from baseline in both tender and swollen joint counts at week 16 qualified for early escape (EE); patients receiving guselkumab continued randomised treatment (receiving placebo at week 16 to maintain blinding), while those in the placebo group received guselkumab at week 16, week 20 and Q8W thereafter (figure 1). After EE, participants could initiate or increase the dose of one permitted concomitant medication up to the maximum allowed dose at the physician's discretion. Sample size estimation is detailed in online supplemental methods.

This study was conducted per the Declaration of Helsinki and Good Clinical Practice guidelines. Each site's ethical body approved the protocol. Patients provided written informed consent.

### Patient and public involvement statement

Patients and the public were not involved in the design or analysis of this study.

### Procedures

Independent assessors evaluated joints for tenderness, swelling and presence/severity of enthesitis (Leeds Enthesitis Index (LEI))[11] and dactylitis (Dactylitis Severity Score (DSS)).[12 13] Patients reported pain and global arthritis activity (0–10 cm visual analogue scale (VAS)), and physical function (Health Assessment Questionnaire-Disability Index (HAQ-DI)).[14] Investigators determined global disease activity (0–10 cm VAS), serum C reactive protein (CRP) and extent (% body surface area (BSA) with psoriasis) and severity of skin symptoms using the Investigator's Global Assessment of psoriasis (IGA)[15] and the Psoriasis Area and Severity Index (PASI).[16] During the study, the protocol was amended to allow self-administration of study agent injections post-week 24 when site visits were not possible due to local COVID-19 restrictions.

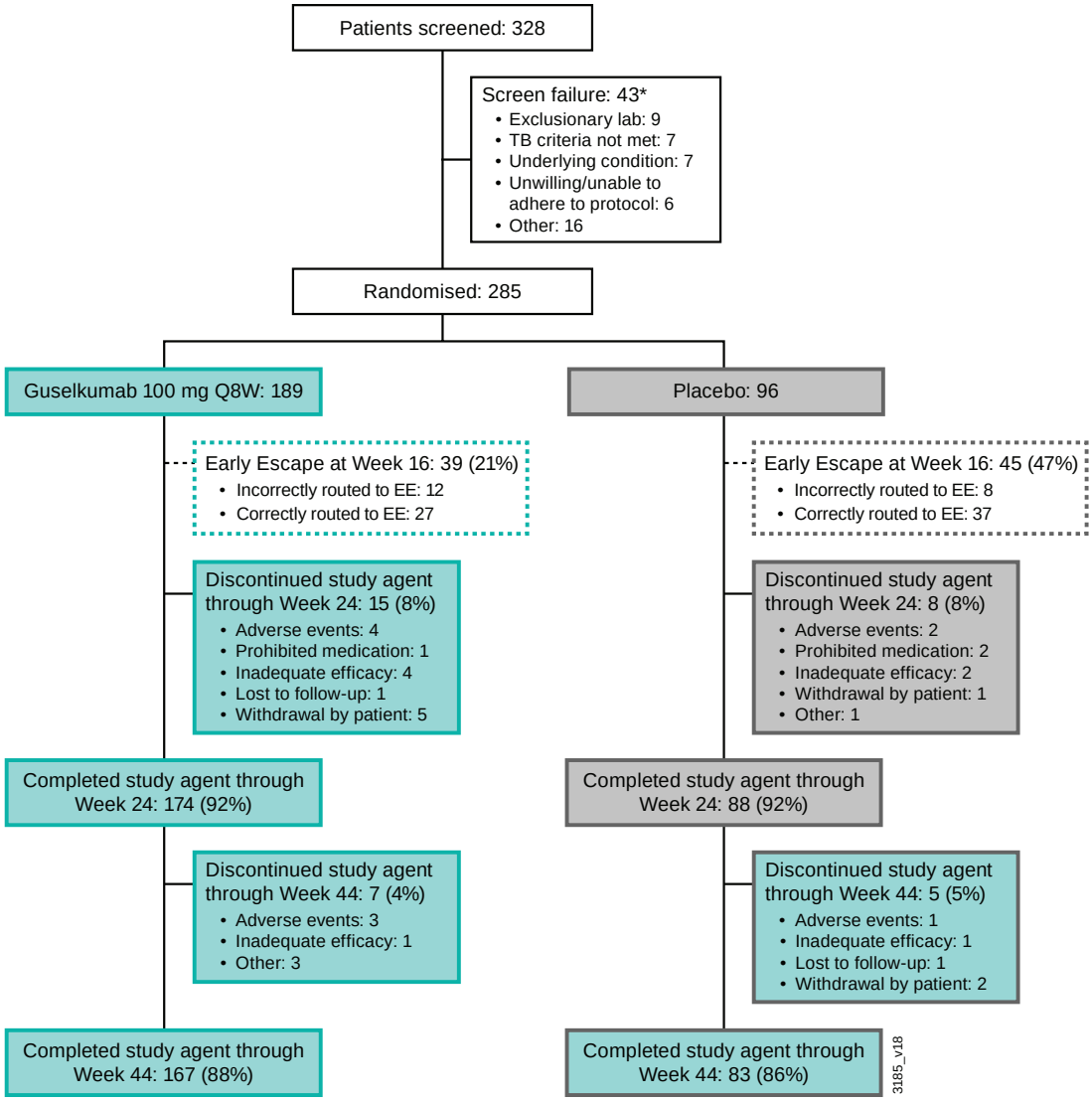
The 36-item Short-Form Health Survey (SF-36) physical and mental component summary (PCS and MCS) scores assessed health-related quality of life (HRQoL).[17] The Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue measured fatigue.[18] Adverse events (AEs) and routine clinical laboratory parameters were monitored.

### Outcomes

The primary endpoint was the proportion of patients with an ACR20 response at week 24. Major secondary endpoints, also at week 24, were (1) mean changes in HAQ-DI, (2) ACR50 response, (3) mean changes in SF-36 PCS and (4) PASI100 response (in patients with ≥3% BSA with psoriasis involvement and IGA ≥2 at baseline). Maintenance of ACR20/50/70 response at week 48 was also assessed in patients who achieved response at week 24. Additional secondary and safety outcomes assessed are shown in online supplemental methods.

### Data analyses

Efficacy results were analysed by randomised treatment group, regardless of actual treatment received. The 'Primary' efficacy analysis included randomised participants who received ≥1 dose of study agent. Patients with missing data and those who met treatment failure (TF) criteria through week 24 (discontinued study agent and/or study participation for any reason, initiated or increased the dose of allowed csDMARDs or oral corticosteroids for PsA, initiated protocol prohibited medications/therapies for PsA or met EE criteria; online supplemental figure 1) were considered non-responders for binary endpoints or having no change for continuous endpoints (non-responder imputation (NRI)). Through week 24, least squares (LS) mean changes from baseline were determined for continuous endpoints using a Mixed-Effect Model Repeated Measures (MMRM) model




* 2 patients were included in >1 category.

**Figure 1** Disposition of patients through 1 year of COSMOS. EE, early escape; Q8W, every 8 weeks; TB, tuberculosis.

including all available data through week 24 (additional details in online supplemental methods). Subgroup analyses evaluated consistency of the primary endpoint based on demographics, baseline disease characteristics and prior medications.

The overall type I error was controlled across the primary and major secondary endpoints at 5% by testing treatment differences (two-sided $\alpha=0.05$) in a fixed sequence (ie, ACR20 response, change from baseline in HAQ-DI, ACR50 response, change from baseline in SF-36 PCS, PASI100 response; online supplemental figure 2), whereby subsequent endpoints were only tested if the previous endpoint achieved statistical significance ($p<0.05$). For endpoints not included in the multiplicity control procedure, the unadjusted (nominal) p values are descriptive in nature.

Supplemental sensitivity analyses, prespecified prior to the week 24 database lock, included a 'Per-Protocol' (PP) analysis (excluded patients with major protocol deviations (MPDs) with potential to impact efficacy assessments; online supplemental figure 3), and an 'EE-Correction' analysis (online supplemental figure 4). The latter analysis was conducted to address 20 patients (12 guselkumab, 8 placebo) incorrectly routed to EE and considered non-responders in the Primary analysis. In the EE-correction analysis, 12 affected patients in the guselkumab group did not meet any other TF criteria (eg, the introduction/change in dose of concomitant therapy) through week 24 and their response was included with those of other guselkumab-treated patients. The eight placebo patients received guselkumab as EE therapy at week 16 and week 20, thus met TF criteria, and were considered non-responders in the EE-correction analysis.

Through week 24, treatment group comparisons for binary endpoints used a Cochran–Mantel–Haenszel test stratified at the study level by baseline csDMARD use (yes/no) and number of prior TNFi (1/2) for binary endpoints or an MMRM model for continuous data (see online supplemental methods). Statistical analyses used SAS (V.9.4), with SAS/STAT (V.14.2; SAS Institute, Inc, Cary, NC, USA).

In post hoc analyses after week 24, results for the placebo→guselkumab group are reported for patients who crossed over to receive guselkumab at week 24. In addition, NRI was applied: patients who discontinued treatment and/or met EE criteria before week 24 (guselkumab group; excluding those who were incorrectly assigned to EE) were imputed as no response for binary endpoints or no change for continuous endpoints; missing data were imputed as no response or using multiple imputation (MI; assumed to be missing-at-random),

respectively. After week 24, changes from baseline are reported as mean (SD). No treatment group comparisons were performed post-week 24.

Safety summaries included participants receiving ≥1 partial or complete administration of study agent, according to actual treatment received; numbers of events/100 patient-years (PY) of follow-up were determined for select AEs of interest.

## RESULTS

### Patient disposition and characteristics

At week 0, 285 patients were randomised to guselkumab (n=189) or placebo (n=96); at week 16, 39 (21%) participants in the guselkumab group and 45 (47%) in the placebo group were assigned to EE. Through week 24, 15 (8%) and 8 (8%) participants, respectively, in the guselkumab and placebo groups discontinued study agent (figure 1). In total, 167 (88%) patients in the guselkumab group and 83 (86%) in the placebo-crossover group completed study treatment.

Although baseline characteristics were generally similar across treatment groups, several numerical imbalances existed, for example, a higher proportion of females and a lower mean body weight in the guselkumab (54%, 84 kg) than placebo (46%, 92 kg) group. The guselkumab group was characterised by more prominent joint symptoms (tender joint count: 21 vs 18) and skin involvement (mean PASI: 11.7 vs 9.2). Prior and concomitant medications were similar across groups (table 1).

Although self-administration was permitted during the COVID-19 pandemic, when site visits were restricted, MPDs related to COVID-19 did occur. These were classified mostly as drug administration or study visit missed or outside of the prespecified window, and most were considered to have no effect on efficacy assessments.

**Table 1** Baseline characteristics of COSMOS participants

| Randomised, treated participants, N | Guselkumab 100 mg Q8W 189 | Placebo 96 | Total 285 |
|---|---|---|---|
| Age, years | 49 [12] | 49 [12] | 49 [12] |
| <65 | 169 (89%) | 89 (93%) | 258 (91%) |
| ≥65 | 20 (11%) | 7 (7%) | 27 (9%) |
| Sex | | | |
| Male | 86 (46%) | 52 (54%) | 138 (48%) |
| Female | 103 (54%) | 44 (46%) | 147 (52%) |
| Weight, kg | 84 [17] | 92 [23] | 86 [20] |
| Body mass index, kg/m$^2$ | 29 [6] | 31 [7]* | 30 [6]† |
| Swollen joint count, 0–66 | 10 [7] | 9 [6] | 10 [6] |
| Tender joint count, 0–68 | 21 [13] | 18 [11] | 20 [12] |
| PsA disease duration, years | 8.3 (7.8) | 8.7 (7.2) | 8.4 (7.6) |
| Patient assessment of pain, 0–10 cm VAS | 6.5 (1.9) | 6.0 (1.8) | 6.3 (1.9) |
| Patient global assessment of arthritis, 0–10 cm VAS | 6.5 (1.7) | 6.2 (1.7) | 6.4 (1.7) |
| Physician global assessment of disease, 0–10 cm VAS | 6.9 (1.5) | 6.4 (1.7) | 6.7 (1.6) |
| HAQ-DI, 0–3 | 1.3 (0.6)‡ | 1.2 (0.6) | 1.3 (0.6)† |
| CRP, mg/dL | 1.2 (2.0)‡ | 1.2 (2.5) | 1.2 (2.2)† |
| Enthesitis (LEI score ≥1) | 126 (67%) | 64 (67%) | 190 (67%) |
| LEI score, 1–6 | 2.9 (1.5) | 2.7 (1.5) | 2.8 (1.5) |
| Dactylitis (DSS ≥1) | 67 (36%) | 36 (38%) | 103 (36%) |
| DSS, 1–60 | 6.7 (6.5) | 7.4 (8.3) | 6.7 (7.1) |
| DAPSA score | 45.5 (19.9) | 40.6 (15.8) | 43.8 (18.7) |
| Psoriatic BSA, % | 17.9 (21.5) | 13.4 (17.7) | 16.4 (20.4) |
| PASI score, 0–72, N | 188 | 96 | 284 |
| Mean (SD) | 11.7 (11.9) | 9.2 (9.4) | 10.9 (11.2) |
| <12 | 119 (63%) | 65 (68%) | 184 (65%) |
| ≥12 and <20 | 33 (18%) | 19 (20%) | 52 (18%) |
| ≥20 | 36 (19%) | 12 (13%) | 48 (17%) |
| IGA score, 0–4, N | 189 | 96 | 285 |
| <2 | 40 (21%) | 29 (30%) | 69 (24%) |
| ≥2 | 149 (79%) | 67 (70%) | 216 (76%) |
| SF-36, standard norm=50 | | | |
| PCS score | 33.0 (7.0)‡ | 33.9 (7.7) | 33.3 (7.3)† |
| MCS score | 47.1 (12.1)‡ | 46.1 (11.5) | 46.8 (11.9)† |
| FACIT-F score, 0–52 | 29.2 (11.3)‡ | 29.2 (10.6) | 29.2 (11.0)† |
| No of prior TNFi | | | |
| 1 | 167 (88%) | 85 (89%) | 252 (88%) |
| 2 | 22 (12%) | 11 (11%) | 33 (12%) |
| Reason for prior TNFi discontinuation | | | |
| Inadequate efficacy | 159 (84%) | 79 (82%) | 238 (84%) |
| Intolerance | 30 (16%) | 17 (18%) | 47 (16%) |
| MTX use at baseline | 105 (56%) | 51 (53%) | 156 (55%) |

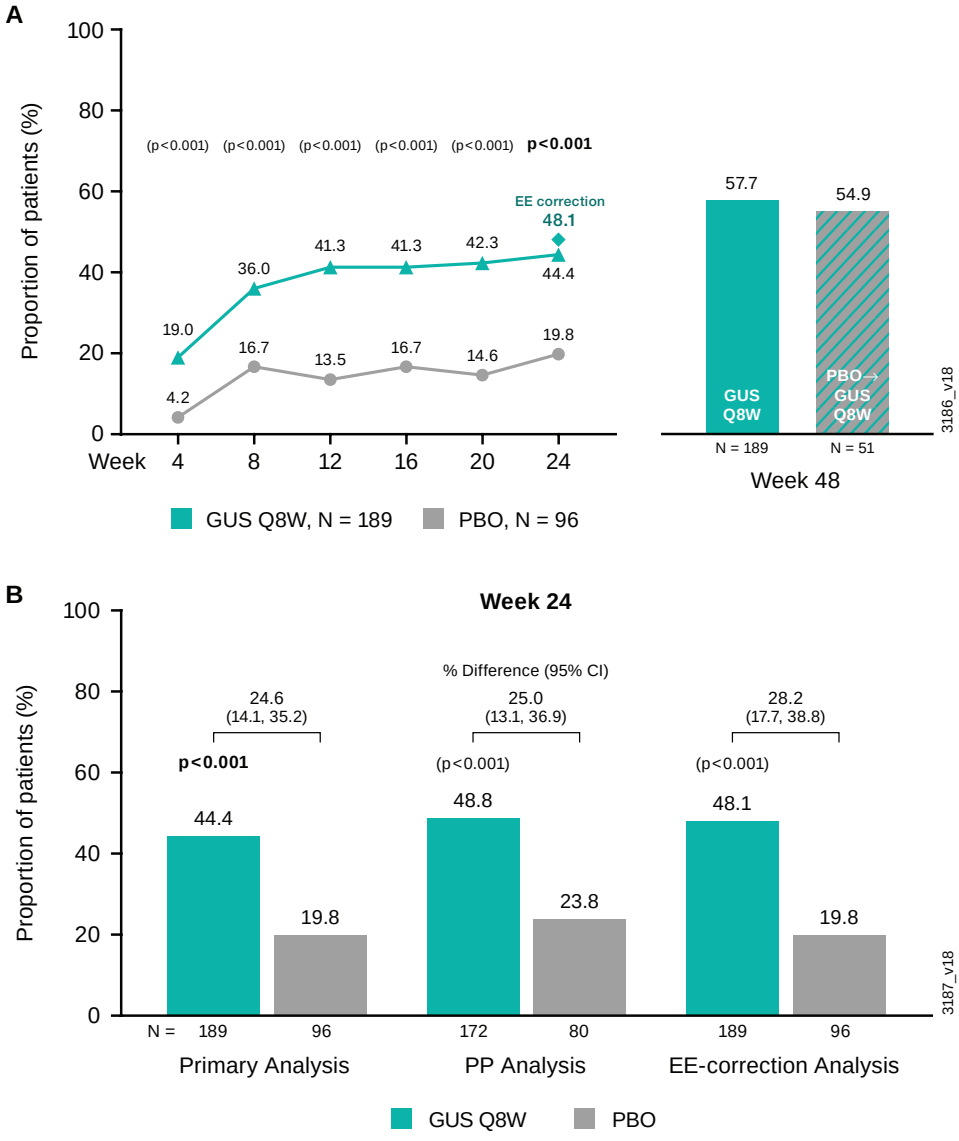
Data are mean (SD) or n (%) unless stated otherwise.
*N=95
†N=284
‡N=188
BSA, body surface area; CRP, C reactive protein; DAPSA, Disease Activity in Psoriatic Arthritis; DSS, Dactylitis Severity Score; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; HAQ-DI, Health Assessment Questionnaire-Disability Index; IGA, Investigator's Global Assessment of psoriasis; LEI, Leeds Enthesitis Index; MCS, mental component summary; MTX, methotrexate; PASI, Psoriasis Area and Severity Index; PCS, physical component summary; PsA, psoriatic arthritis; Q8W, every 8 weeks; SF-36, 36-item Short-Form Health Survey; TNFi, tumour necrosis factor inhibitor; VAS, visual analogue scale.

### Efficacy

The primary endpoint was met. At week 24, based on the Primary analysis population (online supplemental figure 1), 44.4% (84/189) of guselkumab versus 19.8% (19/96) of placebo patients achieved ACR20 (%difference (95% CI): 24.6 (14.1 to 35.2); multiplicity-adjusted p<0.001), with treatment effect seen by week 4 (figure 2A). Results of the PP and EE-correction sensitivity analyses supported the Primary analysis. Specific to the EE-correction analysis, 48.1% (91/189) of guselkumab versus 19.8% (19/96) of placebo patients achieved ACR20 (%difference (95% CI): 28.2 (17.7 to 38.8)) (figure 2B). The benefit of guselkumab over placebo was consistent across subgroups defined by baseline patient, disease and prior/concomitant medication characteristics, including participants who discontinued prior TNFi use due to inadequate efficacy or intolerance (figure 3). Employing NRI, the proportion of guselkumab-randomised patients achieving ACR20 at week 48 was 57.7%. Among 51 placebo patients who crossed over to guselkumab at week 24, 54.9% (n=28) achieved ACR20 at week 48 (figure 2A).

The testing hierarchy did not fail in analyses of the major secondary endpoints; guselkumab was superior to placebo in all four endpoints. At week 24, guselkumab patients demonstrated statistically significantly greater improvements or response rates versus placebo in HAQ-DI score (LSmean (95% CI) change: −0.18 (−0.27 to −0.09) vs −0.01 (−0.12 to 0.10); multiplicity-adjusted p=0.003; figure 4A), ACR50 (19.6% (37/189) vs 5.2% (5/96); multiplicity-adjusted p=0.001; figure 4B), SF-36 PCS score (LSmean (95% CI) change: 3.51 (2.31 to 4.72) vs −0.39 (−1.84 to 1.07); multiplicity-adjusted p<0.001; figure 4C) and PASI100 (in patients with ≥3% BSA with psoriasis and IGA ≥2 at baseline; 30.8% vs 3.8%; multiplicity-adjusted p<0.001; figure 4D). Results of PP and EE-correction sensitivity analyses were consistent with the Primary analysis (Supplemental Figure 5A−D).

Additional secondary endpoints at week 24 also showed benefit of guselkumab over placebo for achieving ACR70 (7.9% vs 1.0%; nominal p=0.018), minimal disease activity (MDA;




Bolded p values are adjusted for multiplicity of testing; p values shown in parentheses are not adjusted for multiplicity of testing

**Figure 2** ACR20 response through week 48 of COSMOS. Proportions of randomised and treated patients achieving ACR20 response through week 24 in the Primary analysis (treatment failure rules applied) (A) and ACR20 response at week 24 across the Primary, PP and EE-correction analyses (B). After week 24, analyses were performed using non-responder imputation methods, including imputation of EE patients as non-responders (see Patients and methods). Results for the placebo→guselkumab group at week 48 are reported for patients who did not enter EE and crossed over to guselkumab at week 24. ACR20, ≥20% improvement in American College of Rheumatology response criteria; EE, early escape; GUS, guselkumab; PBO, placebo; PP, per protocol; Q8W, every 8 weeks.

14.8% vs 3.1%; nominal p=0.003), and PASI75 (59.4% vs 9.4%; nominal p<0.001) and PASI90 (51.1% vs 7.5%; nominal p<0.001) in patients with ≥3% BSA with psoriasis and IGA ≥2 at baseline; 3.7% guselkumab-treated and no placebo-treated patients achieved very low disease activity. At week 24, guselkumab-treated patients also had a greater LSmean change in Disease Activity in Psoriatic Arthritis (DAPSA) score (−14.5 vs −5.7; nominal p<0.001) and a higher DAPSA low disease activity (LDA) response rate (29.6% vs 13.5%, nominal p=0.003) versus placebo; the proportion of patients achieving DAPSA remission was numerically higher in the guselkumab group versus placebo (5.3% vs 2.1%). Among participants affected at baseline, numerically higher proportions of guselkumab than placebo patients had resolved enthesitis (39.7% vs 18.8%; nominal p=0.003) or dactylitis (44.8% vs 25.0%; nominal p=0.040) at week 24. Guselkumab-treated patients also had greater LSmean improvements across all ACR components compared with placebo (Supplemental Figure 6A–G). The LSmean changes in SF-36 MCS were 2.10 and 0.36, respectively, in the guselkumab and placebo groups (table 2). In addition, higher proportions of guselkumab than placebo patients achieved clinically meaningful improvements in HAQ-DI (37.5% vs 16.1%; nominal p<0.001; table 2), FACIT-F (42.9% vs 20.8%; nominal p<0.001; table 2), and SF-36 PCS (42.3% vs 15.6%; nominal p<0.001) and MCS (28.6% vs 15.6%; nominal p=0.016) scores.

After week 24, response rates and mean improvements for secondary endpoints were sustained or numerically improved through week 48 in guselkumab-randomised patients (figure 4A–D and table 2). Among placebo→guselkumab patients, response rates and mean changes in the secondary endpoints increased at week 48 (figure 4A–D and table 2).

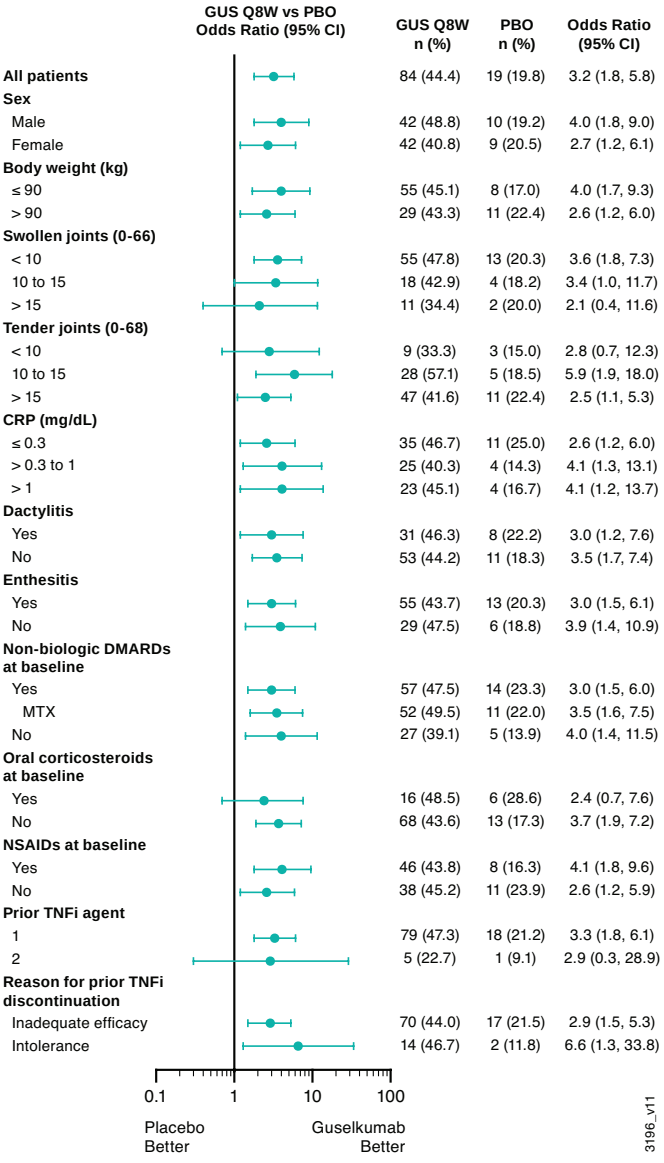



**Figure 3** ACR20 response at week 24 by baseline characteristics of COSMOS participants. ACR20, ≥20% improvement in American College of Rheumatology response criteria; CRP, C reactive protein; DMARD, disease-modifying antirheumatic drug; GUS, guselkumab; MTX, methotrexate; NSAID, non-steroidal anti-inflammatory drug; PBO, placebo; Q8W, every 8 weeks; TNFi, tumour necrosis factor inhibitor.

Maintenance of response was evaluated for guselkumab-randomised patients achieving an ACR20, ACR50 or ACR70 response at week 24; of these patients, 83.3% (70/84), 81.1% (30/37) and 86.7% (13/15), respectively, maintained response at week 48.

## Safety

Through week 24, similar proportions of patients in the guselkumab (42% (80/189)) and placebo (48% (46/96)) groups reported ≥1 AE. Through week 56, 144.9 AEs/100PY were reported among the 279 guselkumab-treated patients (vs 369.8/100PY for placebo). The most common AEs in guselkumab-randomised patients through week 24, ie, nasopharyngitis (5%) and upper respiratory tract infection (4%), occurred with similar incidence in the placebo group (5% and 3%, respectively) (table 3). Infections remained the most common AEs in guselkumab-treated patients through week 56 (37.2/100PY vs 99.6/100PY for placebo).

The incidences of serious AEs (SAEs) and AEs leading to treatment discontinuation were 6.3/100PY and 2.7/100PY, respectively, among guselkumab-treated patients through week 56. One patient experienced a major adverse cardiovascular event at week 44 (non-fatal myocardial infarction (preferred term: acute coronary syndrome)); risk factors included concomitant cyclooxygenase-2-inhibitor therapy and a body mass index of 31. One malignancy occurred: prostatic adenocarcinoma in a guselkumab-randomised patient (4-year history of prostatitis). One patient discontinued study agent (influenza-like illness) after the third guselkumab administration and was diagnosed with suspected inflammatory bowel disease and coeliac disease ~3 weeks and 2 months, respectively, later. Neither diagnosis was confirmed; the patient was lost to follow-up.

One patient (guselkumab group) experienced two events of conversion disorder, requiring hospitalisation; study drug was discontinued after the second instance, which was reported as resolved. Another patient in the guselkumab group (history of previous suicide attempt) reported depression (SAE) 1 week after receiving the second guselkumab administration; study agent was discontinued, with no further follow-up. Other non-serious psychiatric-related AEs were singular events of anxiety and depressed mood in the placebo group (through week 24) and insomnia in the guselkumab group.

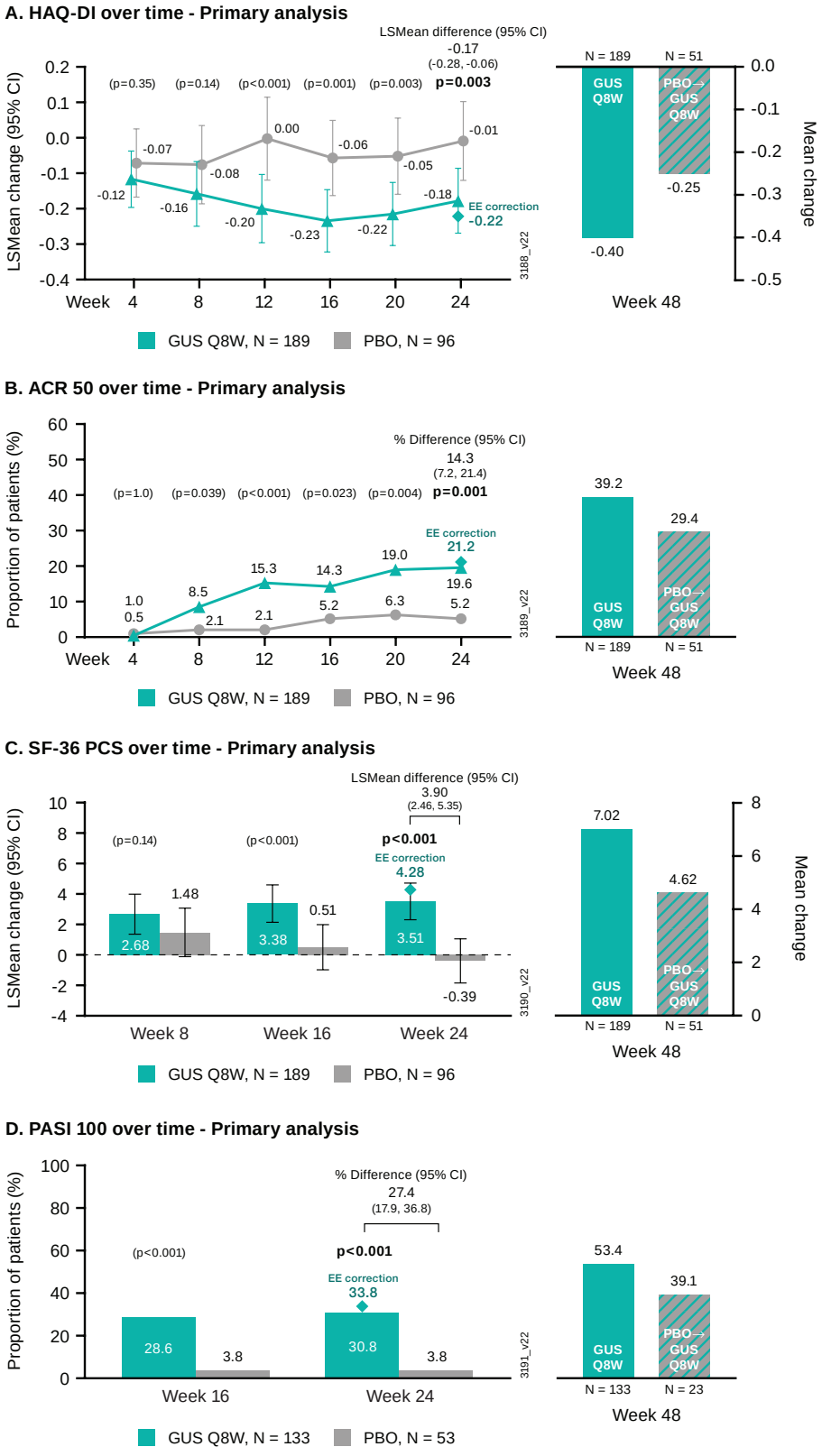
Two serious infections occurred. One guselkumab-randomised patient was hospitalised with community-acquired pneumonia diagnosed at week 12 (history of chronic obstructive pulmonary disease and heart disease); the patient recovered with antibiotic treatment and resumed study agent. A placebo→guselkumab patient was hospitalised with acute pneumonia (week 48); the patient recovered following antibiotic therapy and continued study participation. No opportunistic infections, cases of active TB, or deaths occurred (table 3).

Injection-site reactions, all considered of mild intensity, occurred in 1.8% of guselkumab-treated patients (table 3). No anaphylactic or serum sickness-like reactions occurred through week 56.

Through week 56, AEs of decreased neutrophil and white blood cell counts were uncommon. Neither type of haematological abnormality was reported as an SAE or led to study agent discontinuation, and all were National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) Grade ≤2 (online supplemental table 1). The majority of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) elevations were maximum NCI-CTCAE Grade 1 (online supplemental table 1). Two guselkumab-treated patients had elevated ALT reported as an SAE. The first patient, whose liver enzymes were elevated at baseline, was confirmed to have autoimmune hepatitis via biopsy and imaging studies and discontinued study agent. While ALT levels normalised by week 24, other symptoms (jaundice, nausea) persisted. A second patient had elevated AST and ALT at week 48 and was diagnosed with steatohepatitis; the patient was treated with ademetionine and recovered. ALT and AST elevations occurred in 37% and 28%, respectively, in patients receiving concomitant MTX and in 28% and 24% of those not receiving concomitant MTX.

## DISCUSSION

Guselkumab-treated patients had statistically significant improvements in the signs and symptoms of PsA in TNFi-IR patients compared with placebo. The primary endpoint was




Bolded p values are adjusted for multiplicity of testing; p values shown in parentheses are not adjusted for multiplicity of testing

**Figure 4** Key secondary outcomes through week 48 of COSMOS. Primary analysis through week 24 and post hoc NRI analysis at week 48 of LSmean change and mean change in HAQ-DI score (A), ACR50 response (B), LSmean change and mean change in SF-36 PCS score (C), and PASI100 response (D). After week 24, analyses were performed using NRI (including imputation of EE patients as non-responders in the guselkumab group; see Patients and methods). Results for the placebo→guselkumab group at week 48 are reported for patients who did not enter EE and crossed over to guselkumab at week 24. ACR50, ≥50% improvement in American College of Rheumatology response criteria; GUS, guselkumab; HAQ-DI, Health Assessment Questionnaire-Disability Index; LS, least squares; NRI, non-responder imputation; PASI100, 100% improvement in Psoriasis Area and Severity Index; PBO, placebo; Q8W, every 8 weeks; SF-36 PCS, 36-item Short-Form Health Survey Physical Component Summary.

**Table 2** Additional secondary efficacy assessments at week 24 and week 48 analysed using non-responder imputation*

| | Week 24 | | Week 48 | |
|---|---|---|---|---|
| | Guselkumab 100 mg Q8W | Placebo | Guselkumab 100 mg Q8W | Placebo→guselkumab 100 mg Q8W |
| Treated participants according to randomised group, N | 189 | 96 | 189 | 51 |
| ACR70 response | 15 (7.9%) | 1 (1.0%) | 45 (23.8%) | 9 (17.6%) |
| % difference (95% CI)† | 6.8 (2.6 to 11.1) | | | |
| Unadjusted p value vs placebo‡ | 0.018 | | | |
| Enthesitis resolution (LEI score=0)§ | 50/126 (39.7%) | 12/64 (18.8%) | 70/126 (55.6%) | 14/35 (40.0%) |
| % difference (95% CI)† | 21.6 (8.8 to 34.4) | | | |
| Unadjusted p value vs placebo‡ | 0.003 | | | |
| Dactylitis resolution (DSS=0)¶ | 30/67 (44.8%) | 9/36 (25.0%) | 45/67 (67.2%) | 11/13 (84.6%) |
| % difference (95% CI)† | 19.9 (2.7 to 37.1) | | | |
| Unadjusted p value vs placebo‡ | 0.040 | | | |
| IGA response (IGA 0/1 and ≥2-grade improvement from baseline)** | 64/133 (48.1%) | 5/53 (9.4%) | 87/133 (65.4%) | 14/23 (60.9%) |
| % difference (95% CI)† | 38.8 (27.3 to 50.4) | | | |
| Unadjusted p value vs placebo‡ | <0.001 | | | |
| PASI75 response** | 79/133 (59.4%) | 5/53 (9.4%) | 99/133 (74.4%) | 19/23 (82.6%) |
| % difference (95% CI)† | 49.6 (38.3 to 60.9) | | | |
| Unadjusted p value vs placebo‡ | <0.001 | | | |
| PASI90 response** | 68/133 (51.1%) | 4/53 (7.5%) | 89/133 (66.9%) | 14/23 (60.9%) |
| % difference (95% CI)† | 43.7 (32.7 to 54.7) | | | |
| Unadjusted p value vs placebo‡ | <0.001 | | | |
| HAQ-DI response (≥0.35 improvement from baseline)†† | 66/176 (37.5%) | 14/87 (16.1%) | 94 (53.4%) | 17 (37.0%) |
| % difference (95% CI)† | 21.5 (11.1 to 31.9) | | | |
| Unadjusted p value vs placebo‡ | <0.001 | | | |
| SF-36 MCS score | | | | |
| LSmean change from baseline‡‡ | 2.10 (0.54 to 3.65) | 0.36 (−1.52 to 2.25) | – | – |
| LSmean difference (95% CI)† | 1.73 (−0.14 to 3.61) | | – | – |
| Unadjusted p value vs placebo‡‡ | 0.070 | | | |
| Mean change from baseline (SD)§§ | – | – | 3.05 (9.95) | 3.82 (8.91) |
| FACIT-F response (≥4-point improvement from baseline) | 81 (42.9%) | 20 (20.8%) | 105 (55.6%) | 26 (51.0%) |
| % difference (95% CI)† | 21.9 (11.2 to 32.7) | | | |
| Unadjusted p value vs placebo‡ | <0.001 | | | |
| DAPSA score | | | | |
| LSmean change from baseline‡‡ | −14.5 | −5.7 | – | – |
| LSmean difference (95% CI)† | −8.8 (12.5 to −5.0) | | – | – |
| Unadjusted p value vs placebo‡‡ | <0.001 | | | |
| Mean change from baseline (SD)§§ | – | – | −23.4 (19.8) | −20.3 (15.9) |
| DAPSA LDA (≤14) | 56 (29.6%) | 13 (13.5%) | 84 (44.4%) | 21 (41.2%) |
| % difference (95% CI)† | 16.0 (6.7 to 25.4) | | | |
| Unadjusted p value vs placebo‡ | 0.003 | | | |
| DAPSA remission (≤4) | 10 (5.3%) | 2 (2.1%) | 30 (15.9%) | 6 (11.8%) |
| % difference (95% CI)† | 3.2 (−1.1 to 7.5) | | | |
| Unadjusted p value vs placebo‡ | 0.202 | | | |
| MDA | 28 (14.8%) | 3 (3.1%) | 51 (27.0%) | 14 (27.5%) |
| % difference (95% CI)† | 11.7 (5.6 to 17.7) | | | |
| Unadjusted p value vs placebo‡ | 0.003 | | | |
| VLDA | 7 (3.7%) | 0 | 21 (11.1%) | 2 (3.9%) |
| % difference (95% CI)† | 3.7 (1.0 to 6.4) | | | |
| Unadjusted p value vs placebo‡ | 0.057 | | | |

Data shown are n (%) or n/N (%) unless stated otherwise.
*Through week 24, patients who discontinued study agent/study participation for any reason, initiated or increased the dose of allowed csDMARDs/oral corticosteroids over baseline for PsA, initiated protocol-prohibited medications/therapies for PsA or met EE criteria (including those incorrectly assigned to EE) were considered to be non-responders or to have no improvement from baseline at subsequent timepoints. After week 24, patients who met the EE criteria (excluding those who were incorrectly assigned to EE) and patients who discontinued study agent/study participation for any reason were considered to be non-responders or to have no improvement from baseline at subsequent timepoints; missing data were imputed as non-response or multiple imputation (assumed to be missing-at-random). Among patients randomised to placebo, only those who crossed over to guselkumab at week 24 were included in the week 48 analyses.
†CIs based on Wald statistic.
‡Unadjusted (nominal) p values based on the Cochran–Mantel–Haenszel test, stratified by baseline use of csDMARD (yes/no) and prior exposure to TNFi (1 vs 2).
§In patients with LEI score ≥1 at baseline.
¶In patients with DSS ≥1 at baseline.
**In patients with ≥3% BSA psoriasis involvement and IGA ≥2 at baseline.
††In patients with HAQ-DI score ≥0.35 at baseline.
‡‡LSmeans and unadjusted (nominal) p values based on a mixed model for repeated measures under the missing-at-random assumption for missing data. LSmeans were determined only through week 24.
§§Post-week 24, mean changes from baseline were determined using change of 0 for patients who discontinued or met the EE criteria prior to week 24 (excluding patients incorrectly assigned to EE) and multiple imputation (assumed to be missing-at-random) for missing data.
ACR, American College of Rheumatology; BSA, body surface area; csDMARD, conventional synthetic disease-modifying antirheumatic drug; DAPSA, Disease Activity in Psoriatic Arthritis; DSS, Dactylitis Severity Score; EE, early escape; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; HAQ-DI, Health Assessment Questionnaire-Disability Index; IGA, Investigator's Global Assessment of psoriasis; LDA, low disease activity; LEI, Leeds Enthesitis Index; LS, least squares; MDA, Minimal Disease Activity; PASI75/90, ≥75%/90% improvement in Psoriasis Area and Severity Index; PsA, psoriatic arthritis; Q8W, every 8 weeks; SF-36 MCS, 36-item Short-Form Health Survey Mental Component Summary; TNFi, tumour necrosis factor inhibitor; VLDA, very low disease activity.

**Table 3** Summary of adverse events through week 56 of COSMOS

| | Placebo* | Placebo → guselkumab | | Randomised to guselkumab† | | All guselkumab‡ |
|---|---|---|---|---|---|---|
| | (Weeks 0–24) | (Weeks 16–56)§ | (Weeks 24–56)¶ | (Weeks 0–24) | (Weeks 24–56) | (Weeks 0–56) |
| Randomised patients by treatment received | 96 | 45 | 45 | 189 | 174 | 279 |
| Patient-years of follow-up | 28.1 | 32.9 | 27.2 | 87.7 | 107.6 | 255.4 |
| AEs | | | | | | |
| Events/100PY (95% CI) | 369.8 (302.2 to 448.1) | 127.5 (91.9 to 172.4) | 143.3 (101.9 to 195.9) | 229.2 (198.6 to 263.2) | 81.8 (65.6 to 100.8) | 144.9 (130.5 to 160.4) |
| Patients with ≥1 AE | 46 (47.9%) | 21 (46.7%) | 20 (44.4%) | 80 (42.3%) | 53 (30.5%) | 139 (49.8%) |
| Common AEs (>3% in any group) | | | | | | |
| Nasopharyngitis | 5 (5.2%) | 2 (4.4%) | 0 | 10 (5.3%) | 5 (2.9%) | 16 (5.7%) |
| Upper respiratory tract infection | 3 (3.1%) | 1 (2.2%) | 1 (2.2%) | 7 (3.7%) | 2 (1.1%) | 10 (3.6%) |
| Alanine aminotransferase increased | 4 (4.2%) | 1 (2.2%) | 3 (6.7%) | 5 (2.6%) | 3 (1.7%) | 11 (3.9%) |
| Faecal calprotectin increased | 3 (3.1%) | 0 | 1 (2.2%) | 2 (1.1%) | 1 (0.6%) | 4 (1.4%) |
| Psoriatic arthropathy | 4 (4.2%) | 2 (4.4%) | 0 | 3 (1.6%) | 4 (2.3%) | 10 (3.6%) |
| Hyperglycaemic | 5 (5.2%) | 1 (2.2%) | 0 | 3 (1.6%) | 0 | 4 (1.4%) |
| Hypertension | 3 (3.1%) | 0 | 0 | 1 (0.5%) | 3 (1.7%) | 4 (1.4%) |
| Infections | | | | | | |
| Events/100PY (95% CI) | 99.6 (66.2 to 143.9) | 30.4 (14.6 to 55.9) | 29.4 (12.7 to 57.9) | 63.9 (48.2 to 82.9) | 19.5 (12.1 to 29.8) | 37.2 (30.1 to 45.5) |
| Patients with ≥1 infection | 19 (19.8%) | 7 (15.6%) | 6 (13.3%) | 40 (21.2%) | 16 (9.2%) | 61 (21.9%) |
| Serious infections | | | | | | |
| Events/100PY (95% CI) | 0 | 0 | 3.7 (0.1 to 20.5) | 1.1 (0.03 to 6.4) | 0 | 0.8 (0.1 to 2.8) |
| Patients with ≥1 serious infection | 0 | 0 | 1 (2.2%) | 1 (0.5%) | 0 | 2 (0.7%) |
| SAEs | | | | | | |
| Events/100PY (95% CI) | 10.7 (2.2 to 31.2) | 6.1 (0.7 to 21.9) | 7.4 (0.9 to 26.5) | 8.0 (3.2 to 16.5) | 4.7 (1.5 to 10.8) | 6.3 (3.6 to 10.2) |
| Patients with ≥1 SAE | 3 (3.1%) | 2 (4.4%) | 2 (4.4%) | 7 (3.7%) | 5 (2.9%) | 15 (5.4%) |
| Abdominal pain | 0 | 0 | 0 | 0 | 1 (0.6%) | 1 (0.4%) |
| Acute coronary syndrome | 0 | 0 | 0 | 0 | 1 (0.6%) | 1 (0.4%) |
| Atrial fibrillation | 0 | 0 | 0 | 0 | 1 (0.6%) | 1 (0.4% |
| Buttock injury | 0 | 1 (2.2%) | 0 | 0 | 0 | 1 (0.4%) |
| Conversion disorder | 0 | 0 | 0 | 1 (0.5%) | 1 (0.6%) | 1 (0.4%) |
| Depression | 0 | 0 | 0 | 1 (0.5%) | 0 | 1 (0.4%) |
| Increased alanine aminotransferase | 0 | 0 | 0 | 1 (0.5%) | 0 | 1 (0.4%) |
| Increased liver enzymes | 0 | 0 | 1 (2.2%) | 0 | 0 | 1 (0.4%) |
| Intervertebral disc protrusion | 1 (1.0%) | 0 | 0 | 1 (0.5%) | 0 | 1 (0.4%) |
| Lumbosacral radiculopathy | 0 | 0 | 0 | 1 (0.5%) | 0 | 1 (0.4%) |
| Pneumonia | 0 | 0 | 1 (2.2%) | 1 (0.5%) | 0 | 2 (0.7%) |
| Prostate cancer | 0 | 0 | 0 | 1 (0.5%) | 0 | 1 (0.4%) |
| Pulmonary embolism | 0 | 0 | 0 | 0 | 1 (0.6%) | 1 (0.4%) |
| Umbilical hernia | 1 (1.0%) | 0 | 0 | 0 | 0 | 0 |
| Varicose vein | 0 | 1 (2.2%) | 0 | 0 | 0 | 1 (0.4%) |
| Vomiting | 1 (1.0%) | 0 | 0 | 0 | 0 | 0 |
| AEs leading to study agent discontinuation | | | | | | |
| Events/100PY (95% CI) | 7.1 (0.9 to 25.7) | 0 | 0 | 4.6 (1.2 to 11.7) | 2.8 (0.6 to 8.2) | 2.7 (1.1 to 5.7) |
| Patients with an AE leading to study agent discontinuation | 2 (2.1%) | 0 | 0 | 4 (2.1%) | 3 (1.7%) | 7 (2.5%) |
| Arthralgia | 1 (1.0%) | 0 | 0 | 0 | 0 | 0 |
| Conversion disorder | 0 | 0 | 0 | 0 | 1 (0.6%) | 1 (0.4%) |
| Fatigue | 0 | 0 | 0 | 0 | 1 (0.6%) | 1 (0.4%) |
| Increased alanine aminotransferase | 0 | 0 | 0 | 1 (0.5%) | 0 | 1 (0.4%) |
| Influenza-like illness | 0 | 0 | 0 | 1 (0.5%) | 0 | 1 (0.4%) |
| Prostate cancer | 0 | 0 | 0 | 1 (0.5%) | 0 | 1 (0.4%) |
| Psoriatic arthropathy | 0 | 0 | 0 | 0 | 1 (0.6%) | 1 (0.4%) |
| Urticaria | 0 | 0 | 0 | 1 (0.5%) | 0 | 1 (0.4%) |
| Vomiting | 1 (1.0%) | 0 | 0 | 0 | 0 | 0 |
| Participants with ≥1 malignancy | 0 | 0 | 0 | 1 (0.5%) | 0 | 1 (0.4%) |
| Participants with ≥1 ISR | 1 (1.0%) | 0 | 1 (2.2%) | 4 (2.1%) | 0 | 5 (1.8%) |

Highlighted SAEs also led to study agent discontinuation in the same patient.
*AEs that occurred during placebo treatment in placebo-randomised patients.
†Includes guselkumab-randomised patients who received an EE placebo injection at week 16.
‡AEs that occurred in all patients who received at least one administration of guselkumab, including those randomised to placebo.
§AEs that occurred during guselkumab treatment in placebo-randomised patients who crossed over to guselkumab prior to week 24.
¶AEs that occurred in placebo-randomised patients who crossed over to guselkumab at week 24.
AE, adverse event; EE, early escape; ISR, injection-site reaction; PY, patient-years; SAE, serious adverse event.

achieved (ACR20: guselkumab, 44% vs placebo, 20%). Guselkumab 100 mg Q8W afforded higher ACR20 and ACR50 response rates, as early as week 4 and week 8, respectively. Furthermore, >80% of patients who achieved ACR20/50/70 at week 24 maintained response at week 48. In addition, this study demonstrated the efficacy of guselkumab in resolving enthesitis and dactylitis, achieving clear skin and achieving MDA in patients with TNFi-IR PsA. The guselkumab group also had greater improvements in fatigue, physical function and HRQoL scores than placebo at week 24, with approximately 30%–40% of guselkumab-randomised patients achieving an improvement greater than the minimal clinically important differences at week 24.

Importantly in this TNF-IR population, improvements in signs and symptoms of PsA were maintained or numerically increased through week 48 among guselkumab-randomised patients. Among placebo→guselkumab patients, response rates and mean improvements increased through week 48. Thus, guselkumab 100 mg Q8W demonstrated efficacy through 1 year across the diverse symptoms in patients with TNFi-IR PsA.

Prespecified sensitivity analyses (eg, excluding patients with MPDs relevant to efficacy outcomes and correcting errors in EE patients thus providing a more accurate assessment of treatment effect) confirmed those of the primary endpoint (ACR20 at week 24). Although absolute response rates tended to be numerically lower in COSMOS patients relative to the primarily biologic-naïve populations in previous studies, the treatment effect of guselkumab as measured by the difference between the Q8W group and placebo at week 24 (ACR20 %differences: 25—28% across primary and sensitivity analyses) was generally consistent with that observed for guselkumab 100 mg Q8W in largely biologic-naïve patients with active PsA in the similarly designed pivotal DISCOVER-1 and DISCOVER-2 studies (ACR20 %differences: 30—31%).[8 9]

Guselkumab was well tolerated by participants, demonstrating a safety profile similar to placebo. Two guselkumab-treated patients had a serious infection. Two placebo-treated patients and three guselkumab-treated patients reported psychiatric disorders; two were SAEs, one occurring in a patient with a prior history of suicide attempt. One case of suspected, but unconfirmed, inflammatory bowel disease was reported ~1 month after the patient discontinued guselkumab due to an influenza-like illness. Abnormal clinical laboratory findings were uncommon; no participant died or developed an opportunistic infection or TB. Thus, these safety findings in patients with TNFi-IR PsA through week 56 of COSMOS expand on, and are consistent with, the accumulated guselkumab safety profile established in patients with psoriasis receiving guselkumab through 5 years[19] and that seen in DISCOVER-1 (1 year)[20] and DISCOVER-2 (2 years).[21 22]

Although head-to-head trials comparing guselkumab with other targeted or biologic therapies have not been conducted in patients with PsA, results from a recent network meta-analysis found that guselkumab had comparable efficacy with TNFi and IL-17A inhibitors in achieving ACR response in biologic-naïve patients.[23] In addition, the rates of AEs and SAEs were generally similar across treatment modalities; however, comparisons were limited by the significant uncertainty in the comparisons.[23] It is generally recommended to switch to an alternate mechanism of action following biologic treatment failure,[1 2] with only one switch between TNFi now recommended by the European Alliance of Associations for Rheumatology.[2] The demonstrated efficacy of therapies targeting the IL-12/23p40-subunit, IL-17A, and Janus kinases in TNFi-experienced patients with PsA[24–27] further supports the use of novel therapies to target alternative disease pathways.

However, patients who have experienced IR with a biologic, such as those enrolled in COSMOS, are at continued risk of treatment failure with subsequent therapies, thus highlighting the recalcitrant nature of the disease course in some patients with PsA.[4–6] Of note, 88% of guselkumab-randomised patients in COSMOS remained on treatment, and 94% of placebo patients who received guselkumab after week 24 completed study treatment through week 44. High study retention in COSMOS may thus reflect a positive benefit–risk profile for patients who had inadequate response to previous TNFi therapy. Patients who did not achieve an ACR20 response may have experienced substantial improvement in other symptoms (eg, skin disease). Other factors, such as comorbidities and limited availability or concerns about adverse effects of alternative treatment options in this refractory population, may have also contributed to patient retention.

Numerical imbalances in baseline characteristics (eg, gender, weight, joint counts and severity of skin disease) and errors in EE assignment may have influenced efficacy, although predominately not in favour of guselkumab. The slight imbalance between the treatment groups in the proportion of women is noteworthy considering research demonstrating that among patients with PsA, women tend to report having a higher disease burden and lower levels of response to treatment compared with men.[28] In addition, a separate analysis of 855 patients with PsA treated at a single rheumatology clinic found that being overweight was associated with not achieving treatment goals, specifically for women; however, no information was provided on the specific treatments these patients received.[29]

The COSMOS study was conducted across Europe, limiting ethnic diversity. COVID-related regulations during the latter half of study conduct may have increased MPDs; however, most were related to timing of study visits and did not impact efficacy. While the positive guselkumab benefit–risk profile observed through week 24 was maintained through 1 year, real-world evidence will further inform long-term guselkumab persistence in TNFi-IR patients.

In conclusion, guselkumab 100 mg Q8W was effective in patients with TNFi-IR PsA and demonstrated a favourable benefit–risk profile through 1 year. The statistically significant improvements observed with guselkumab across multiple clinical disease domains suggest a broad impact of targeting the p19 subunit of IL-23 in TNFi treatment-resistant PsA.

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EPIDEMIOLOGICAL SCIENCE

# Flares after hydroxychloroquine reduction or discontinuation: results from the Systemic Lupus International Collaborating Clinics (SLICC) inception cohort

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## ABSTRACT

**Objectives** To evaluate systemic lupus erythematosus (SLE) flares following hydroxychloroquine (HCQ) reduction or discontinuation versus HCQ maintenance.

**Methods** We analysed prospective data from the Systemic Lupus International Collaborating Clinics (SLICC) cohort, enrolled from 33 sites within 15 months of SLE diagnosis and followed annually (1999–2019). We evaluated person-time contributed while on the initial HCQ dose ('maintenance'), comparing this with person-time contributed after a first dose reduction, and after a first HCQ discontinuation. We estimated time to first flare, defined as either subsequent need for therapy augmentation, increase of ≥4 points in the SLE Disease Activity Index-2000, or hospitalisation for SLE. We estimated adjusted HRs (aHRs) with 95% CIs associated with reducing/discontinuing HCQ (vs maintenance). We also conducted separate multivariable hazard regressions in each HCQ subcohort to identify factors associated with flare.

**Results** We studied 1460 (90% female) patients initiating HCQ. aHRs for first SLE flare were 1.20 (95% CI 1.04 to 1.38) and 1.56 (95% CI 1.31 to 1.86) for the HCQ reduction and discontinuation groups, respectively, versus HCQ maintenance. Patients with low educational level were at particular risk of flaring after HCQ discontinuation (aHR 1.43, 95% CI 1.09 to 1.87). Prednisone use at time-zero was associated with over 1.5-fold increase in flare risk in all HCQ subcohorts.

**Conclusions** SLE flare risk was higher after HCQ taper/discontinuation versus HCQ maintenance. Decisions to maintain, reduce or stop HCQ may affect specific subgroups differently, including those on prednisone and/or with low education. Further study of special groups (eg, seniors) may be helpful.

## Key messages

**What is already known about this subject?**

- In clinical practice, patients often ask physicians about hydroxychloroquine (HCQ) reduction or discontinuation.
- The literature and clinical experience suggest that HCQ reduction/withdrawal may be safe in some stable patients, but in other settings it may be associated with disease flare.

**What does this study add?**

- Using real-world data from an international systemic lupus erythematosus (SLE) inception cohort, maintaining HCQ was associated with a lower flare risk than when reducing or stopping HCQ, even in patients with low disease activity or remission.
- Low education was associated with increased flare risk among patients discontinuing HCQ.
- Patients with SLE on prednisone or immunosuppressors were at higher risk for flare.
- The crude flare rate was over 30 flares per 100 person-years, even while maintaining HCQ.
- Over the interval of follow-up, most patients experienced a flare.
- This emphasises the ongoing need to optimise therapeutic options in SLE.

## INTRODUCTION

Hydroxychloroquine (HCQ) is a cornerstone of systemic lupus erythematosus (SLE) treatment.[1–3] However, physicians and patients often consider

## Key messages

**How might this impact on clinical practice or future developments?**

- If a patient were to ask "if someone decreases HCQ, what are the chances of flaring sooner than if they stay on the same dose?" the physician could reply: "According to this research, there is a 54% probability that a given person decreasing HCQ will flare sooner than someone staying on the same dose."
- Similarly, our results suggest that overall, a given patient who stops HCQ has a 61% probability of flaring sooner than a given patient who continues on HCQ. (Ssee Spruance et al (PMC478551) on how to interpret hazard ratioHRs in terms of chances).
- This translates back to the crude flare rates: maintaining HCQ had about 30–31 events per 100 person- years, while those that reduced or stopped HCQ had about 40–41 events per 100 person-years.
- Decisions to maintain, reduce or stop HCQ may affect specific subgroups differently, including those on prednisone and/or with low education.

reducing or discontinuing HCQ over the decades-long course of SLE, sometimes in order to limit cumulative exposure and avoid important HCQ-induced toxicity.[4 5]

Over 20 years ago, a pivotal HCQ withdrawal randomised trial suggested that sustained HCQ might greatly reduce disease flares, leading to the suggestion that all patients should remain on HCQ 'indefinitely'.[6 7] However, it is hard to know if results from that trial apply to patients in whom physicians would want to taper treatment, notably those in remission or very low activity, since many of the patients in the trial did not have completely controlled disease at study entry (40% were using prednisone and the average SLE Disease Activity Index score was 8).[7] For years, physicians have attempted to identify a subgroup of patients in whom it would be safe to stop or reduce HCQ, such as seniors.[8]

The aims of our study were to determine (1) the extent to which HCQ reduction or discontinuation is associated with increased risk of SLE flares, and (2) the predictors of a flare once HCQ is reduced or discontinued, using a longitudinal international SLE inception cohort.

## METHODS

### Data source

The Systemic Lupus International Collaborating Clinics (SLICC) cohort is a multinational inception cohort for SLE outcomes research.[9] From 1999 to 2011, a cohort of recently diagnosed patients with SLE was recruited from 33 SLICC sites in Europe, Asia and North America.[10] Briefly, patients meeting American College of Rheumatology revised classification criteria for SLE[11] were enrolled within 15 months of diagnosis. Data are collected per protocol at enrolment and annually and entered into a centralised database.

### Study population and design

We selected all patients on HCQ therapy at baseline (cohort entry) or during the follow-up up to April 2019. At each annual follow-up visit, average HCQ daily dose since the last assessment was recorded. We evaluated outcomes in patients reducing/stopping HCQ and compared them with those remaining on therapy. Patients contributed person-time in the HCQ maintenance cohort until they either reduced the dose, discontinued treatment, had the outcome of interest or were censored (death, lost to follow-up or end of study, April 2019), whichever came first. If HCQ was reduced, patients contributed person-time in the HCQ reduction cohort until they either discontinued HCQ, had the outcome or were censored. If HCQ was discontinued, patients contributed person-time in the HCQ discontinuation cohort until they had the outcome or were censored. A given patient could contribute person-time to one or more cohorts.

Time-zero among those reducing HCQ was the first date recording HCQ reduction and time-zero in the HCQ discontinuation cohort was the first date recording discontinuation. To create the comparison HCQ maintenance groups (one for reduction, one for discontinuation), each patient reducing or discontinuing HCQ was randomly matched on prior HCQ use duration to up to two individuals remaining on HCQ treatment (figure 1). A time-zero was then assigned to the matched maintenance group on the day of matching. This approach balances the groups on the length of previous treatment at the beginning of follow-up and avoids immortal person-time.[12]

Patients who discontinued HCQ but started chloroquine immediately were not included in the discontinuation cohort, as they were still on an antimalarial; these were censored at the time of switching.

The reasons underlying HCQ dose change were not recorded, but dose reduction may have been due to the following scenarios: (a) physician or patient may have been concerned about cumulative use of HCQ and/or lowered dosing to reflect guidelines (particularly the 2016 American Academy of Ophthalmology (AAO) guidelines, which cautions against dosage >5 mg/kg/day)[5]; (b) low SLE activity; (c) other reasons (eg, intolerance, patient request). Reasons for stopping HCQ may include (a) retinal toxicity; (b) clinical disease remission; (c) non-adherence; (d) intolerance, pigmentary skin changes or other adverse effect; (e) other reasons (eg, cost, healthcare access issues, drug insurance issues, patient choice).

We explored ways to categorise these possible reasons. Among patients who reduced HCQ dose, we identified how many had their dose reduced to 5 mg/kg/day after the 2016 AAO guidelines, and, of the remainder, how many had low disease activity state[13] (SLE Disease Activity Index-2000 (SLEDAI-2K) <4 and current prednisone dose ≤7.5 mg/day). Patients not falling into one of these groups were classified into 'other reasons'. Similarly, among those who stopped HCQ, we first identified those who had retinal damage on the SLICC Damage Index (SDI). Of those without retinal damage, we identified how many were in remission[14] (SLEDAI-2K=0 and no prednisone or immunosuppressives in the last year). For the remainder, reasons were unclear but may reflect non-adherence or other unknown reasons.

### Outcome

The primary composite outcome was time to the first of the following events indicating a SLE flare: (a) increase of at least four points (above the score at time-zero) in the SLEDAI-2K[15]; (b) hospitalisation for SLE (eg, skin and joint flare, nephritis, pericarditis and pneumonia) and/or (c) augmented SLE therapy, defined as increased HCQ (or restart if discontinued) or a new start/increase in prednisone, immunosuppressive agents (azathioprine, methotrexate, mycophenolate mofetil, cyclophosphamide), biologics (rituximab or belimumab) or start of chloroquine. Quinacrine was used by only nine patients in our sample and was not considered as augmented SLE therapy. Since

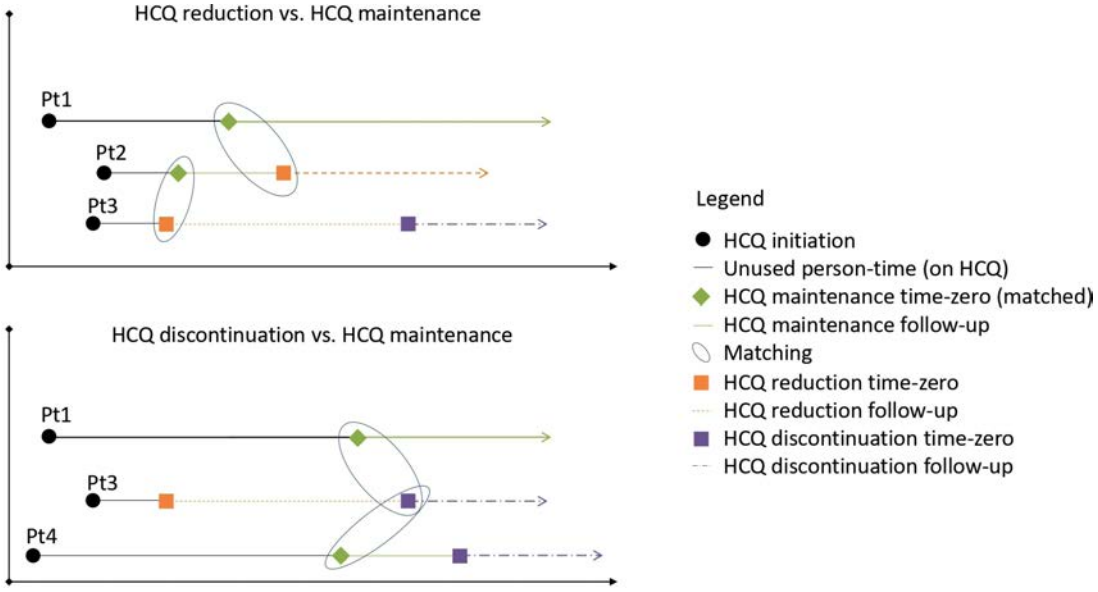


**Figure 1** Example of four cohort patients (Pt1–4). A given patient could contribute person-time to one or more cohorts. Hydroxychloroquine (HCQ) maintenance person-time was matched (2:1) to the reduction or discontinuation cohorts on HCQ duration at time-zero.

immunosuppressive agents may be given in addition to or instead of steroid therapy to lower the dose of steroids,[16] we did not compute an event when patients increased/started an immunosuppressor (azathioprine, mycophenolate or methotrexate) but decreased their prednisone dose at the same visit. Hospitalisation data were available for 60% of patients and the composite outcome for patients without hospitalisation data was based on increase in disease activity and therapy augmentation only. Sensitivity analyses excluding hospitalisation from the composite outcome for all patients were also performed.

### Covariates

Decisions to reduce, stop or maintain HCQ may be driven by patient or clinical characteristics that are also associated with the outcome. Therefore, we considered potential confounders or effect modifiers, assessed at time-zero: sociodemographic variables (sex, Caucasian vs non-Caucasian race/ethnicity, high school education or less vs college/university education), age at SLE diagnosis (continuous) and geographic location (North America, Europe or Asia). Other variables assessed at time-zero included body mass index (BMI, continuous), current smoking (yes/no), high disease activity (≥4 points on SLEDAI-2K, a validated definition of active SLE),[15 17] SLE duration (continuous, years), current prednisone (yes/no), current immunosuppressive agents (azathioprine, methotrexate or mycophenolate mofetil) and current biological agents (rituximab or belimumab), and presence of renal damage, based on the SDI.[18]

### Statistical analysis

In descriptive analysis at time-zero, we described the means and SDs for continuous variables and frequency distributions for categorical variables.

For the HCQ reduction/discontinuation cohorts and their respective maintenance control cohorts, we calculated crude incidence rates (first flare) with 95% CIs. A multivariable Cox proportional hazards (PH) model was used to estimate the adjusted HRs (HRs), with 95% CIs, for time to first flare in patients who reduced or discontinued HCQ (vs the maintenance groups), while controlling for the covariates listed above. Hazard proportionality was assessed using Schoenfeld and Martingale residuals.

Separate multivariable Cox PH models were estimated in the reduction, discontinuation and maintenance cohorts to assess which characteristics were associated with increased risk for first flare.

As a secondary analysis, we aimed to assess how disease activity status influence the risk of SLE flares after HCQ reduction or discontinuation (vs HCQ maintenance). Thus, we stratified the absolute flare rates and adjusted Cox models by low disease activity state or remission.

We also conducted sensitivity analysis. Since the same patient could contribute person-time to different cohorts being compared, we accounted for potential clustering by using random effects in our Cox models. Also, to evaluate the impact of having patients without complete outcome information (ie, missing hospitalisation data), we considered only increase in disease activity and therapy augmentation in the computation of the composite outcome for all patients.

All analyses were conducted with SAS V.9.4 (SAS Institute, Cary, North Carolina, USA).

### Patient and public involvement

Patients with SLE, patient advocates and organisations such as the Canadian Network of Improved Outcomes in SLE and the Canadian Rheumatology Association were engaged as partners since the early phases of our project, providing feedback on the protocol, interpretation of findings and dissemination. For instance, this study was planned and designed based on focus groups conducted in 2017 with patients with rheumatic disease,[19] which identified that uncertainties about risks and benefits of stopping/continuing drugs were a primary concern. Our patient-partner assisted in the development of questionnaires and provided feedback regarding interpretation of findings. We also conducted interviews of individual patient with SLE to explore experiences and preferences with HCQ dose changes[20] and defined potential reasons underlying HCQ changes, and incorporated this in our analyses, as mentioned before.

**Table 1** Characteristics at time-zero of patients with SLE who maintained, reduced or discontinued HCQ

| Characteristics at time-zero* | HCQ reduction n=564 | HCQ maintenance n=778 | HCQ discontinuation n=389 | HCQ maintenance n=577 |
|---|---|---|---|---|
| Female (%) | 90.6 | 87.9 | 90.2 | 87.0 |
| N missing | 0 | 0 | 0 | 0 |
| Race/Ethnicity (%) | | | | |
| Caucasian | 51.6 | 55.1 | 42.9 | 55.6 |
| Asian | 24.3 | 14.7 | 19.3 | 13.9 |
| Black | 12.4 | 16.1 | 15.4 | 15.9 |
| Others | 10.6 | 13.3 | 21.4 | 13.9 |
| N missing | 6 (1.1) | 6 (0.8) | 4 (1.0) | 4 (0.7) |
| Age at SLE diagnosis (years, mean±SD) | 34.1±13.4 | 35.6±13.3 | 33.6±13.4 | 35.9±13.6 |
| N missing | 0 | 0 | 0 | 0 |
| No college/university education (%) | 34.0 | 38.9 | 38.8 | 39.9 |
| N missing | 6 (1.1) | 16 (2.0) | 3 (0.8) | 8 (1.4) |
| Geographic location (%) | | | | |
| North America | 56.2 | 63.2 | 59.6 | 62.6 |
| Europe | 26.1 | 27.9 | 26.5 | 29.3 |
| Asia† | 17.7 | 8.9 | 13.9 | 8.1 |
| N missing | 0 | 0 | 0 | 0 |
| Time on HCQ (years, mean±SD) | 3.4±2.6 | 3.2±2.5 | 4.2±3.2 | 3.9±3.1 |
| N missing | 0 | 0 | 0 | 0 |
| HCQ daily dosage (mg, mean±SD) | 240±73 | 347±83 | 0 | 349±81 |
| N missing | 0 | 0 | 0 | 0 |
| SLE duration (years, mean±SD) | 5.5±3.0 | 5.4±3.0 | 6.7±3.5 | 6.1±3.4 |
| N missing | 0 | 0 | 0 | 0 |
| SLEDAI-2K ≥4 (%) | 39.9 | 35.7 | 38.0 | 36.0 |
| N missing | 15 (2.6) | 15 (1.9) | 11 (2.8) | 19 (3.3) |
| Renal damage (%) | 6.4 | 5.7 | 10.7 | 5.4 |
| N missing | 3 (0.5) | 5 (0.6) | 5 (1.3) | 2 (0.3) |
| Current smoker (%) | 25.9 | 33.2 | 29.6 | 31.5 |
| N missing | 3 (0.5) | 4 (0.5) | 7 (1.8) | 2 (0.3) |
| BMI (mean±SD) | 24.1±5.1 | 25.6±5.9 | 25.1±5.7 | 25.7±5.9 |
| N missing | 16 (2.8) | 30 (3.8) | 7 (1.8) | 23 (4.0) |
| Current prednisone (%) | 58.0 | 55.4 | 51.9 | 52.8 |
| N missing | 0 | 0 | 0 | 0 |
| Current immunosuppressors‡ (%) | 44.1 | 47.0 | 41.6 | 46.8 |
| N missing | 0 | 0 | 0 | 0 |
| Current biological agents§ (%) | 3.0 | 2.6 | 3.6 | 4.0 |
| N missing | 0 | 0 | 0 | 0 |

*Time-zero of each subcohort (not inception cohort entry).
†Asia was represented by a single country, South Korea.
‡Immunosuppressors included mycophenolate, azathioprine and methotrexate.
§Biologics included belimumab and rituximab.
BMI, body mass index; HCQ, hydroxychloroquine; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index-2000.;

Lupus Canada and the Arthritis Society have pledged support to disseminate findings via websites, communiques and their provincial chapters. The Singer Family Fund for Lupus Research will help with knowledge dissemination through newsletters mailed twice yearly to patients with SLE.

## RESULTS

Among the 1711 patients enrolled in the SLICC cohort, we included 1460 (85.3%) who initiated HCQ. We identified 592 patients who reduced HCQ (564 were matched to 778 patients maintaining HCQ) and 407 patients who discontinued HCQ (389 were matched to 577 patients remaining on HCQ). There were few differences in patient characteristics at time-zero between the matched groups: patients reducing HCQ were more likely to be from Asia and patients discontinuing HCQ were less likely to be Caucasian (table 1).

The HCQ reduction or discontinuation was further classified according to the possible reasons for the respective changes. Specifically, we estimated that 5.0% may have reduced HCQ therapy as result of the AAO guidelines (daily dose changed from >5 mg/kg to 5 mg/kg after July 2016, based on real body weight), 54.8% because of low disease activity state and the remainder (40.2%) presumably reduced due to other reasons (eg, intolerance, patient preference, etc). Among those who discontinued HCQ, 4.4% had retinal damage, 15.2% were in remission and 80.5% may have stopped HCQ due to other reasons, including non-adherence and intolerance.

### SLE flares

The HCQ reduction cohort was followed for an average of 2.0 years per patient (with 78.7% flaring over follow-up, table 2)

**Table 2** Incidence rates of the first flare in patients with SLE who maintained, reduced or discontinued HCQ

| | HCQ reduction n=564 | HCQ maintenance n=778 | HCQ discontinuation n=389 | HCQ maintenance n=577 |
|---|---|---|---|---|
| First flare (any) | | | | |
| Number of events (%) | 444 (78.7) | 413 (53.1) | 280 (72.0) | 292 (50.6) |
| Therapy augmentation only | 399 (70.7) | 325 (41.8) | 252 (64.8) | 239 (41.4) |
| Increase in disease activity only | 61 (17.0) | 127 (16.3) | 68 (17.5) | 81 (14.0) |
| Hospitalisation only | 1 (0.2) | 1 (0.2) | 0 | 2 (0.5) |
| Total person-years in follow-up | 1110.2 | 1294.7 | 677.9 | 973.4 |
| Crude rate/100 person-years (95% CI) | 40.0 (36.4 to 43.9) | 31.9 (29.0 to 35.1) | 41.3 (36.7 to 46.4) | 30.0 (26.7 to 33.6) |

HCQ, hydroxychloroquine; ;SLE, systemic lupus erythematosus.

while the average follow-up in the other cohorts was about 1.7 years (with 72% flaring in the HCQ discontinuation cohort, and about 50% flaring in the maintenance cohorts). Need for therapy augmentation was frequent and hospitalisation due to lupus flares was relatively uncommon. The crude incidence rate of the first flare was considerably higher among those who reduced or stopped HCQ versus those who remained on the drug (table 2). Compared with HCQ maintenance, the adjusted HRs for SLE flare were 1.20 (95% CI 1.04 to 1.38) and 1.56 (95% CI 1.31 to 1.86) for the HCQ reduction and discontinuation cohorts, respectively. The mean doses of those reducing HCQ and flaring versus those reducing but not flaring were similar (data not shown).

### Risk factors

Separate multivariable Cox PH models were fit in each of the HCQ reduction, discontinuation and maintenance cohorts to estimate HRs for potential risk factors (table 3). Use of prednisone and immunosuppressives were both associated with higher risks of SLE flares in all cohorts (although in the discontinuation cohort, the 95% CI for the immunosuppressives HR just barely included the null value). We also observed a lower flare risk among patients reducing HCQ who live in Asia (South Korea) versus North American patients. Lower education was associated with an increased risk of SLE flares among patients who discontinued HCQ.

### Secondary and sensitivity analyses

Table 4 presents the results from the prespecified secondary analysis restricted to subgroups of patients on disease activity status. We observed that maintaining HCQ was associated with lower SLE flare risk even for patients in low disease activity state or in remission at time-zero (table 4). Patients not in remission tended to have relatively higher crude flare rates, about 46–48 events per 100 patient-years when lowering or stopping HCQ, and about 39–41 events per 100 patient-years when maintaining HCQ.

Accounting for clustering and removing hospitalisation from the composite outcome led to small changes in the SEs, but had little or no effect on HR estimates (data not shown).

## DISCUSSION

Ours is the first study in incident SLE to demonstrate that patients who reduced or discontinued HCQ had an increased risk of flaring versus those who maintained therapy. Other

**Table 3** HRs and 95% CIs for the first SLE flare, according to HCQ cohort

| | HCQ reduction | HCQ maintenance | HCQ discontinuation | HCQ maintenance |
|---|---|---|---|---|
| **Characteristics at time-zero** | **aHR (95% CI)** | **aHR (95% CI)** | **aHR (95% CI)** | **aHR (95% CI)** |
| Male sex | 0.93 (0.66 to 1.32) | 0.96 (0.68 to 1.34) | 0.97 (0.64 to 1.46) | 0.77 (0.52 to 1.15) |
| Non-Caucasians | 1.27 (1.00 to 1.61) | 1.02 (0.81 to 1.28) | 0.96 (0.70 to 1.32) | 0.96 (0.73 to 1.27) |
| Age at SLE diagnosis in years | 1.00 (0.99 to 1.01) | 1.00 (0.99 to 1.01) | 0.99 (0.98 to 1.00) | 1.01 (1.00 to 1.02) |
| No college/university education | 1.01 (0.82 to 1.24) | 1.10 (0.90 to 1.36) | **1.43 (1.09 to 1.87)** | 0.92 (0.72 to 1.18) |
| Geographic location | | | | |
| North America | Reference | Reference | Reference | Reference |
| Europe | 1.24 (0.98 to 1.59) | 1.16 (0.91 to 1.48) | 1.02 (0.75 to 1.37) | 0.99 (0.75 to 1.31) |
| Asia | **0.70 (0.51 to 0.94)** | 1.00 (0.69 to 1.43) | 0.73 (0.49 to 1.08) | 0.87 (0.56 to 1.34) |
| SLE duration | 1.00 (0.96 to 1.04) | 1.01 (0.98 to 1.06) | 1.00 (0.96 to 1.04) | 1.02 (0.98 to 1.06) |
| Active disease (SLEDAI-2K ≥4) | 1.17 (0.95 to 1.44) | 1.22 (0.98 to 1.51) | 1.25 (0.95 to 1.64) | 1.22 (0.95 to 1.56) |
| Renal damage | 0.88 (0.57 to 1.37) | 0.94 (0.58 to 1.53) | 0.88 (0.60 to 1.30) | 0.88 (0.49 to 1.56) |
| Body mass index | 1.02 (1.00 to 1.05) | 0.99 (0.98 to 1.01) | 0.99 (0.97 to 1.01) | 1.00 (0.97 to 1.02) |
| Smoker | 1.07 (0.85 to 1.35) | 0.88 (0.70 to 1.11) | 1.02 (0.78 to 1.35) | 0.94 (0.71 to 1.23) |
| On prednisone | **1.49 (1.16 to 1.91)** | **1.65 (1.28 to 2.13)** | **1.58 (1.15 to 2.17)** | **1.87 (1.38 to 2.54)** |
| On immunosuppressives | **1.37 (1.09 to 1.72)** | **1.84 (1.46 to 2.32)** | 1.31 (0.96 to 1.77) | **1.84 (1.39 to 2.44)** |
| On biologics | 0.72 (0.39 to 1.35) | 1.00 (0.51 to 1.95) | 0.70 (0.35 to 1.39) | 0.77 (0.33 to 1.79) |

Renal damage was defined as a score ≥1 in the SLICC/ACR Damage Index renal item (low glomerular filtration rate, proteinuria or end-stage renal failure). Prednisone, immunosuppressives and biologics were dichotomous variables (yes/no). Immunosuppressive drugs included azathioprine, mycophenolate and methotrexate. Biologics included belimumab, rituximab and abatacept.
Bolded values are those whose 95% CI excludes the null value.
ACR, American College of Rheumatology; aHR, adjusted HR; HCQ, hydroxychloroquine; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index-2000; SLICC, Systemic Lupus International Collaborating Clinics.

**Table 4** Adjusted HRs with 95% CIs for SLE flares associated with HCQ reduction/discontinuation versus maintenance: main and stratified analyses

| | HCQ reduction versus maintenance | | | HCQ discontinuation versus maintenance | | |
|---|---|---|---|---|---|---|
| | No. of patients | Absolute flare rate/100 person-years (95% CI) | Adjusted HR (95% CI)* | No. of patients | Absolute flare rate/100 person-years (95% CI) | Adjusted HR (95% CI)* |
| Main analysis | 1342 | 40.0 (36.4 to 43.9) vs 31.9 (29.0 to 35.1) | 1.20 (1.04 to 1.38) | 966 | 41.3 (36.7 to 46.4) vs 30.0 (26.7 to 33.6) | 1.56 (1.31 to 1.86) |
| Stratified analyses: | | | | | | |
| Low disease activity‡ state at time-zero | | | | | | |
| Yes | 815 | 37.5 (33.2 to 42.4) vs 27.8 (24.5 to 31.6) | 1.32 (1.10 to 1.60) | 592 | 35.5 (30.4 to 41.3) vs 26.6 (22.8 to 30.9) | 1.62 (1.28 to 2.05) |
| No | 527 | 43.9 (38.0 to 50.6) vs 39.8 (34.3 to 46.1) | 1.04 (0.84 to 1.29) | 374 | 53.6 (44.7 to 64.2) vs 36.4 (30.5 to 43.5) | 1.60 (1.22 to 2.09) |
| Remission† at time-zero | | | | | | |
| Yes | 196 | 26.2 (20.1 to 34.1) vs 13.2 (9.5 to 18.4) | 2.14 (1.34 to 3.42) | 133 | 24.7 (17.7 to 34.6) vs 12.2 (8.0 to 18.8) | 2.77 (1.46 to 5.26) |
| No | 1146 | 46.3 (41.9 to 51.1) vs 41.7 (37.8 to 46.0) | 1.14 (0.98 to 1.32) | 833 | 47.9 (42.3 to 54.2) vs 39.2 (35.0 to 43.9) | 1.50 (1.25 to 1.81) |

*Adjusted for sex, race, age at SLE diagnosis, education, geographic residence and the following variables assessed at time-zero: SLE duration, renal damage according to SLICC Damage Index, body mass index, smoking, prednisone, immunosuppressives and biologics. The main analysis was additionally adjusted by disease activity at time-zero.
†Remission was defined as SLEDAI-2K=0 and no prednisone or immunosuppressives use during the last year.
‡Low disease activity state was defined as SLEDAI-2K <4 and current prednisone dose ≤7.5 mg/day.
HCQ, hydroxychloroquine; SLE, systemic lupus erythematosus; SLEDAI-2K, SLE Disease Activity Index-2000; SLICC, Systemic Lupus International Collaborating Clinics.

medications, geographic location and education were associated with flare risk. Age was not a clear risk factor, which is interesting given a recent paper that suggested HCQ discontinuation may be relatively safe in seniors (although the time-frame for flare risk was 1 year only).[8] When stratifying our own results by age >50, power was decreased, but there remained a trend for HCQ maintenance being associated with a lower crude flare rate (25.3 events per 100 person-years, 95% CI 20.4 to 31.3) versus HCQ reduction (36.9 events per 100 person-years, 95% CI 30.0 to 45.4). The same trend was seen for HCQ discontinuation during person-time for age >50, again with imprecision (HCQ maintenance flare 31.0 events per 100 person-years, 95% CI 24.6,39.2 and HCQ discontinuation flare rate 42.4 events per 100 person-years, 95% CI (37.2 to 48.4).

Patients using immunosuppressives or prednisone at time-zero were at higher risk of flare after either HCQ maintenance, reduction or discontinuation. At least two other cohort studies have shown that patients under immunosuppressives (who generally have fairly severe SLE) have a twofold higher flare risk overall (vs patients not on immunosuppressives, who generally have less severe SLE).[21 22] In addition to immunosuppressives, steroids are also markers of severe and active SLE.[23–25]

We observed some geographical differences in SLE treatment management and flare risks. Patients from Asia were more likely to reduce HCQ than maintain the dose (table 1). A survey showed that, compared with Europeans, Asian physicians were more likely to taper HCQ even in in patients with severe disease.[26] Another study conducted in South Korea observed that polymorphisms in CYP2D6*10, an allele that is more common in Asians than in Caucasians,[27 28] were associated with higher blood concentrations of HCQ's metabolite.[29] Together with recent results suggesting that Asian patients are more adherent to HCQ than Caucasians,[30] this evidence may correlate with our finding that patients living in Asia had a lower risk of flaring after HCQ reduction than those living in North America and Europe. Since data from Asia came from a single tertiary centre in South Korea, these findings may reflect local practices or factors inherent to that population.

Low education was associated with increased flare risk among patients discontinuing HCQ. Low education is a well-known predictor of poor adherence to long-term therapies including in SLE.[31–34] Subjects who discontinued HCQ (particularly those with low education) may have been non-adherent with other medications and physician advice, perhaps due to mistrust or not understanding physician recommendations.[31 35]

Our results suggest that HCQ maintenance typically results in lower flare risks, even in patients in disease remission. This finding is interesting in view of a small survey which suggested some (though not all) rheumatologists attempt to taper or discontinue HCQ in patients in remission[26] and indicates that current disease activity alone may not sufficiently predict who will flare after HCQ is tapered. Incomplete adherence may explain some of our findings.[36] However, flares occur even in patients with HCQ blood levels above the therapeutic threshold,[37] reinforcing the relapsing-remitting nature of SLE, with durable remission being rare.[38 39]

The potential benefits of tapering or discontinuing HCQ must be balanced with the subsequent risk of a flare. Need for therapy augmentation occurred in 65%, 71% and ~40% of patients after HCQ discontinuation, reduction or maintenance, respectively. Of subjects needing therapy augmentation, over 65% augmented/started prednisone after HCQ reduction or discontinuation. Although the potential for antimalarial-induced toxicity (including retinopathy and cardiomyopathy) is of concern for patients and physicians,[4 40] the adverse effects of glucocorticoids are severe and well established in patients with SLE[41] and most physicians and patients would certainly prefer maintaining HCQ than augmenting prednisone.[42 43]

We studied a large international inception cohort with almost 20 years of follow-up and a well-characterised study population. However, some potential limitations should be mentioned. Patients and physicians did not explicitly provide the reason(s) for HCQ reduction or discontinuation. If decisions to reduce/discontinue HCQ are based on the patient's current or past disease activity, long-term SLE remission may be more likely in the HCQ reduction/discontinuation cohorts, which may bias estimates towards the null. Despite this, our results still suggest that lowering/discontinuing HCQ is associated with higher flare risk versus maintaining HCQ.

Another potential limitation is that our composite outcome includes some interval-censored endpoints (those assessed only at annual clinic visits). However, simulations reported in the study by Huszti *et al*,[44] for example, indicate that this will induce only minor bias towards the null in the estimated HRs. Moreover, our

composite outcome is a practical approach similar to that used in clinical trials and, in addition to the accepted minimal clinically significant SLEDAI-2K change (important but not always sensitive), we included SLE-related hospitalisations (detecting the most serious SLE flares), as well as drug changes (a potentially more enduring marker of flares). Unfortunately, our definition of flare cannot clearly separate mild from moderate or severe flares.

It is interesting that the HCQ reduction and discontinuation cohorts had similar flares rates. Among those who reduced HCQ, the mean doses of those flaring versus not flaring were similar. This may reflect individual differences in drug metabolism or even in the amount of HCQ stored in body tissues. It has been suggested that doses under the maximum 400 mg/day (eg, 200 and 300 mg/day) still are potentially associated with less activity, thrombosis and survival.[6 45 46] We did not evaluate HCQ levels (which are not part of usual care at most of our centres) or self-reported adherence. Nevertheless, in adjusting for sex, age, race/ethnicity, education and multiple medications, we accounted for factors that are themselves strong predictors of adherence.

The implications of our study findings are complex, with the decision to maintain or taper HCQ still being up to the patients and their physicians, through discussion of the trade-offs between the risk of disease flare, with the potential benefits of tapering HCQ. Our results should help facilitate this, by providing information about risks of flare associated with maintaining, reducing or stopping HCQ, and how and demographic factors (eg, disease activity, medications, education) may influence outcomes. These carefully quantified risks could be translated to improve patient education materials and discussions between healthcare providers and patients. Last but not least, the fact that there are over 30 flares per 100 person-years, even while remaining on HCQ, emphasises the ongoing need to optimise therapeutic options in SLE.

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TRANSLATIONAL SCIENCE

# Rheumatoid arthritis, systemic lupus erythematosus and primary Sjögren's syndrome shared megakaryocyte expansion in peripheral blood

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## ABSTRACT

**Objectives** Rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS) share many clinical manifestations and serological features. The aim of this study was to identify the common transcriptional profiling and composition of immune cells in peripheral blood in these autoimmune diseases (ADs).

**Methods** We analysed bulk RNA-seq data for enrichment of biological processes, transcription factors (TFs) and deconvolution-based immune cell types from peripheral blood mononuclear cells (PBMCs) in 119 treatment-naive patients (41 RA, 38 pSS, 28 SLE and 12 polyautoimmunity) and 20 healthy controls. The single-cell RNA-seq (scRNA-seq) and flow cytometry had been performed to further define the immune cell subsets on PBMCs.

**Results** Similar transcriptional profiles and common gene expression signatures associated with nucleosome assembly and haemostasis were identified across RA, SLE, pSS and polyautoimmunity. Distinct TF ensembles and gene regulatory network were mainly enriched in haematopoiesis. The upregulated cell-lineage-specific TFs *PBX1*, *GATA1*, *TAL1* and *GFI1B* demonstrated a strong gene expression signature of megakaryocyte (MK) expansion. Gene expression-based cell type enrichment revealed elevated MK composition, specifically, $CD41b^{+}CD42b^{+}$ and $CD41b^{+}CD61^{+}$ MKs were expanded, further confirmed by flow cytometry in these ADs. In scRNA-seq data, MKs were defined by TFs *PBX1/GATA1/TAL1* and pre-T-cell antigen receptor gene, *PTCRA*. Cellular heterogeneity and a distinct immune subpopulation with functional enrichment of antigen presentation were observed in MKs.

**Conclusions** The identification of MK expansion provided new insights into the peripheral immune cell atlas across RA, SLE, pSS and polyautoimmunity. Aberrant regulation of the MK expansion might contribute to the pathogenesis of these ADs.

## Key messages

**What is already known about this subject?**

► Rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS) share many clinical and serological features. The peripheral blood mononuclear cells (PBMCs) are the common origin for immune cells infiltrating specific targeted organs in these autoimmune diseases (ADs). However, the initial immune cells regulated by core transcription factors (TFs) in the PBMC remain unknown.

**What does this study add?**

► This study uncovers common gene expression signatures in platelet activation, consisting of increased megakaryocyte (MK) composition with upregulated expression of cell-lineage specific TFs *PBX1*, *GATA1*, *TAL1* and *GFI1B* in PBMC across RA, SLE, pSS and polyautoimmunity. In peripheral blood, MKs are a heterogeneous cell population that comprises a subpopulation with distinct immune characteristics in these ADs. Speculatively, this subpopulation of immunologically active MKs might be an initial stimulus for T-cell initiation of these diseases.

**How might this impact on clinical practice?**

► Our results elucidate MK expansion for the atlas of peripheral immune cells across RA, pSS and SLE and support the hypothesis that regulatory events in MK expansion might act as pivotal components, which could be an entry point toward developing targeted treatment for patients with these ADs.

## INTRODUCTION

Rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS) are common autoimmune disease (ADs) in women, which preferentially affect specific target organs. Indeed, these ADs share several clinical manifestations, serological profiles and immunological characteristics. Furthermore, the co-occurrence of these ADs within a single patient (polyautoimmunity) and within members of a nuclear family (familial autoimmunity) indicate that they have common aetiological components, including genetic and epigenetic factors and sex hormones. The genetic variants in the T-cell receptor (TCR) pathway and *TNFAIP3*[1 2] and DNA methylation signatures had been previously uncovered across RA, SLE and pSS.[3] However, it is still unknown which antigen initially primes autoimmune T cells.

Beyond affected organs, peripheral blood represents the main highway for the immune system for RA, SLE and pSS. Peripheral blood mononuclear cells (PBMCs) in this context are the immune cells which initiate the autoimmune inflammatory process directed against target organs. Therefore, the gene expression signatures of PBMC could shed light on the molecular features of the immune cells in the targeted organs in these ADs. Shared type I interferon (IFN)-stimulated genes were identified via meta-analysis of PBMC transcriptomes across RA, SLE and pSS.[4] However, PBMC comprises several cell types and each cellular subtype expresses a unique set of genes; thus, cell-specific signatures may further define immune cell composition for AD pathogenesis. On the other hand, underlying immune responses are the developmental trajectories that determine immune cells' fates.[5] The transcription factor (TF) network controlling cell lineage commitment in the bone marrow could determine the landscape for immune cell expansion in the peripheral blood in pathogenesis of these ADs.

Herein, we combined bulk RNA-seq and single-cell RNA-seq (scRNA-seq) of gene expression signatures, immune cell subsets and TF networks to identify potential common mechanisms in the immunopathogenesis of SLE, RA and pSS.

## Patients and methods

### Subjects

PBMCs were obtained from 42 RA, 41 pSS, 28 SLE and 12 polyautoimmunity patients and 21 gender-matched healthy controls (online supplemental table S1). Patients met the 2002 American-European Consensus Group for pSS, the 2012 Systemic Lupus Collaborating Clinics for SLE and the 2010 ACR/EULAR for RA, respectively. Polyautoimmunity was defined as patients with two ADs, RA/pSS or SLE/pSS.[6] Fully informed consent was obtained from all participants prior to sample collection. For more details about the study design, experimental and bioinformatic methods, see online supplemental methods (online supplemental figure S1).

## RESULTS

### RA, pSS and SLE shared common gene expression signatures enriched in coagulation and nucleosome assembly

Using bulk RNA-seq data, we initially assessed clustering of RA, SLE, pSS and polyautoimmunity by principal component analysis that demonstrated that these ADs were similar (figure 1A). Compared with healthy controls, these ADs had similar transcriptional profiles, sharing differentially expressed genes ((DEGs) figure 1B–C and online supplemental figures S2–6 and table S2), including type I IFN-stimulated gene *IFI27* plus chemokine receptors *CXCR1* and *CXCR2*.[4] Indeed, 446 common upregulated and 165 downregulated genes overlapped across these ADs (figure 1D and online supplemental table S2). Among the upregulated genes, the major biological processes that were enriched were related to nucleosome assembly and coagulation cascades (figure 1E). The most impacted pathway was the 'SLE' pathway (figure 1F). To further illustrate this point, haemostasis and megakaryocyte (MK) differentiation had been identified via gene ontology (GO) term networks (online supplemental figure S6A). Protein–protein interactions were demonstrated among histone genes H2A and H2B, including *H2AC11*, *H2AC13*, *H2BC11* and *H2BC12*, which were consistent with the GO term of nucleosome assembly (online supplemental figure S6B). Gene set enrichment analysis showed significant enrichment of platelet activation in these ADs as well (online supplemental figure S6C). Collectively, transcriptional profiling suggested potential regulation of MK/platelet-related processes emerged as the gene expression signatures in these ADs.

### Common TFs highlighted MK expansion responding to the gene expression signatures

We next sought to identify TFs linked with gene enrichment involved in biological processes in ADs. Transcriptional regulatory networks indicated *GATA1* as the top-ranked regulator by enrichment analysis of upregulated genes (figure 2A). Only 17 common upregulated and 8 downregulated TFs were identified (figure 2B and online supplemental figure S6G) and were mainly enriched in embryonic haematopoiesis and granulocyte differentiation (figure 2C–D). We further determined correlations among TFs, reasoning that the distinct TF ensembles could be correlated with expression. The correlated expression pattern was comprised of: *GRHL1, MEIS1, THRB, PBX1, GATA1, TAL1, GFI1B* and *E2F1* (figure 2E). Focusing on TF function, estradiol promotes haematopoietic stem cell (HSC) division by enrichment of cell cycle genes, harbouring a binding motif for the TF *E2F1*.[7] In addition, oestrogen receptor (ER) interacted with *MEIS1*, *THRB* and *GRHL1*;[8] consequently, interaction of *MEIS1* and *PBX1* acts upstream of *GATA1* to regulate primitive haematopoiesis and induce lineage commitment toward a MK-erythroid progenitor cell.[9 10] Thus, *GRHL1, MEIS1, THRB* and *PBX1* formed a compound linking oestrogen to haematopoietic and MK-erythroid commitment. We mapped TFs *GATA1*, *TAL1* and *GFI1B* to their source immune cell lineage according to the order of expression of haematopoietic transcriptional networks[11] and identified a strong gene expression signature in MK expansion (figure 2F). The upregulated expression of *MEIS1, PBX1*, *GATA1, TAL1* and *GFI1B* in ADs was validated by real-time quantitative PCR (online supplemental figure S7A).

We also observed downregulated TFs, including *EGR1*, *EGR2*, *EGR3* and *CEBPE*. Egr2 and Egr3 have long been regarded as negative regulators of T-cell activation.[12] *CEBPE* is expressed in a stage-specific manner during myeloid differentiation and is an essential TF for granulocytic differentiation.[13] Therefore, the TF network highlighted MK expansion responding to the gene expression signatures.

### Immune cell composition further supported the MK expansion

To dissect the MK in the PBMC, reasoning that the gene signature of MK was enriched in the bulk RNA-seq data, we integrated three central algorithms of deconvolution: xCell, CIBERSORT and ABIS (figure 2G and online supplemental figure S7B). The xCell results demonstrated that MKs and erythrocytes were positively enriched ($p<0.001$), while neutrophils, eosinophils and basophils were negatively enriched in ADs versus healthy individuals ($p<0.001$). Consistent with the results of xCell, the ABIS results identified decreased absolute deconvolution values of low-density neutrophil and basophil in ADs ($p<0.001$). Furthermore, the CIBERSORT results confirmed decreased neutrophils in ADs ($p<0.001$, figure 2G). We identified well-known MK marker genes, including *PPBP*, *PF4*, *GNG11* and *GP9* (*CD42b*), which were upregulated in ADs (online supplemental figure S7C). Thus, in accordance with gene expression signatures and TF regulation networks, the composition of immune cells demonstrated MK expansion in PBMC from ADs. To validate cellular composition, we analysed the MKs by flow cytometry. The percentage of $CD41b^+CD42b^+$ and $CD41b^+CD61^+$ MKs was significantly elevated in ADs compared with healthy controls (figure 2H and online supplemental figure S8A). Thus,

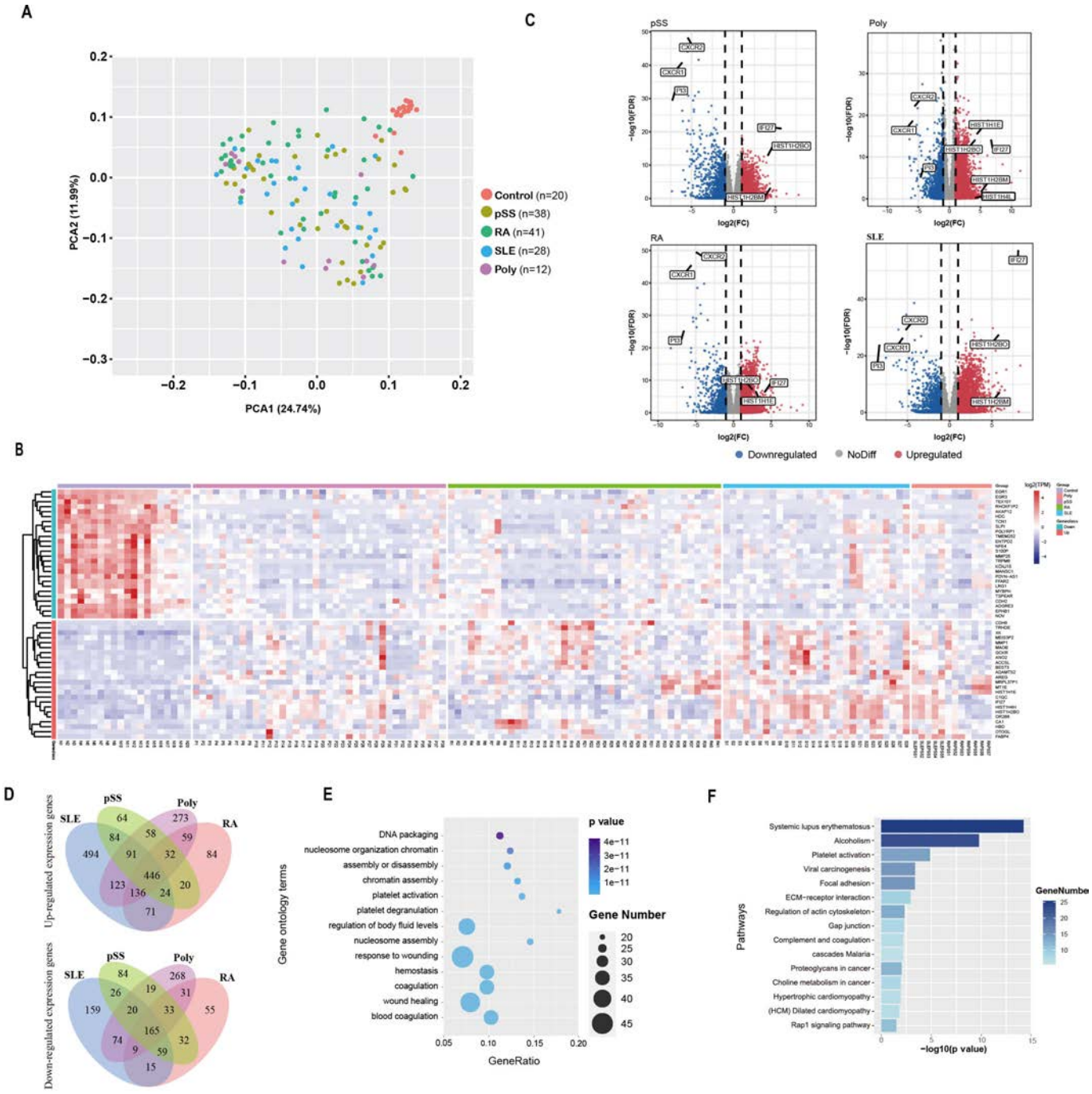


**Figure 1** Shared transcriptional profiling and platelet activation across RA, SLE and pSS. (A) Principal component analysis (PCA) of gene expression profiles for PBMCs from RA, SLE, pSS and polyautoimmunity, indicating absence of a clear differentiation among these ADs. Each point is assigned a location to illustrate potential clusters of neighbouring samples, which contain similar gene expression patterns. (B) Heatmap illustrating the top differentially expressed genes (DEGs) across RA, SLE, pSS and polyautoimmunity. (C) Volcano plots showing DEGs across RA, SLE, pSS and polyautoimmunity, in which some representative genes were highlighted. (D) Venn diagram showing 446 upregulated (top panel) and 165 downregulated genes (bottom panel) in common across RA, SLE, pSS and polyautoimmunity. (E) Gene ontology term enrichment of biological processes for common 446 upregulated genes showing nucleosome assembly and platelet degranulation. (F) Kyoto Encyclopedia of Genes and Genomes pathway enrichment highlighted 'systemic lupus erythematosus' and platelet activation pathways. Controls denote healthy controls. ADs, autoimmune diseases; FC, fold change; poly, polyautoimmunity; PBMCs, peripheral blood mononuclear cells; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

these findings were consistent with our original gene expression signatures indicating the MK expansion.

### MK and cellular heterogeneity identified by scRNA-seq

To map the MKs in the immune cell population of PBMC, we initially combined 57 486 individual cells from pSS (n=3), SLE (n=3, datasets obtained from Mistry *et al* 2019; GSE139360),[14] patients with RA (n=1) and a healthy control (n=1). MKs were identified by type-specific markers of *PPBP*, *PF4* and *PTCRA* (figure 3A–C) and TFs, *PBX1*, *MEIS1*, *GATA1* and *TAL1* (online supplemental figure S8B-E and table S3). GO analysis of MKs further indicated enrichment of upregulated genes related to

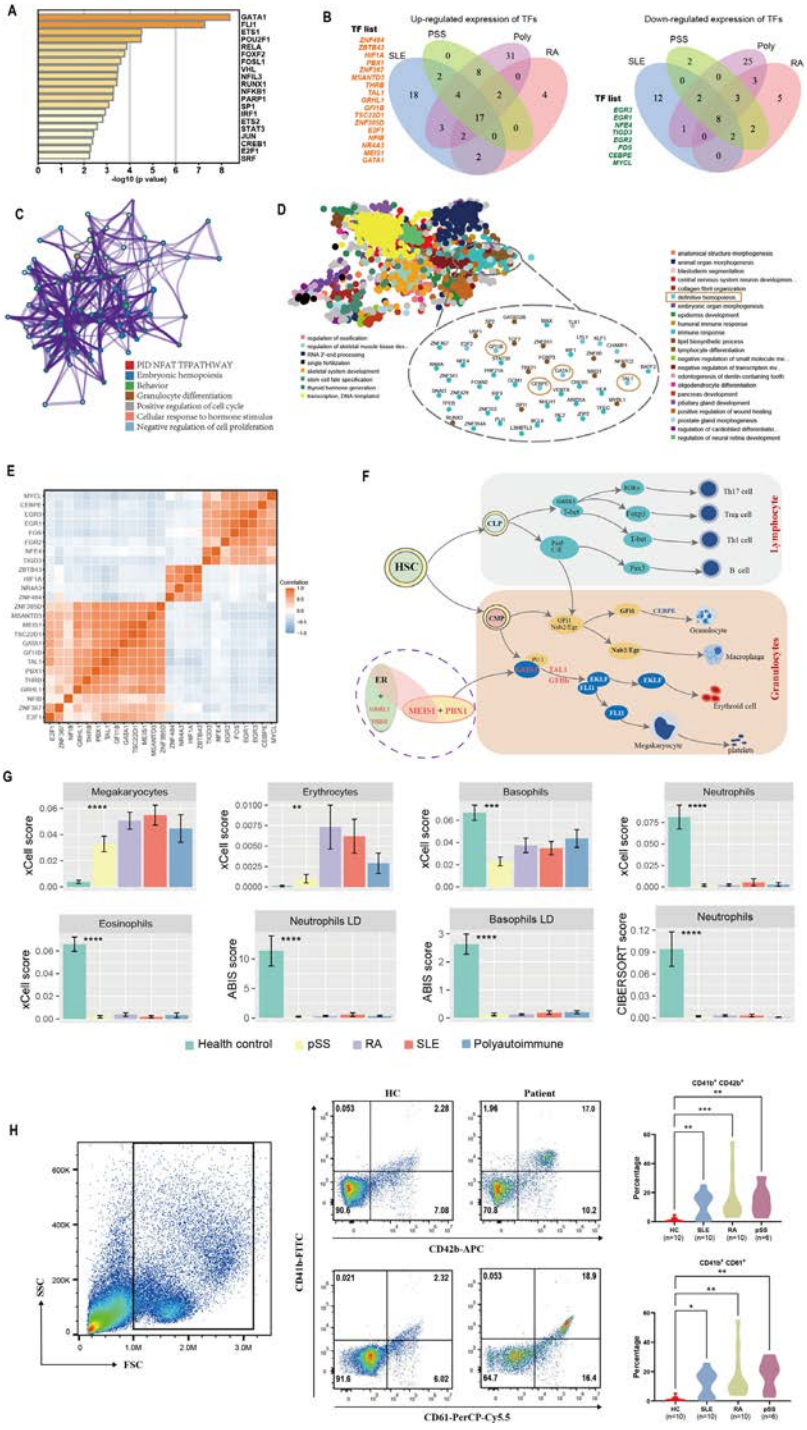


**Figure 2** Core transcription factors presented the megakaryocyte (MK) expansion. (A) Enrichment of regulator by Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TRRUST) showing *GATA1* as the top-ranking TF. (B) Venn diagram showing 25 common TFs across RA, SLE, pSS and polyautoimmunity. Left panel, upregulated expression of TFs, right panel, downregulated expression of TFs. (C) Gene ontology term for 25 common TFs significantly enriched in biological process of haematopoiesis. (D) TF enrichment had been performed by ChIP-X Enrichment Analysis 3 (ChEA3), which offers associations among involved TFs. TFs that are covered by the ChEA3 database, including *GATA1*, *TAL1*, *GFI1B* and *CEBPE*, are significantly related to definitive haematopoiesis. (E) TF correlation heatmap generated by the upregulated coexpression of TFs. Red colour indicates correlation. (F) TFs defining, showing MK expansion. Oestrogen interacted with *MEIS1*, *THRB* and *GRHL1* and *MEIS1* and *PBX1* act upstream of *GATA1* to regulate primitive haematopoiesis with *TAL1* and *GFI1B* to determine MK lineage. TF in red means upregulated expression, while in blue means downregulated expression. (G) Immune cell composition generated by xCell-inferred, ABIS-inferred and CIBERSORT-inferred enrichment score of cell types across ADs and healthy controls. *p<0.05; **p<0.01; ***p<0.001 by Kruskal-Wallis test. (H) Flow cytometry and its quantification of MKs from PBMC. Representative fluorescence-activated cell sorting plots for the identification of MKs. After gating for MKs by forward versus side scatter (FSC vs SSC), MKs were characterised as $CD41b^{+}CD42b^{+}$ and $CD41b^{+}CD61^{+}$. ***p<0.001 by Mann-Whitney U test. ADs, autoimmune diseases; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; ER, oestrogen receptor; HC, healthy controls; HSCs, haematopoietic stem cells; LD, low-density; PBMC, peripheral blood mononuclear cell; poly: polyautoimmunity; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TF, transcription factor.

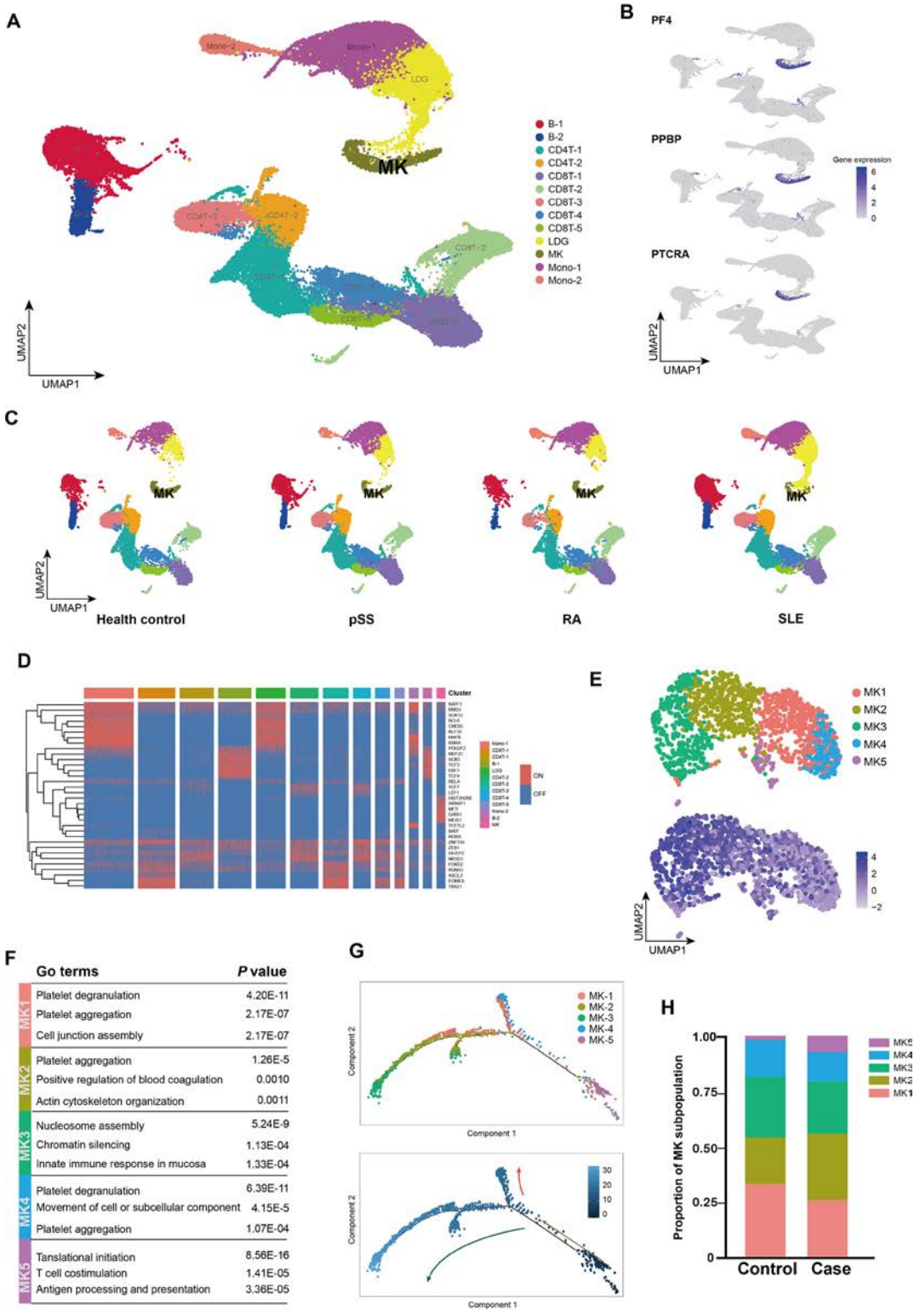


**Figure 3** Cell type confirmed the megakaryocyte (MK) and subset with high expression of *PTCRA*. (A) Uniform manifold approximation and projection (UMAP) embedding of the entire dataset coloured by orthogonally generated clusters labelled by cell type annotation. Thirteen putative cell clusters were identified from all profiled samples (n=57 486 cells). (B) The expression of cell-lineage marker genes for MK, including *PF4*, *PPBP* and *PTCRA*. (C) UMAP embedding split by ADs and healthy control highlighted the MK cluster. (D) Heatmap showing the activity of top five cell type-specific transcription factors (rows) in each cell type (columns) as identified by single-cell regulatory network inference and clustering. Term of ON indicates activity exceeds a regulon-specific area under the curve threshold. ON, active; OFF, inactive. (E) Five putative MK subpopulations were identified and subpopulations with high expression of *PTCRA* were highlighted (bottom). (F) The representative terms of gene ontology (GO) (biological processes) term enriched in each MK subpopulation. (G) Monocle pseudotime trajectory analysis of MKs, indicating two developmental directions. MK subpopulations along the branching trajectories (top). Inferred pseudotime for each cell is shown (bottom). (H) Stacked bar charts showing the percentage of each MK subpopulation among total MKs in ADs and healthy control. ADs, autoimmune diseases; B, B cell; CD4T, $CD4^+$ T cell; CD8T, $CD8^+$ T cell; mono, monocyte; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

platelet degranulation and aggregation (online supplemental figure S8F). We constructed a gene regulatory network among the TFs predicted by single-cell regulatory network inference and clustering and revealed that *MEIS1* and *GATA1* were active in the MK compared with other immune cell types (figure 3D and online supplemental figure S8G). GO enrichment in biological processes of DEGs of MKs in ADs compared with healthy controls revealed that upregulated genes were associated with translation and translational initiation, while downregulated genes were associated with platelet degranulation and aggregation (online supplemental figure S9A).

To dissect MK heterogeneity, five putative subpopulations (MK1–MK5) of MKs were identified by subclustering (figure 3E–F and online supplemental table S4). We then characterised the gene sets enriched in these MK subpopulations. MK1, MK2 and MK4 mainly showed enrichment of GO terms related to platelet degranulation and aggregation, whereas MK3 exhibited enriched GO terms affecting nucleosome assembly. MK5 highly expressed genes were associated with translational initiation and antigen processing and presentation. The GO term 'translational initiation' was perhaps indicative of a less mature MK population.[15] Cellular trajectory analysis revealed distinct differentiation trajectories underpinning MK heterogeneity with a major bifurcation and highlighted MK5 at the origin of the trajectory (figure 3G and online supplemental figure S9B-E). Furthermore, the proportion of the MK5 subpopulation was increased in ADs, compared with healthy control (figure 3H). Thus, the subpopulation proportion of MKs coincided with the GO terms of DEGs in ADs.

Given the immune characteristics of MKs, we characterised cell communication by ligand–receptor interactions between MK and other immune cell subsets. We identified ligand–receptor interactions between MKs and $CD4^+$ and $CD8^+$ T cells such as a PF4–CXCR3 pair (online supplemental figure S9F). We next focused on the granulocytes; low-density granulocytes were identified by genes highly specific for neutrophils, including *FCGR3B* and *CMTM2*. Biological processes of GO enrichment suggested that upregulated genes in ADs were associated with viral transcription and cellular response to type I interferon (online supplemental figure S10). We also used peripheral TCR repertoire sequencing to find clonotype in RA and healthy control. TCR gene rearrangement and variable gene usage are presented in online supplemental figure S11.

## DISCUSSION

Using transcriptomic profiling, we demonstrated common gene expression signatures relating to haemostasis via the regulation of transcriptomes by the TF network, *PBX1/GATA1/TAL1/GFI1B*. This provides novel evidence of MKs expansion in PBMC in treatment-naive RA, SLE and pSS. It is in this context that we examined patients with polyautoimmunity, seeking to elucidate gene expression signatures and TFs common across ADs; through these patients we feel we have supported such an AD-associated pathway.

In this study, bulk RNA-seq was used to find the gene expression signature, TFs and composition of immune cells, while the scRNA-seq was used to identify the cell type of interest in the PBMC. Bulk RNA-seq TF and composition studies confirmed the presence of MKs expansion in these ADs. scRNA-seq data further defined the MK subpopulations. We observed similar transcriptional profiles linking RA, SLE, pSS and polyautoimmunity and noted across-disease upregulated expression of the type I IFN-stimulated gene *IFI27*, as well as downregulated expression of chemokine receptors, *CXCR1* and *CXCR2*. These genes play significant roles in the suppression of megakaryopoiesis.[16] Importantly, the gene expression signatures enriched in haemostasis might explain common immunological characteristics via regulation with transcriptomic reprogramming.

It is noteworthy that dysregulated transcriptomic reprogramming might introduce disturbances in immune homeostasis leading to ADs.[17] Transcriptomic data of bone marrow (BM)-derived haematopoietic stem and progenitor cells from SLE mice showed myeloid skewing, with granulocytic differentiation arrest and a positive correlation with platelet degranulation that indicated expansion of stem cell-like MK-committed cells.[18] In accordance with BM observations, we further detected upregulated expression of MK-lineage TFs *PBX1, GATA1*, *TAL1* and *GFI1B* and downregulated expression of granulocytic-lineage TF *CEBPE*.

Furthermore, via deconvolution of bulk RNA-seq data, we identified increased MKs, accompanied by decreased neutrophils, eosinophils and basophils in these ADs. Thus, we have expanded the previously documented MK expansion in the BM to peripheral blood in ADs. Conventional antigen-presenting cells (APCs) are essential for AD progression, but it is unknown what initially primes autoimmune T cells. MKs express MHC I and II molecules, thus acting as professional APC that enhance Th17 and Th1/Th17 responds to lupus autoantigens.[19 20] We reasoned, preliminary, that elevated levels of MKs enhance their intrinsic antigen presenting function in peripheral blood across RA, SLE and pSS.

In scRNA-seq, we identified MK across patients with pSS, SLE and RA. MK expansion had been previously observed[21 22] and was a critical peripheral source of cytokine storms in COVID-19.[23] We described MKs with highly expressed *PTCRA*, encoding the pre-TCRα chain (pTα). Normally, pTα along with TCRβ and CD3 form the pre-TCR, which are exclusively expressed in immature thymocytes during early T-cell development.[24] *PTCRA* (pTα) is also required for TCR rearrangement for extrathymic T-cell development.[25] Cell surface $PTCRA^+$ MKs had been identified in early human embryonic yolk sacs.[15] Furthermore, a less mature immune MK subpopulation had been found to be enriched in ADs. This subpopulation of MKs presenting immune characteristics with antigen processing and presentation had been previously demonstrated in yolk sac and fetal liver cells.[15] Therefore, we speculated that MKs act as specific endogenous APC, resulting in abnormal TCR arrangements which, in turn, trigger the initial autoimmune T cell for AD pathogenesis.

We also speculate that there might be a connection between sex hormones and megakaryocytopoieses. A predominant role of sex hormones has been suggested as the main cause of sex-biased ADs.[26] Oestrogen stimulates HSC self-renewal, megakaryocytopoiesis and erythropoiesis in females.[7 27] Megakaryopoiesis is dynamic and adaptive to biological needs, termed as 'emergency haematopoiesis' that biases toward the MK lineage.[28] *MEIS1* interacts with ER[8] and *PBX* acts upstream of *GATA1* to regulate primitive haematopoiesis.[9] Oestrogen promotes MK polyploidisation via ERβ-mediated transcription of *GATA1*.[29] Therefore, an upregulated TF network *MEIS1/PBX1/GATA1/TAL1/GFI1B* might connect estrogens and MK expansion in RA, SLE and pSS.

To summarise, we have presented evidence for peripheral MK expansion across RA, SLE and pSS. Our discovery provides clues that MK expansion might initially prime autoimmune T cells in the pathogenesis of these ADs.

**Correction notice** This article has been corrected since it published Online First. Figure 3 has been amended.

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**Contributors** GZ and YW designed the study. YW, XX, CZ, MS, JW, SG and CL performed experiments and analysed data. GZ, DEF and MM-C wrote the manuscript. JL and QL helped with sample collection.

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**Data availability statement** All sequencing data have been deposited in public, open access repository of the Genome Sequence Archive for Human (http://bigd.big.ac.cn/gsa-human/) with the accession ID. HRA000911 and HRA000916. Data are available in a public, open accessrepository. Data are available upon reasonable request.



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TRANSLATIONAL SCIENCE

# A unique circulating miRNA profile highlights thrombo-inflammation in Behçet's syndrome

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## ABSTRACT

**Objectives** Behçet's syndrome (BS) is a rare systemic vasculitis often complicated by thrombotic events. Given the lack of validated biomarkers, BS diagnosis relies on clinical criteria.
In search of novel biomarkers for BS diagnosis, we determined the profile of plasmatic circulating microRNAs (ci-miRNAs) in patients with BS compared with healthy controls (HCs).

**Methods** ci-miRNA profile was evaluated by microarray in a screening cohort (16 patients with BS and 18 HCs) and then validated by poly(T) adaptor PCR (PTA-PCR) in a validation cohort (30 patients with BS and 30 HCs). Two disease control groups (30 patients with systemic lupus erythematosus (SLE) and 30 patients with giant cell arteritis (GCA) were also analysed.

**Results** From the microarray screening, 29 deregulated (differentially expressed (DE)) human ci-miRNAs emerged. A hierarchical cluster analysis indicated that DE ci-miRNAs clearly segregated patients from controls, independently of clinical features. PTA-PCR analysis on the validation cohort confirmed the deregulation of miR-224-5p, miR-206 and miR-653-5p. The combined receiver operating characteristic (ROC) curve analyses showed that such ci-miRNAs discriminate BS from HCs (and BS with active vs inactive disease), as well as BS from patients with SLE and GCA.
The functional annotation analyses (FAAs) showed that the most enriched pathways affected by DE ci-miRNAs (ie, cell–matrix interaction, oxidative stress and blood coagulation) are related to thrombo-inflammatory mechanisms. Accordingly, the expression of the three ci-miRNAs from the validation cohort significantly correlated with leucocyte reactive oxygen species production and plasma lipid peroxidation.

**Conclusions** The ci-miRNA profile identified in this study may represent a novel, poorly invasive BS biomarker, while suggesting an epigenetic control of BS-related thrombo-inflammation.

## Key messages

**What is already known about this subject?**
- The pathogenesis of Behçet's syndrome (BS) is still unclear and no unique feature or specific laboratory test is available in the clinical setting.
- Circulating miRNAs (ci-miRNAs) play a role in both vascular and immune-mediated diseases, acting both as potential candidate biomarkers and pathogenic pathway clues.
- ci-miRNAs may represent valuable poorly invasive biomarkers in vasculitides such as BS.

**What does this study add?**
- Thanks to a microarray screening and subsequent results validation by RT-qPCR, we identified a unique ci-miRNA profile which segregates patients with BS from healthy subjects, as well as BS from other disease control groups.
- The identified ci-miRNA profile potentially controls pathways related to thrombo-inflammation.

**How might this impact on clinical practice or future developments?**
- The obtained ci-miRNA profile could be exploited as a novel, poorly invasive, candidate biomarker and could allow the design of novel therapeutic strategies in BS.

## INTRODUCTION

Behçet's syndrome (BS) is chronic systemic vasculitis of unknown aetiology and unique geographical distribution.[1] BS is a rare disease, with higher prevalence limited to countries across the ancient Silk Route. Given the lack of validated biomarkers, disease definition is based on clinical criteria, which leads to difficult and often delayed diagnosis, especially in areas with low BS prevalence.[1] BS pathophysiology is poorly understood, but both genetic predisposition and infectious triggers seem to have a causative role in the derangement of adaptive and innate immune responses.[2]

Besides mucocutaneous and ocular involvement, vascular events represent one of the major BS manifestations, with venous thrombosis, aneurysms and arterial occlusions being the main factors accounting for BS-associated morbidity and mortality.[3 4] Consistently, BS represents a model of inflammation-induced thrombosis. The inflammatory nature of the vascular BS manifestations is suggested both by the pathogenetic mechanisms identified so far (eg, oxidative modifications of coagulation proteins, the formation of neutrophil extracellular traps, endothelial dysfunction and enhanced adhesiveness)[5–9] and by clinical studies supporting the use of immunosuppressants rather than anticoagulants



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for the management of thrombotic events.[3 4 8 10] The hypercoagulability state which characterises BS seems to be associated with inflammation-driven alterations of the thrombotic balance. The hyperactivation and perivascular infiltration of neutrophils with the ensuing production of reactive oxygen species (ROS) apparently links immune response deregulation with thrombotic events.[5 7] However, the exact cellular and molecular mechanisms underlying BS-associated thrombotic diathesis still lack clarity.

miRNAs are a class of small non-coding RNAs that act as post-transcriptional regulators of gene expression by base-pairing to specific sites of target mRNAs, causing their degradation or translational inhibition. Thirty per cent of all human genes are under epigenetic modulation by miRNAs, through the control of the expression of multiple mRNA targets. Therefore, miRNAs act as pathogenetic pathway clues, reflecting pathophysiological processes in several human diseases.[11] Some miRNAs are actively secreted into extracellular spaces (circulating miRNAs, ci-miRNAs), either through microvesicles or as free non-encapsulated RNAs, and may hence behave as cell-to-cell molecular communication devices, under both physiological and pathological conditions, by specific cell uptake.[12]

Plasma is the main repository of extracellular miRNAs, originating from endothelial or blood cells.[13] Extracellular ci-miRNAs display several properties that make them appealing candidates as biomarkers for various human diseases,[14–16] including vascular and immune-mediated disorders.[17–24] To date, some miRNA-based diagnostic products are in clinical development for a wide range of diseases,[25–27] but no miRNA-based diagnostic has reached the market, yet.

The aim of this study was to determine whether a peculiar ci-miRNA profile is specifically associated with BS, hence representing a novel biomarker candidate, with potentially relevant implications in BS pathophysiology.

## MATERIALS AND METHODS

### Study design and population

A prospective derivation and validation study was performed at the Behçet Center of the Careggi University Hospital (Florence, Italy). Two independent cohorts of adult patients with BS fulfilling the International Criteria for Behçet Disease (ICBD) were included.[28] Of them, one cohort was used as screening setting and one as validation setting. Both cohorts were matched by age and sex with a healthy control (HC) cohort. Demographic, clinical and therapeutic data related to the medical history and to the time of enrolment were collected. Subjects with other autoimmune, neoplastic or active infectious diseases were excluded. Disease activity was evaluated by Behçet's Disease Activity Form (BDCAF). Two disease control groups were also included in the study: systemic lupus erythematosus (SLE) and giant cell arteritis (GCA). SLE was chosen as the prototype of systemic autommune diseases, and because affected patients may show some clinical manifestations similar to BS. On the other hand, GCA along with BS represents a systemic vasculitis with prevalent medium and large vessels involvement.

Sample size was determined by power analysis

The experimental workflow is described in figure 1. For the screening phase, plasma samples were subjected to microarray analysis, whereas the expression levels of the six most deregulated miRNAs emerging from microarray data were quantified by real-time PCR in the validation phase.

Hierarchical cluster analysis and functional annotation analysis (FAA) were applied to microarray data to assess the ability to segregate patients with BS from HCs, and the biological meaning of differentially expressed (DE) ci-miRNAs. PCR data from the validation cohort were used to perform ROC curve analysis to assess DE ci-miRNAs' discriminatory power. Moreover, functional analysis (both bioinformatic, by FAA, and experimental, determining oxidative stress in circulating leucocytes and plasma lipid peroxidation) was performed to evaluate the potential impact of the selected ci-miRNAs on BS pathogenetic mechanisms (figure 1).

### Plasma preparation and RNA extraction

Eight millilitres of peripheral blood was collected from each subject (BS and HC) in K2-EDTA anticoagulant by standard venipuncture. Platelet-free plasma (PFP) was obtained from peripheral blood samples by a double centrifugation protocol (1500 g for 15 min at room temperature followed by supernatant centrifugation at 13 000×g for 3 min). Total RNA was extracted from 250 µL fresh plasma aliquots, using TRIzol LS

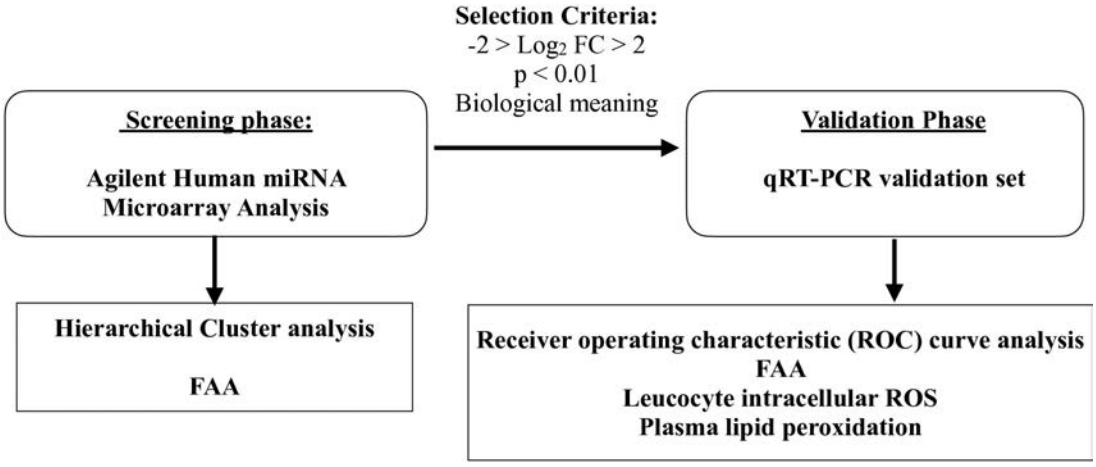


**Figure 1** Flow chart of the study design. The study was composed of two different sequential phases: a screening phase followed by a validation phase (indicated by the rounded boxes). In the first phase, miRNA microarray technology was applied to an initial study cohort and top differentially expressed (DE) circulating microRNAs (ci-miRNAs) were then selected for further poly(T) adaptor PCR (PTA-PCR) validation in a larger and cohort according to the following criteria: $-2>\log_2 FC>2$, $p<0.01$ (Limma differential expression t-test) and biological meaning (see online supplemental methods). The analyses performed on the results deriving from each phase are indicated in the square boxes. FAA, functional annotation analysis; FC, normalised expression fold change values in log2 scale; ROC, receiver operating characteristic; ROS, reactive oxygen species.

reagent (Invitrogen, Carlsbad, California, USA) following the manufacturer's protocol. Only RNA samples showing acceptable quality and concentration values were included (see online supplemental methods).

### Archival plasma collection

For disease control groups, available archival PFP plasma samples were collected at the Lupus and Vasculitis Unit of the University of Florence. RNA extraction was performed using the same protocol described for fresh plasma samples.

Comparability between archival and fresh plasma samples was checked by comparing PCR amplification results (online supplemental figure 1).

### miRNA microarray

ci-miRNA profiling was performed using Agilent Human miRNA 8×15 k Microarray kit v3.0 and miRNA Complete Labelling and Hyb Kit (Agilent Technologies, Santa Clara, California, USA) following manufacturer's protocol. Microarray data analysis is detailed in the online supplemental methods.

### Real-time quantitative PCR (PTA-PCR)

Selected ci-miRNA expression was validated by PTA-PCR using mature miRNA-specific primers and the stringent thermal protocol[29] (see the online supplemental methods, table 1 and figure 2).

### Functional annotation analysis

Predicted miRNA targets FAA was performed on the comprehensive (ie, the 29 DE human ci-miRNAs, see the Results section) ci-miRNA profile using the online tool DIANA-miRPath (see the online supplemental methods).[30]

### Assessment of leucocyte ROS production and plasma lipid peroxidation

Lymphocyte, monocyte and neutrophil ROS production, as well as plasma lipid peroxidation, were measured as described in Becatti *et al*.[31]

### Statistics

Categorical variables are presented with counts and proportions, while continuous ones as the mean±SE of the mean (SEM) or median with IQR. Statistical analysis was performed using GraphPad Prism V.6.0 (GraphPad Software, San Diego, California, USA). Differential expression analysis of Agilent microRNA array data was performed using the AgiMicroRna Bioconductor library (see the online supplemental methods). ROC curve analysis was performed using Matlab built-in function perfcurve V.2019a. All statistical tests were two tailed with a significance level of 0.05. Power analysis was performed using the software STATA (StataCorp V.14).

## RESULTS

### Patients' characteristics

The study included a screening cohort of 34 subjects (16 BS and 18 HC) and a validation cohort of 60 subjects (30 BS and 30 HC). Clinical and demographic characteristic of both cohorts are reported in table 1. In both cohorts, the two sexes were equally distributed. Notably, 6 out of 16 (37.5%) and 14 out of

**Table 1** Main demographics and clinical features of patients with Behçet's syndrome and healthy controls included in the screening and validation cohorts

| | Screening cohort | | Validation cohort | |
|---|---|---|---|---|
| | BS (n, % out of 16) | HC (n, % out of 18) | BS (n, % out of 30) | HC (n, % out of 30) |
| Female sex | 7 (43.8%) | 8 (44.4%) | 17 (56.7%) | 17 (56.7%) |
| Age at enrolment, years | 42.5 (36–44.5) | 43 (36–45) | 43.5 (37–51) | 44 (36.5–52) |
| **Overall disease manifestations** | | | | |
| Oral ulcers | 16 (100%) | – | 29 (96.6%) | – |
| Genital ulcers | 9 (56.3%) | – | 19 (63.3%) | – |
| Cutaneous | 11 (68.8%) | – | 21 (70%) | – |
| Articular | 6 (37.5%) | – | 16 (53.3%) | – |
| Ocular | 10 (62.5%) | – | 13 (43.3%) | – |
| Vascular | 6 (37.5%) | – | 14 (46.6%) | – |
| Gastrointestinal | 6 (37.5%) | – | 14 (46.6%) | – |
| Neurological | 7 (48.3 %) | – | 8 (26.6%) | – |
| **Disease manifestations at enrolment** | | | | |
| Oral ulcers | 4 (25.0%) | – | 6 (20.0%) | – |
| Genital ulcers | 2 (12.5%) | – | 3 (10.0%) | – |
| Cutaneous | 2 (12.5%) | – | 4 (13.3%) | – |
| Articular | 2 (12.5%) | – | 1 (3.3%) | – |
| Ocular | 1 (6.3%) | – | 1 (3.3%) | – |
| Vascular | 1 (6.3%) | – | 1 (3.3%) | – |
| Gastrointestinal | 1 (6.3%) | – | 1 (3.3%) | – |
| Neurological | 1 (6.3%) | – | 0 | – |
| **Active disease at enrolment** | 8 (50.0%) | – | 12 (40.0%) | – |
| **Immunomodulating therapy at enrolment** | 13 (81.3%) | – | 29 (96.7%) | – |

Data are presented as mean±SD, median with IQR or number (n) and relative percentage when applicable. No statistically significant differences were found between groups when analysing mean age and sex ratio evaluated by Student's t-test and $\chi^2$ test, respectively.
BDCAF, Behçet's Disease Activity Form; BS, Behçet's syndrome; HC, healthy controls; ICBD, International Criteria for Behçet's Disease.

30 (46.6%) patients had history of vascular events following BS onset, in the screening and validation cohorts, respectively.

In the screening cohort, 50% of patients had active BS at time of enrolment, defined as a BDCAF score ≥1, and 13 out of 16 (81.3%) were on active immunomodulating therapy, either for active manifestations or for remission maintenance. In the validation cohort, 40% of patients had active BS at enrolment, and 29 out of 30 (96.7%) patients were receiving immunomodulators.

Disease control groups comprised 30 patients with SLE (mean age at enrolment 47.6±2.36, 29 females) and 30 patients with GCA (mean age 71.8±2.18, 21 females). The two groups were representative of the overall SLE and GCA populations in terms of demographic characteristics.

### The DE ci-miRNA microarray profile segregates patients with BS from HC

RNA samples included in the screening phase were subjected to miRNA profiling using dedicated Agilent technology. Complete microarray data are available at GEO (accession number GSE145191) and included in Bagni *et al*.[32] Statistical analysis of microarray data revealed the presence of 36 DE ($p<0.05$; $-1>\log_2 FC>1$) ci-miRNAs between patients with BS and HC (see table 2).

The identified profile mainly comprised human sequences (29 out of 36, indicated by the 'hsa' prefix), only seven mi-RNAs being of viral origin. Considering only human sequences, 16 out of the 29 DE ci-miRNAs were upregulated and 13 downregulated. Unsupervised hierarchical cluster analysis performed on the DE ci-miRNAs showed that 94% of samples co-segregate according to their different clinical status (BS vs HC), with the presence or absence of the disease causing the variation itself (figure 2). No significant association of the pattern of DE ci-miRNAs with specific clinical or demographic features (including disease activity state) emerged from microarray analysis.

To strengthen microarray results, the differential expression of 6 ci-miRNAs (selected on the basis of most relevant fold

**Table 2** DE ci-miRNAs identified by microarray analysis in the screening phase

| | miRNA ID | MIMATID | FC | P value | |
|---|---|---|---|---|---|
| UP | ***hsa-miR-653-5p*** | MIMAT0003328 | 2.4544 | 0.0005 | Human |
| | ***hsa-miR-224-5p*** | MIMAT0000281 | 2.0148 | 0.0027 | |
| | ***hsa-miR-206*** | MIMAT0000462 | 2.0056 | 0.0037 | |
| | **hsa-miR-558** | MIMAT0003222 | 1.9013 | 0.0072 | |
| | **hsa-miR-573** | MIMAT0003238 | 1.8562 | 0.0222 | |
| | **hsa-miR-593** | MIMAT0003261 | 1.7143 | 0.0433 | |
| | **hsa-miR-425-3p** | MIMAT0001343 | 1.6772 | 0.0133 | |
| | **hsa-miR-189** | MIMAT0000079 | 1.5837 | 0.0144 | |
| | **hsa-miR-525*** | MIMAT0002839 | 1.4999 | 0.0152 | |
| | **hsa-miR-200a** | MIMAT0000682 | 1.4419 | 0.0055 | |
| | **hsa-miR-601** | MIMAT0003269 | 1.4341 | 0.0100 | |
| | **hsa-miR-100** | MIMAT0000098 | 1.4236 | 0.0054 | |
| | **hsa-miR-608** | MIMAT0003276 | 1.4009 | 0.0245 | |
| | **hsa-miR-569** | MIMAT0003234 | 1.3756 | 0.0399 | |
| | **hsa-miR-376a** | MIMAT0000729 | 1.1229 | 0.0166 | |
| | **hsa-miR-627** | MIMAT0003296 | 1.0759 | 0.0329 | |
| DOWN | **hsa-miR-302b** | MIMAT0000715 | −1.1783 | 0.0449 | |
| | **hsa-miR-98** | MIMAT0000096 | −1.2594 | 0.0329 | |
| | **hsa-miR-520e** | MIMAT0002825 | −1.4332 | 0.0287 | |
| | **hsa-miR-340** | MIMAT0004692 | −1.6206 | 0.0363 | |
| | **hsa-miR-566** | MIMAT0003230 | −1.6358 | 0.0155 | |
| | **hsa-miR-423** | MIMAT0001340 | −1.7271 | 0.0330 | |
| | **hsa-miR-519e*** | MIMAT0002828 | −1.8331 | 0.0130 | |
| | **hsa-miR-432** | MIMAT0002814 | −1.8483 | 0.0144 | |
| | **hsa-miR-31** | MIMAT0000089 | −1.9111 | 0.0111 | |
| | ***hsa-miR-411-5p*** | MIMAT0003329 | −2.1903 | 0.0013 | |
| | ***hsa-miR-187-3p*** | MIMAT0000262 | −2.1927 | 0.0037 | |
| | ***hsa-miR-27a-3p*** | MIMAT0000084 | −2.2675 | 0.0034 | |
| | **hsa-miR-600** | MIMAT0003268 | −2.3197 | 0.0033 | |
| UP | ebv-miR-BHRF1-2* | MIMAT0000996 | 1.4385 | 0.0229 | Viral |
| | ebv-miR-BART1-5p | MIMAT0000999 | 1.3104 | 0.0217 | |
| | ebv-miR-BART14-3p | MIMAT0003426 | 1.1894 | 0.0376 | |
| DOWN | ebv-miR-BART6-3p | MIMAT0003415 | −1.6170 | 0.0069 | |
| | kshv-miR-K12-7 | MIMAT0002187 | −1.6632 | 0.0222 | |
| | kshv-miR-K12-9 | MIMAT0002185 | −1.8083 | 0.0187 | |
| | kshv-miR-K12-1 | MIMAT0002182 | −2.0371 | 0.0026 | |

miRNAs in bold italic were selected for the technical validation phase. miRNAs in bold were included in the FAA. Only human miRNAs were taken into account (indicated by 'hsa' prefix) considering that the observed presence of DE miRNA sequences originating from DNA viruses has been widely found in plasma samples (also from healthy subjects) but may only account for infection latency and reportedly failed to associate with active infectious state. P values were calculated by two-tailed Student's t-test.

BS, Behçet's syndrome; DE ci-miRNA, differentially expressed circulating microRNA; DOWN, downregulated miRNAs; n=34 (16 patients with BS vs 18 HCs); FAA, Funcional annotation analysis ; FC, normalised expression fold change values in log2 scale; HC, healthy control; MIMATID, unique mature miRNA accession number; p, Limma (linear models for microarray data) differential expression t-test p value; UP, upregulated miRNAs.

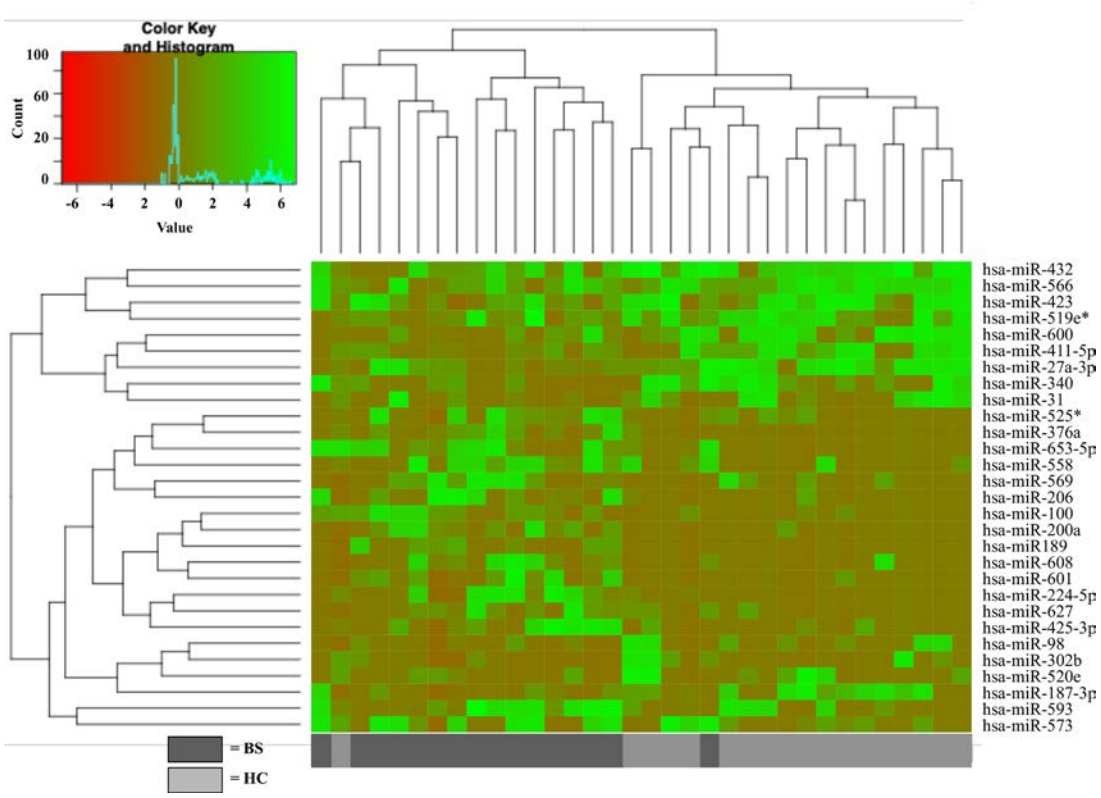


**Figure 2** Differentially expressed (DE) circulating microRNA (ci-miRNA) microarray profile hierarchical clustering analysis. Comprehensive DE ci-miRNA profile in patients with BS and HC: DE ci-miRNAs showed in the heatmap were selected according to the following statistical conditions: p<0.05 (Limma differential expression t-test), −1>log2 FC>1. Each heatmap column represents the expression profile of one sample (n=34, 16 patients ith BS vs 18 HC); green colour indicates high expression levels, while red colour indicates low expression levels. The heatmap dendrogram is representative of the unsupervised hierarchical cluster analysis. BS, Behçet's syndrome; HC, healthy control.

change values, p value and potential biological meaning, see online supplemental methods) emerging from microarray (hsa-miR-206, hsa-miR-224-5p, hsa-miR-653-5p, hsa-miR-187-3p, hsa-miR-411-5p and hsa-miR-27a-3p, highlighted in bold italic in table 2) was assessed by PTA-PCR, in a small sample subset (5 patients with BS vs 5 HCs, online supplemental table 2). The obtained PCR fold change values showed a significant correlation (Pearson's correlation coefficient r=0.828, two-tailed p=0.0418) with those from the microarray screening (online supplemental figures 3 and 4).

### The FAA of the DE ci-miRNAs indicates the involvement of pathways related to thrombo-inflammation

FAA was then performed on the 29 DE hsa ci-miRNAs, applying DIANA-miRPath analysis. A list of significantly (FDR-corrected p value <0.05) enriched pathways was identified. As expected, from both KEEG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis and Gene Ontology (GO), biological mechanisms related to the immune system emerged. In particular, KEEG terms potentially converging on T regulatory (Treg) cell function and development (eg, FOXO-related terms) (figure 3A, online supplemental figure 5),[33] and Toll-like receptors (TLR) signalling pathways GO:BP (Gene Ontology Biological Process) terms were also significantly enriched (figure 3B). However, the KEEG terms which displayed significant enrichment scores were 'focal adhesion', 'regulation of actin cytoskeleton', 'MAPK signalling pathway', 'ECM (extracellular matrix) receptor interaction' (figure 3A). In addition, the GO:BP term 'cellular nitrogen compound metabolic process' showed the highest enrichment score (figure 3B). Another significantly enriched GO:BP term was the 'cellular protein modification process' (figure 3B), which comprises mechanisms relative to protein oxidation and oxidative carbonylation. Finally, the 'blood coagulation' term also showed significant enrichment in the comprehensive DE human ci-miRNA profile analysis (figure 3B). Interestingly, part of the DE ci-miRNA targets included in the 'blood coagulation' GO:BP term overlapped with those involved in the most enriched 'ECM-receptor interaction' KEEG pathway. These include genes encoding for integrin alpha and beta subunits, thrombospondin, collagen type 1 alpha 2 chain, fibronectin, von Willebrand factor and platelet glycoprotein VI.

Overall, threemain processes comprise the most enriched terms emerging from the FAA analysis: cell–matrix interaction, oxidative stress and blood coagulation (figure 3C). Notably, such three processes can be considered together as affecting the thrombotic balance at the vessel level. Collectively, terms and pathways suggestive of thrombo-inflammatory mechanisms emerged from the FAA analysis of the DE ci-miRNAs.

### The combination of hsa-miR-224-5p, hsa-miR-206 and hsa-miR-653-5p shows diagnostic potential for BS

We then analysed plasma samples belonging to the validation cohort, applying the PTA-PCR protocol to determine the expression levels of the following ci-miRNAs: hsa-miR-27a-3p, hsa-miR-187-3p, hsa-miR-411-5p, hsa-miR-224-5p, hsa-miR-206 and hsa-miR-653-5p. Such miRNAs were previously used to assess the reliability of microarray data (online supplemental figures 3 and 4), and were here chosen since they were the most upregulated or downregulated human sequences (based on $-2>\text{Log}_2$ FC>12 with p<0.01) and had already been reported

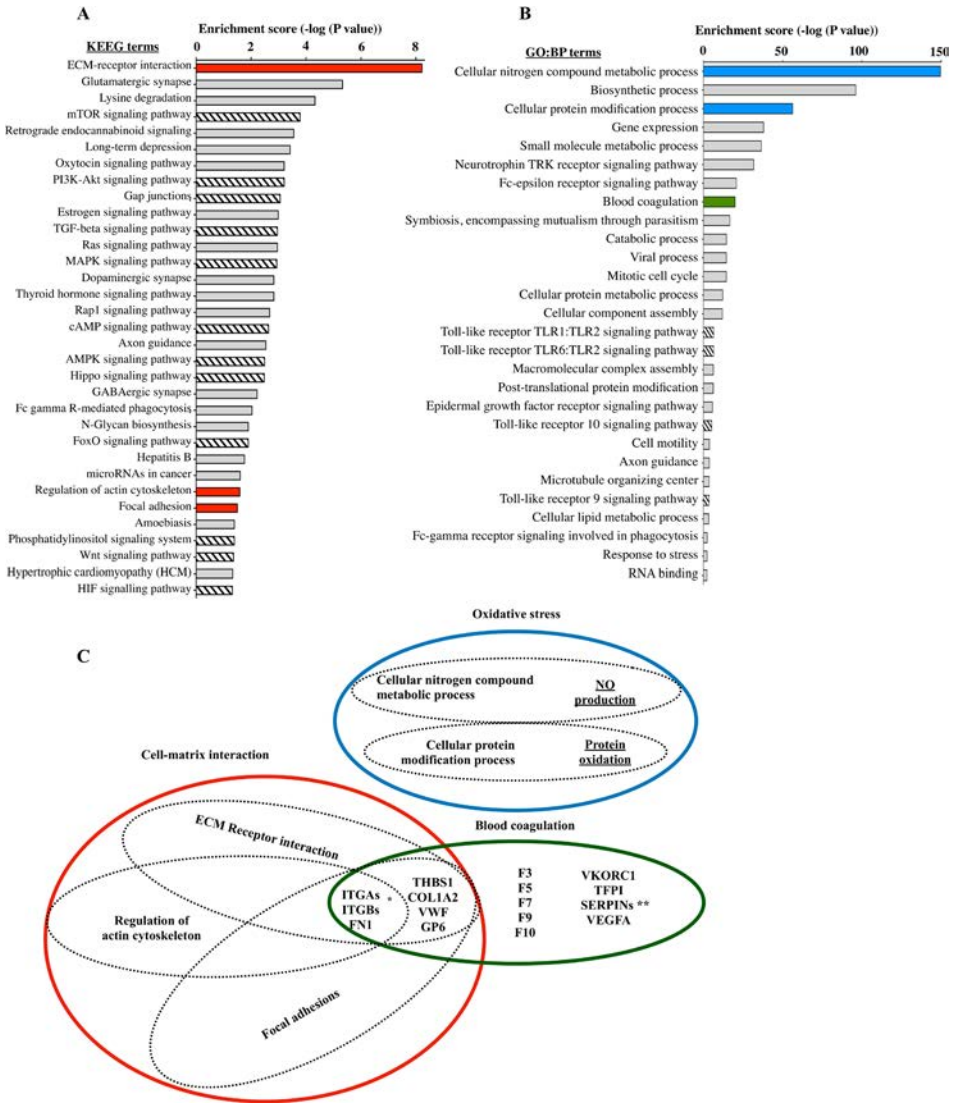


**Figure 3** KEEG (Kyoto Encyclopedia of Genes and Genomes) pathways and GO (Gene Ontology) enrichment analysis. (A) Top significantly enriched KEEG terms. Terms relative to cell–matrix interaction are shown in red, the ones linked to innate and adaptive immunity are in black and white pattern. Highlighted KEEG terms: 'ECM receptor interaction' (hsa04512) (p=0.008), 'mTOR signaling pathway' (hsa04150) (p=0.0005), 'PI3K-Akt signaling pathway' (hsa04151) (p=0.0006), 'gap junctions' (hsa04540) (p=0.0008), 'TGF-beta signaling pathway' (hsa04350) (p=3.80E-05), 'MAPK signaling pathway' (hsa04010) (p=0.0011), 'cAMP signaling pathway' (hsa04024) (p=0.0022), 'AMPK signaling system' (hsa04152) (p=3.95E-05), 'Hippo signaling pathway' (hsa04390) (p=0.0032), 'FoxO signaling pathway' (hsa04068) (p=1.24E-06), 'regulation of actin cytoskeleton' (hsa04810) (p=0.024), 'focal adhesion' (hsa04510) (p=0.00028), 'phosphatidylinositol signaling system' (hsa04070) (p=0.039), 'Wnt signaling pathway' (hsa04310) (p=0.0027), 'HIF signaling pathway' (hsa04066) (p=0.006). (B) Top significantly enriched GO BP terms. Bars corresponding to terms involved in oxidative stress are shown in blue, the ones related to blood coagulation are in green, while the ones related to innate and adaptive immunity are in black and white pattern. Highlighted GO:BP terms: 'cellular nitrogen compound metabolic process' (GO:0034641) (p=1.92E-150), 'cellular protein modification process' (GO:0006464) (p=2.25E-52), 'blood coagulation' (GO:0007596) (p=7.09E-31), 'toll-like receptor TLR1:TLR2 signaling pathway' (GO:0038123) (p=2.49E-17), 'toll-like receptor TLR6:TLR2 signaling pathway' (GO:0038124) (p=2.49E-17), 'toll-like receptor 10 signaling pathway' (GO:0034166) (p=4.04E-17), 'toll-like receptor 9 signaling pathway' (GO:0034162) (p=0.0002683). Both KEEG pathway and GO annotation analysis was performed taking into account the comprehensive DE human miRNA profile using DIANA-miRPath v.3.0 (genes union mode, FDR-corrected p value threshold=0.05, microT threshold=0.07). The larger -log10(p value) (enrichment score) indicates a smaller p value. (C) Proposed ci-miRNA-driven pathogenetic mechanisms grouping terms and target genes outlined in (A) and (B). COL1A2, collagen type 1 alpha 2 chain; F3, coagulation factor 3 or tissue factor (TF); F5, coagulation factor 5; F7, coagulation factor 7; F9, coagulation factor 9; F10, coagulation factor 10; FN1, fibronectin 1; GP6, platelet glycoprotein VI; ITGAs, integrins alpha subunit genes; ITGBs, integrins beta subunit genes; NO, nitric oxide; SERPIN, serine protease inhibitor; TFPI, tissue factor pathway inhibitor; THBS1, thrombospondin 1; VEGFA, vascular endothelial growth factor; VKORC1, vitamin K epoxide reductase complex subunit 1; VWF, von Willembrand factor. *ITGA1, ITGA10, ITGA2, ITGA3, ITGA5, ITGA6, ITGAV, ITGB1. **SERPINB2, SERPIND1, SERPINE1, SERPINE2, SERPINF2, SERPING1.

as ci-miRNAs with available experimentally validated targets (see the online supplemental methods, tables 3 and 4). Considering a p value ≤0.05 to assess significance, three of six selected miRNAs (hsa-miR-224-5p, hsa-miR-206 and hsa-miR-653-5p) showed statistically significant differences between the two study groups (BS and HC), with a positive fold change direction (expressed as relative quantification, or 'RQ', performed by ΔΔCq method) consistent with microarray screening results

(figure 4A). On the contrary, those ci-miRNAs resulting downregulated in the microarray screening phase failed to reach significance when analysed by PTA-PCR in the validation cohort.

ROC curve analyses, both single and multiple marker combinations, were then performed on the three selected miRNAs which reached the statistical significance (hsa-miR-206, hsa-miR-224-5p and hsa-miR-653-5p). Both single miRNAs and two miRNA combinations (figure 4B,C) failed to reach area under the curve (AUC) values corresponding to an acceptable marker discriminating power. On the contrary, the combination of all three miRNAs (figure 4D) revealed a fair but highly significant AUC value (0.72, p=0.0005), with a specificity of 0.83 and a sensitivity of 0.57.

To further support the potential relevance of the identified ci-miRNA profile as potential disease biomarker, we also determined the expression levels of the three selected ci-miRNAs in two disease control groups. Figure 5A,B shows the results of the analysis of BS versus SLE and BS versus GCA, respectively: in any case, values of the relative quantification (RQ) were greater than 2, and the corresponding p values were statistically significant.

This indicates that the three ci-miRNAs levels significantly differ between patients with BS and the two disease control groups. This conclusion was corroborated by the results of the combined ROC curve analysis (online supplemental figure 6A,B), which showed significant AUC values (AUC=0.81 for both disease control groups).

Comparison between both disease control groups and HC showed no significant deregulation in the expression of the three selected ci-miRNAs. Coherently, no valuable AUC value emerged from the combined ROC curve analysis of either SLE and GCA when compared with HC (online supplemental figure 7).

Finally, we also tested the ability of the identified three-ci-miRNA panel in discriminating patients with BS with active versus inactive disease state. The relative combined ROC curve analysis showed a significant AUC value of 0.71, with a specificity of 0.94 and a sensitivity of 0.42 (online supplemental figure 8).

### The validated ci-miRNAs confirm their biological meaning potentially related to thrombo-inflammation: correlation with leucocyte ROS and plasma lipid peroxidation

The FAA performed on the three miRNAs hsa-miR-206, hsa-miR-224-5p and hsa-miR-653-5p revealed a significant enrichment in several of the pathways which also emerged from the comprehensive ci-miRNA profile emerging from the screening phase (figure 6A,B). Specifically, both GO and KEEG terms involved in thrombo-inflammation ('blood coagulation', 'platelet activation', 'cellular nitrogen compound metabolic process' and 'ECM-receptor interaction') as well as in native and adaptive immunity ('TGF-beta signaling pathway', 'Hippo signaling pathway' and 'phosphatidylinositol signaling pathway') showed the highest enrichment scores (figure 6A,B and online supplemental tables 5 and 6).

Furthermore, the expression levels of the three selected ci-miRNAs were compared with ROS levels in the main circulating leucocyte populations in patients with BS belonging to the validation cohort. Neutrophil ROS levels showed direct correlation with hsa-miR-224-5p ($R^2$=0.1706, p=0.0233) (figure 6C). A significant direct correlation also emerged between lymphocyte ROS and upregulated hsa-miR-206 ($R^2$=0.2039, p=0.0123) and hsa-miR-224-5p ($R^2$=0.1712, p=0.0231) (figure 6D). In addition, monocyte ROS showed a significant direct correlation with hsa-miR-224-5p ($R^2$=0.1620, p=0.0275) and hsa-miR-653-5p ($R^2$=0.1412, p=0.0407) (figure 6E). Finally, plasma lipid peroxidation was directly correlated with hsa-miR-224-5p ($R^2$=0.2544, p=0.0045) and hsa-miR-206 expression levels ($R^2$=0.1853, p=0.0176) (figure 6F).

Overall, these findings suggest that the selected three ci-miRNA profiles which emerged from the validation phase of the study is potentially connected with the thrombo-inflammatory aspects uniquely associated with BS, further strengthening its role as candidate biomarker.

## DISCUSSION

The present study provides evidence that a peculiar profile of ci-miRNAs might serve to segregate patients affected by BS from HCs. Notably, the ci-miRNA profile we identified highlights the relevance of thrombo-inflammation in BS, hence suggesting an epigenetic regulation of thrombo-inflammatory mechanisms in the disease.

The DE ci-miRNA profile which first emerged from a microarray screening was clinically validated in a larger cohort by PCR. From the latter, the combination of three specific human ci-miRNAs (hsa-miR-224-5p, hsa-miR-206 and hsa-miR-653-5p) emerged, capable of segregating patients with BS from HC. Based on the specificity and sensitivity values derived from the combined ROC (CombiROC) curves, the above three ci-miRNAs might represent a supplemental diagnostic step following clinical evaluation. In other words, they might be exploited as a final tool to confirm a clinically based BS suspected diagnosis. The identified three ci-miRNA profiles were also able to discriminate patients with BS with active versus patients with BS with inactive disease. Finally, and more clinically relevant, the ci-miRNA profile could discriminate patients with BS from two disease control groups (SLE and GCA), further confirming its specificity for BS. Using the identified three ci-miRNAs in clinical practice would require additional testing with other control groups, such as isolated erythema nodosum, recurrent aphthous stomatitis, idiopathic uveitis and deep venous thrombosis. Nevertheless, the ci-miRNA profile emerging from our study paves the way to a valuable diagnostic support based on a novel biomarker, which is still strongly needed. Indeed, several potential biomarkers, either genetic or circulating have been described in BS, but none has effectively reached the clinical setting.[34]

In the last few years, specific miRNAs have been reported to be deregulated in peripheral blood mononuclear cells (PBMCs) from patients with BS compared with controls.[20–24] Interestingly, none of the DE ci-miRNAs emerging from our analysis overlapped with those reported as differentially expressed in BS PBMCs, suggesting a more relevant involvement of endothelial cells and/or platelets in defining the plasmatic ci-miRNAs profile of patients with BS by active secretion. Notably, the FAA performed on the intracellular miRNA profile identified in BS PBMCs only partly covers the pathways that we identified in our study, with no emerging association with terms potentially linking thrombosis to inflammation.[21]

This potential origin acquires further strength from the functional analysis we performed, which indicated that the DE ci-miRNAs emerging from our study, besides being capable to discriminate patients from HC, contribute to highlight pathophysiological pathways underlying the disease, as expected by a disease biomarker.[35]

Indeed, both the bioinformatic analysis, focused on target genes of the DE ci-miRNAs, and the biochemical analysis on blood cells and plasma lipids identified molecular pathways potentially linked to BS pathogenesis. In particular, the FAA

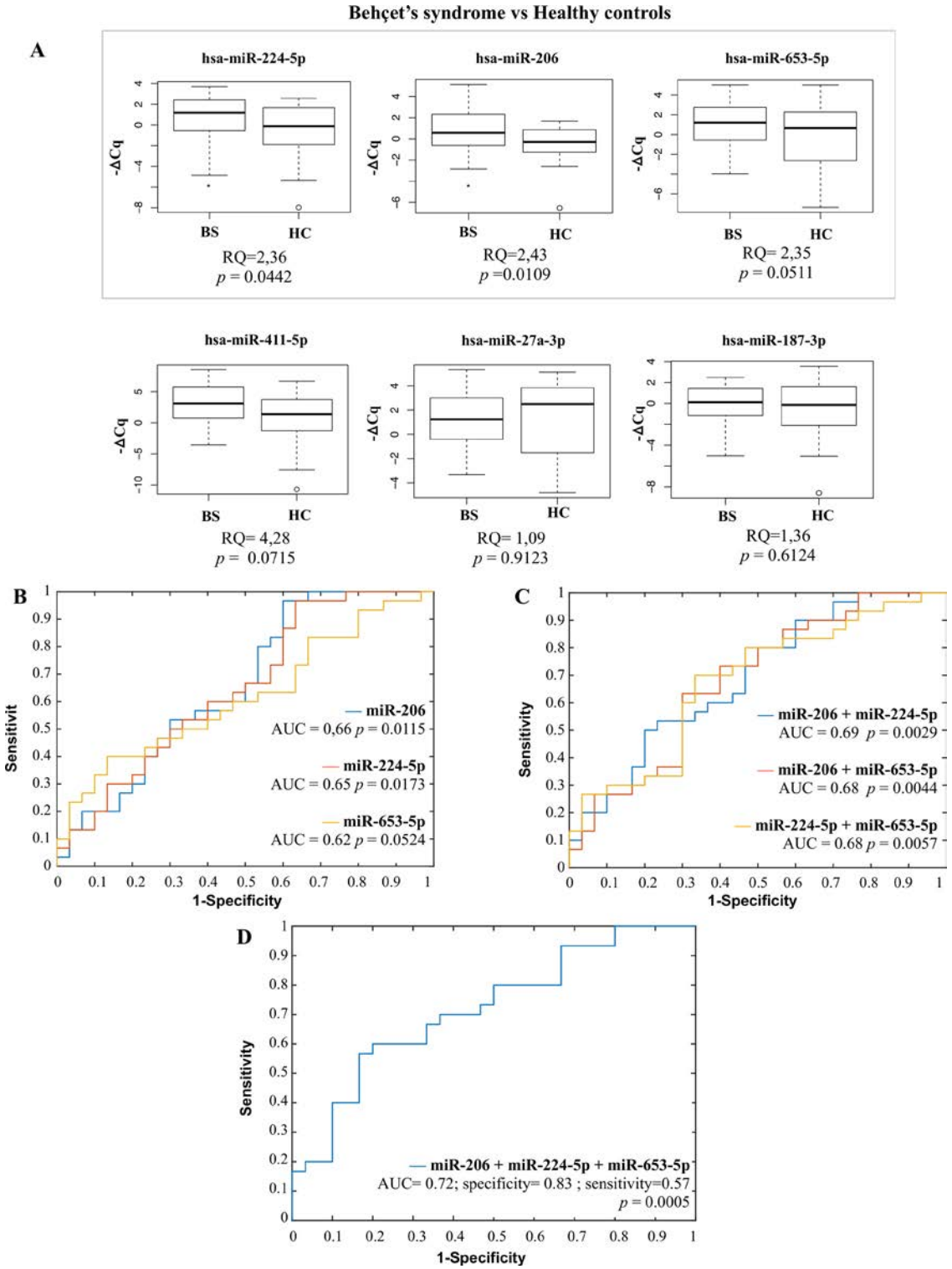


**Figure 4** Validation phase results. (A) Selected ci-miRNAs poly(T) adaptor PCR (PTA-PCR) results in BS compared with HC. hsa-miR-653-5p (RQ=2.35, p=0.05), hsa-miR-224-5 p (RQ=2.35, p=0.04) and hsa-miR-206 (RQ=2.43, p=0.01) showed an upregulation in accordance with microarray results. hsa-miR-27a-3p and hsa-miR187-3p showed RQ of 1.09 (p=0.91) and 1.36 (p=0.61), respectively, while hsa-miR-411-5p reached a value of RQ=4.28 still without reaching statistical significance (p=0.07). Box and whiskers plots (95% CI). BS, Behçet's syndrome; Cq, threshold cycle; HC, healthy control; RQ, relative quantification (ΔΔCq method); p, p value (Mann-Whitney test). n=60 (30 BS and 30 HC). Selected miRNAs ROC curve analysis. (B) Single miRNA ROC curves (hsa-miR-206: AUC=0.66, p=0.0115, 95% CI 0.60 to 0.97; hsa-miR-224-5p: AUC=0.65, p=0.0173, 95% CI 0.63 to 0.97; hsa-miR-653-5 p: AUC=0.62, p=0.0524, 95% CI 0.13 to 0.40); (C) two miRNA combination ROC curves (hsa-miR-206+hsa-miR-224-5 p: AUC=0.69, p=0.0029, 95% CI 0.50 to 0.80; hsa-miR-206+hsa-miR-653-5p: AUC=0.68, p=0.0044, 95% CI=0.30–0.63; hsa-miR-224–5p+hsa-miR-653–5 p: AUC=0.68, p=0.0057, 95% CI=0.33–0.70); (D) three miRNA combination ROC curve (hsa-miR-206+hsa-miR-224-5p+hsa-miR-653-5p): AUC=0.72, p=0.0005, 95% CI 0.50 to 0.93, specificity=0.83, sensitivity=0.57). AUC, area under the curve; p=p value (z-test)al. n=60 (30 BS and 30 HC). The receiver operating characteristic (ROC) curves and the optimal values of sensitivity and sensibility have been computed using the built-in Matlab function perfcurve.m (V.R2021b).

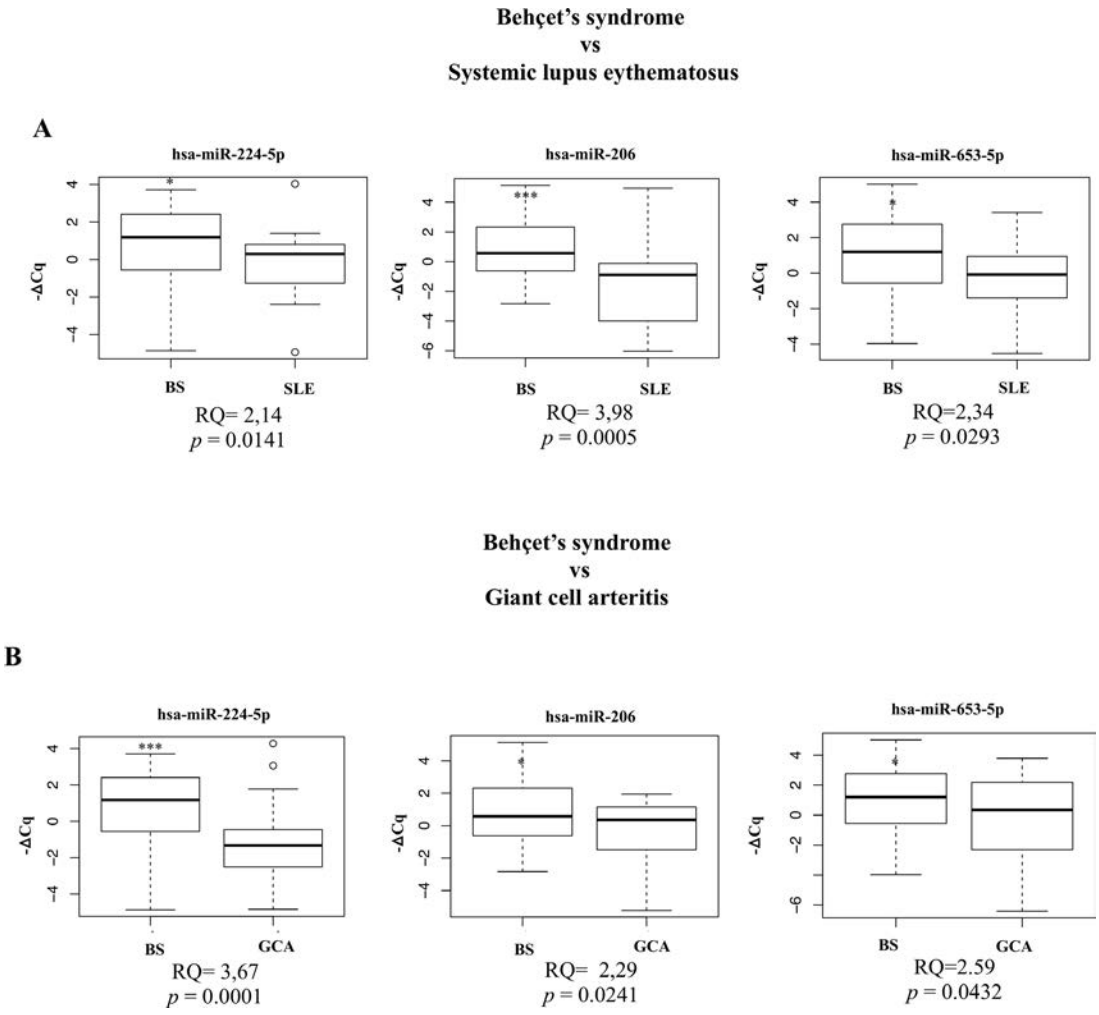


**Figure 5** Selected miRNA expression in BS compared with disease control groups. (A, B). Selected circulating microRNAs (ci-miRNAs) poly(T) adaptor PCR (PTA-PCR) results in BS compared with disease control groups (SLE, GCA). hsa-miR-653-5p (BS vs SLE, RQ=2.35, p=0.02; BS vs GCA, RQ=2.59 p=0.04), hsa-miR-224-5p (BS vs SLE, RQ=2.14, p=0.01; BS vs GCA, RQ=3.67, p=0.0001) and hsa-miR-206 (BS vs SLE, RQ=3.98, p=0.0005; BS vs GCA, RQ=2.29, p=0.02) showed significant upregulation in BS when compared with both disease control groups. Box and whiskers plots (95% CI). BS, Behçet's syndrome; Cq, threshold cycle; GCA, giant cell arteritis; RQ, relative quantification (ΔΔCq method); p, p value (Mann-Whitney test); SLE, systemic lupus erythematosus. n=60 (30 BS vs 30 SLE or 30 GCA).

confirmed the undebated pathogenic role of native and adaptive immunity in BS,[2] providing evidence that both TLRs and Treg-related pathways, in particular those converging on FOXP3 regulation,[33 36–38] are deregulated in patients with BS. However, the most innovative finding emerging from our functional analyses indicated that most of the target genes of the DE ci-miRNAs are related to cellular and molecular processes underlying thrombo-inflammation. Notably, the latter is one of the main clinical aspects which characterises BS and strongly affects its morbidity and mortality.[3] Our conclusion derived by the fact that the most enriched terms emerging from either KEEG or GO analyses on both microarray data and on the three validated ci-miRNAs, can be grouped into three main processes: cell–matrix interaction, oxidative stress and blood coagulation (figure 3). The first process is suggestive of tissue infiltration and interaction of blood cells with the lying endothelium, both signs of the inflammatory burden which characterises the perivascular milieu in BS and underlies its clinical features.[2] The enriched pathway terms involved in oxidative stress processes are in agreement with recent evidence that an oxidative damage, produced, for example, by neutrophil activation,[7 39] indeed occurs in BS. This evidence is further supported by our data showing a correlation of the three ci-miRNAs emerging from the validation phase (hsa-miR-206, hsa-miR-653-5p, hsa-miR-224-5p) with leucocyte ROS and plasma lipid peroxidation. Interestingly, such miRNAs directly or indirectly target those genes (collectively grouped in terms such 'cellular nitrogen compound metabolic processes' or 'cellular protein modification process'), involved in antioxidant defenses, which hence contribute to cellular ROS accumulation.[40–44] Both cell–matrix interaction and oxidative stress can be related to the extravascular tissue damage and endothelial dysfunction, which may contribute to the alterations of the thrombophilic profile characterising BS.[6 45 46] This comprises, for example, oxidation-induced modifications of fibrinogen, which produces high resistance to fibrinolytic digestion.[5] Hence, it is not surprising that the third process emerging from the FAA on microarray data as well as on the three ci-miRNAs emerging from the validation study (figures 3A,B and 6A,B, respectively) is related to blood coagulation. Genes related to the most relevant coagulation factors (tissue factor, factor IX, X and co-factor V) and coagulation inhibitors (serpins, tissue factor pathway inhibitor) are controlled by the ci-miRNAs emerging from our analysis, suggesting that these phenomena are potentially under epigenetic control.

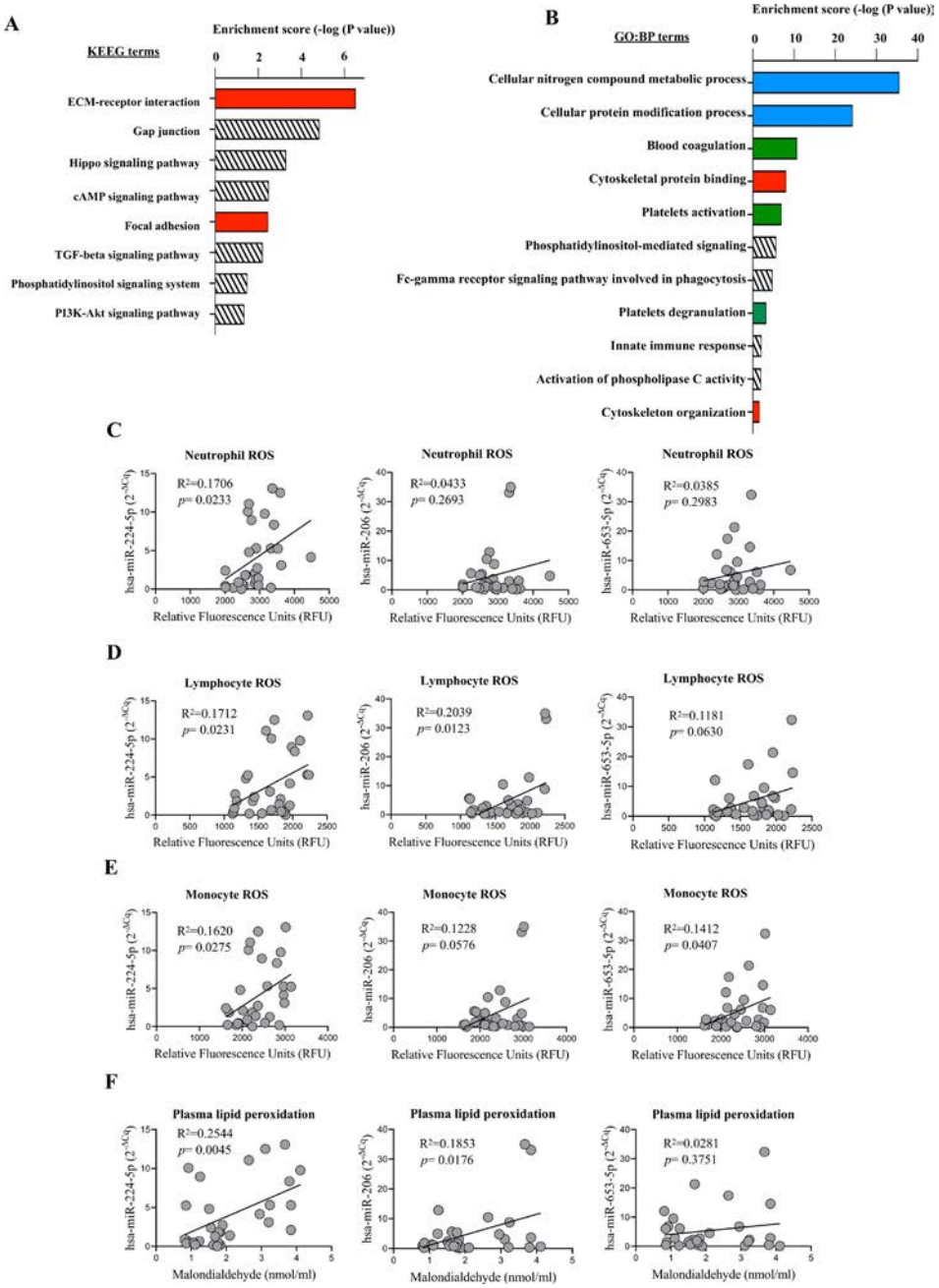


**Figure 6** Selected miRNA functional annotation analysis (FAA) and correlation with intracellular and plasmatic oxidative stress markers. FAA was performed using the DIANA-miRPath v.3.0 (genes union mode, FDR-corrected p value threshold=0.05, microT threshold=0.07). (A) Top significantly enriched KEEG (Kyoto Encyclopedia of Genes and Genomes) terms. Bars corresponding to terms involved in cell–matrix interaction are shown in red, the ones related to innate and adaptive immunity are in black and white pattern. Highlighted KEEG terms: 'ECM-receptor interaction' (hsa04512) (p=2.97E-07), 'gap junction' (hsa04540) (p=1.45E-05), 'Hippo signaling pathway' (hsa04390) (p=0.0005), 'cAMP signaling pathway' (hsa04024) (p=0.0032), 'focal adhesion' (hsa04510) (p=0.0035), 'TGF-beta signaling pathway' (hsa04350) (p=0.0062), 'phosphatidylinositol signaling pathway' (hsa04070) (p=0.0318), 'PI3K-Akt signaling pathway' (hsa04151) (p=0.0422). (B) Top significantly enriched Gene Ontology (GO) BP terms. Bars corresponding to terms involved in cell–matrix interaction are shown in red, the ones related to innate and adaptive immunity are in black and white pattern, the ones related to blood coagulation are in green, while the ones linked to oxidative stress are in blue. Highlighted GO:BP terms: 'cellular nitrogen compound metabolic process' (GO:0034641) (p=2.73E-45), 'cellular protein modification process' (GO:0006464) (p=5.55E-25), 'blood coagulation' (GO:0007596) (p=2.023E-11), 'cytoskeletal protein binding' (GO:0008092) (p=8.44E-09), 'platelets activation' (GO:0030168) (p=1.04E-07), 'phosphatidylinositol-mediated signaling pathway' (GO:0048015) (p=2.24E-06), 'Fc-gamma receptor signaling pathway involved in phagocytosis' (GO:0038096) (p=1.80E-05), 'platelets degranulation' (GO:0002576) (p=0.0005), 'innate immune response' (GO:0045087) (p=0.0082), 'activation of phospholipase C activity' (GO:0007202) (p=0.0103), 'cytoskeleton organization' (GO:0007010) (p=0.0230). The larger -log10(p value) (enrichment score) indicates a smaller p value (p=FDR-corrected p value). Comprehensive significantly enriched KEEG and GO terms lists are reported in online supplemental tables 5 and 6, respectively. Validated circulating microRNA (ci-miRNA) expression level correlation with leucocytes' oxidative stress levels and plasma lipid peroxidation: (C) neutrophil ROS; (D) lymphocyte ROS; (E) monocyte ROS; (F) plasma lipid peroxidation. BS, Behçet's syndrome; Cq, threshold cycle; RFU, relative fluorescence units; ROS, reactive oxygen species; R, Pearson's correlation coefficient: p, p value (Pearson's correlation analysis). n=30 BS.

Overall, the unique ci-miRNA profile described here can be considered a novel candidate biomarker in patients with BS and reinforces the hypothesis that BS represents a model of thrombo-inflammation.[7] This may lead to uncover novel and still unexplored diagnostic and therapeutic strategies for the management of BS.

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**Contributors** AA, DP, GE and LE provided concept and designed of the study. GB and EL performed microarray experiments and data acquisition. GB performed RT-qPCR experiments and data acquisition. GB, EL, AB and FDP performed microarray and RT-qPCR data analysis and interpretation. EL and GB performed plasma samples preparation. GE, ES, AB and MLU collected the samples and interpreted relative clinical data. FDP and AB performed data statistical analysis. CF and MB performed ROS measurement experiments and the relative data analysis. DP, AA and GE revised the results and gave final approval of the manuscript. AA served as overall content guarantor. All authors have read and approved the final manuscript. First authorship is shared by GE and GB as they contributed equally to this study. Senior authorship is shared by AA and DP.

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**Competing interests** None declared.

**Patient and public involvement statement** We provided patients with understandable information about the study at the time of informed consent signing. With the exception of clinical sample donation, patients were not involved in the study, since it revolves around basic and translational research.

**Patient consent for publication** Not applicable.

**Ethics approval** This study was approved by the local ethical committee for Azienda Ospedaliero-Universitaria (AOU) Careggi in accordance with the 1975 Declaration of Helsinki ethical guidelines (protocol number CE 13972). A signed written informed consent was obtained from each participant enrolled in the study.

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CLINICAL SCIENCE

# Serious infections in patients with rheumatoid arthritis and psoriatic arthritis treated with tumour necrosis factor inhibitors: data from register linkage of the NOR-DMARD study

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## ABSTRACT

**Objectives** To estimate the incidence of serious infections (SIs) in patients with rheumatoid arthritis (RA) and psoriatic arthritis (PsA) treated with tumour necrosis factor inhibitor (TNFi), and compare risk of SIs between patients with RA and PsA.

**Methods** We included patients with RA and PsA from the NORwegian-Disease Modifying Anti-Rheumatic Drug registry starting TNFi treatment. Crude incidence rates (IRs) and IR ratio for SIs were calculated. The risk of SIs in patients with RA and PsA was compared using adjusted Cox-regression models.

**Results** A total of 3169 TNFi treatment courses (RA/PsA: 1778/1391) were identified in 2359 patients. Patients with RA were significantly older with more extensive use of co-medication. The crude IRs for SIs were 4.17 (95% CI 3.52 to 4.95) in patients with RA and 2.16 (95% CI 1.66 to 2.81) in patients with PsA. Compared with the patients with RA, patients with PsA had a lower risk of SIs (HR 0.59, 95% CI 0.41 to 0.85, p=0.004) in complete set analysis. The reduced risk in PsA versus RA remained significant after multiple adjustments and consistent across strata based on age, gender and disease status.

**Conclusions** Compared with patients with RA, the risk of SIs was significantly lower in patients with PsA during TNFi exposure.

## Key messages

**What is already known about this subject?**

► Previous studies have assessed serious infection (SI) in rheumatoid arthritis (RA) populations treated with tumour necrosis factor inhibitor (TNFi), but data are scarce regarding the risk of SI in patients with psoriatic arthritis (PsA) treated with TNFi and the comparative risk of infection in TNFi treated RA versus patients with PsA.

**What does this study add?**

► We observed that the risk of SI is significantly lower in patients with PsA compared with patients with RA treated with a TNFi.

**How might this impact on clinical practice or future developments?**

► Although the results need to be interpreted with caution given the many important differences between the RA and PsA population, our findings indicate that the clinician should consider the rheumatological diagnoses when assessing the risk of future SI in patients starting a TNFi.

## INTRODUCTION

Treatment of rheumatoid arthritis (RA) and psoriatic arthritis (PsA) has advanced considerably over the past two decades. Tumour necrosis factor inhibitors (TNFis) are pivotal in the management of RA and PsA.[1–3] Given their immunosuppressive effects, infections related to TNFi treatment is a concern. In patients with RA, TNFi therapy is associated with an increased risk of serious infections (SIs) compared with conventional synthetic disease modifying antirheumatic drugs (DMARDs).[4–7] Few observational studies have addressed incidence rates (IRs) of SIs in PsA[8–11] and studies comparing the risk of SIs between patients with RA and PsA are sparse.[11 12] The future risk of infections should be considered when making treatment decisions.[13]

We aimed to estimate the incidence of SIs in patients with RA and PsA treated with TNFi and compare the risk of SIs between these two disease populations, and across strata.

## METHODS

### Data sources

Data from the prospective observational multicentre NORwegian-Disease Modifying Anti-Rheumatic Drug (NOR-DMARD) study were used.[14] We included adult patients diagnosed with clinical RA or PsA, starting treatment with a TNFi between January 2009 and December 2018. All were diagnosed by a rheumatologist. In addition, diagnoses were defined according to international classification criteria (American College of Rheumatology/European Alliance Of Associations For Rheumatology (ACR/EULAR)) n=773, ACR n=550, ClASsification criteria for Psoriatic ARthritis (CASPAR) n=597). Each patient could contribute more than one treatment course. Start of

observation was the start of treatment. End of observation was the first occurrence of following; last visit or withdrawal from NOR-DMARD, death, emigration or censor date. A 30-day observation period was added to capture infections registered after the last visit.

### Register linkages

To identify events (SIs), we linked NOR-DMARD to the Norwegian Patient Registry (NPR) and Norwegian Cause of Death Registry. Comorbidities were identified through linkage to the Norwegian Control and Payment of Health Reimbursement database and NPR, receiving data from primary and specialist healthcare services respectively. At discharge from hospital stay, diagnoses are reported to the NPR by the attending physician according to the International Classification of Diseases version 10 (ICD-10). The NPR is considered reliable from 2008, and 2009 was thus selected as the first year included in the analyses.[15] Patients signed informed consent.

### Outcomes

The outcome, SI, was defined as an infection requiring hospital admission with at least one-night hospital stay and/or as an infection causing death according to a predefined list of ICD-10 diagnoses (online supplemental table 1). The infection had to be listed as the primary diagnosis at discharge, or as the first contributory diagnosis given that the primary diagnosis was RA or PsA. Only the first SI for each treatment course was included in our analyses.

### Covariates

#### Disease activity

At each NOR-DMARD visit, disease activity measures and markers of inflammation were recorded and the Disease Activity Score for 28 joints (DAS28) was calculated. Comprehensive questionnaires including the use of medication and the modified Health Assessment Questionnaire were completed.[14]

#### Comorbidities

The following were considered potential confounders; diabetes, chronic obstructive pulmonary disease (COPD) or asthma.[16]

### Statistical analyses

Baseline demographics are presented as means (SD), medians (IQR) or frequencies (%) and compared between cohorts by appropriate bivariate methods. Crude IRs of SI for RA and PsA were presented as events per 100 person-years and the IR ratio (IRR) of IR between RA and PsA was estimated. Robustness of results was examined in models adjusted for multiple confounders. To ensure comparable models, cases without missing values for included variables were used in the main results. IRs and risk of SI in RA versus PsA were estimated in the stratum. Analyses were made in STATA V.16.

### Sensitivity analyses

Baseline variables were compared between patients with complete dataset and those who had missing data for key variables. Cox regressions were performed in cohorts with missing versus not missing for key variables. The linear relationship between time and risk of SI was explored in models censored at 12-month and 24-month follow-up.

**Table 1** Baseline characteristics for the treatment courses

| Variable | RA (n=1778) | PsA (n=1391) | P value |
|---|---|---|---|
| Age in years, mean (SD) | 53.2 (13.8) | 48.2 (11.9) | <0.001 |
| Age, n (%) | | | |
| <50 years | 651 (36.6) | 755 (54.3) | <0.001 |
| ≥50 years | 1127 (63.4) | 636 (45.7) | |
| Female gender, n (%) | 1341 (75.4) | 797 (57.3) | <0.001 |
| Years on treatment, median (IQR) | 1.1 (0.4–2.6) | 1.1 (0.5–2.7) | 0.65 |
| Disease duration, years, median (IQR)* | 6.9 (2.3, 14.5) | 5.2 (1.6, 11.8) | <0.001 |
| Current smoking, n (%) | 252 (14.2) | 225 (16.2) | 0.12 |
| DAS28-CRP, mean (SD)† | 4.0 (1.3) | 3.5 (1.2) | <0.001 |
| MHAQ, median (IQR)‡ | 0.6 (0.3, 1.0) | 0.6 (0.3, 1.0) | 0.22 |
| MTX co-medication, n (%)§ | 1265 (73.2) | 798 (59.1) | <0.001 |
| Prednisolone co-medication, n (%)§ | 976 (56.5) | 400 (29.6) | <0.001 |
| Prednisolone dose, n (%) | | | |
| >0–5 mg | 412 (23) | 135 (10) | <0.001 |
| >5–10 mg | 264 (15) | 68 (5) | <0.001 |
| >10 mg | 269 (16) | 87 (6) | <0.001 |
| Comorbidities | | | |
| COPD and/or asthma, n (%) | 180 (10.1) | 93 (6.7) | 0.001 |
| Diabetes, n (%) | 127 (7.1) | 116 (8.3) | 0.209 |

Continuous variables presented as mean (SD) or median (IQR), dichotomous variables presented as number (%).
*Disease duration missing in 266 patients with RA, and 286 patients with PsA.
†DAS28-CRP missing in 228 patients with RA and 200 patients with PsA.
‡MHAQ missing in 58 patients with RA and 50 patients with PsA.
§MTX and prednisolone co-medication missing in 50 patients with RA and 41 patients with PsA.
COPD, chronic obstructive pulmonary disease; CRP, C reactive protein; DAS28, Disease Activity Score for 28 joints; MHAQ, Modified Health Assessment Questionnaire; MTX, methotrexate; PsA, psoriatic arthritis; RA, rheumatoid arthritis; TNFi, tumour necrosis factor inhibitor.

## RESULTS

### Population characteristics

A total of 3169 TNFi treatment courses were identified (RA/PsA 56/44%), in 2359 patients (RA/PsA 1352/1007). Patients with PsA were younger and more frequently male. Patients with RA had significantly longer disease duration, a higher baseline DAS28-CRP (C reactive protein) score, more likely to receive co-medication at baseline and more often had COPD (table 1).

### Incidence and risk of SIs

A total of 187 cases of SIs occurred during the study period, 131 with RA versus 56 with PsA. The majority (37%) were respiratory tract infections. The IRR between PsA and RA was 0.52 (95% CI 0.37 to 0.71) (table 2). Patients with PsA had a lower risk of SI (HR 0.59, 95% CI 0.41 to 0.85) compared with patients with RA when adjusted for age and gender, and across subgroups, except in those using methotrexate as sole co-medication (table 3, online supplemental table 2).

### Sensitivity analyses

The HR for SI was explored across cohorts of patients with missing versus not-missing data for key variables (online supplemental table 3 and figure 1) and after adjustment for components

**Table 2** Incidence of serious infection

| | RA | PsA |
|---|---|---|
| Treatment courses TNFi, n | 1778 | 1391 |
| Person-years | 3139 | 2590 |
| Serious infection, n | 131 | 56 |
| Crude IR/100 PY (95% CI) | 4.17 (3.52 to 4.95) | 2.16 (1.66 to 2.81) |
| Incidence rate ratio (95% CI) | 0.52 (0.37 to 0.71) | |

IR, incidence rate; PsA, psoriatic arthritis; PY, person years; RA, rheumatoid arthritis; TNFi, tumour necrosis factor inhibitor.

of DASs (online supplemental table 4). In sensitivity analyses with 12-month and 24-month follow-up, the risk of SI remained significantly lower in PsA versus patients with RA (HR 0.47, 95% CI 0.28 to 0.78) at 12 months and (HR 0.46, 95% CI 0.30 to 0.71) at 24 months.

## DISCUSSION

In this register linkage data study, we found a significantly lower risk of SI for patients with PsA compared with patients with RA receiving TNFi therapy. This result remained significant in the adjusted models with complete cases only, supporting the robustness of our results. Patients with RA were older, more often female, with higher DAS28-CRP and more frequent users of co-medication at baseline. Adjustment for multiple factors, including the above-mentioned differences, were made in multivariate analyses, and did not alter the risk-difference. However, the additive effect of multiple risk factors in the RA population, including more frequent prednisolone use, may explain some of the increased risk of SIs in patients with RA. Another explanation could be the RA disease itself, through disease-related alterations in host defence.[17]

**Table 3** Adjusted HRs of serious infection in patients with PsA versus RA treated with tumour necrosis factor inhibitor

| | Number | HR (95% CI) | P value |
|---|---|---|---|
| Model A: adjusted for age and gender | | | |
| PsA vs RA | 2675 | 0.59 (0.41 to 0.85) | 0.004 |
| Model B: adjusted for age, gender, DAS28-CRP, MHAQ | | | |
| PsA vs RA | 2675 | 0.58 (0.40 to 0.84) | 0.004 |
| Model C: adjusted for age, gender, concomitant MTX, baseline prednisolone | | | |
| PsA vs RA | 2675 | 0.69 (0.47 to 1.00) | 0.049 |
| Model C1: adjusted for age, gender, concomitant MTX | | | |
| PsA vs RA | 2675 | 0.59 (0.41 to 0.85) | 0.005 |
| Model C2: adjusted for age, gender, baseline prednisolone any dose | | | |
| PsA vs RA | 2675 | 0.69 (0.48 to 1.00) | 0.048 |
| Model C3: adjusted for age, gender, baseline prednisolone low dose | | | |
| PsA vs RA | 2675 | 0.60 (0.42 to 0.86) | 0.006 |
| Model C4: adjusted for age, gender, baseline prednisolone intermediate dose | | | |
| PsA vs RA | 2675 | 0.64 (0.44 to 0.92) | 0.017 |
| Model C5: adjusted for age, gender, baseline prednisolone high dose | | | |
| PsA vs RA | 2675 | 0.62 (0.43 to 0.90) | 0.011 |
| Model D: adjusted for age, gender, COPD and/or asthma, diabetes | | | |
| PsA vs RA | 2675 | 0.58 (0.40 to 0.83) | 0.003 |
| Model E: adjusted for all variables in models A–D | | | |
| PsA vs RA | 2675 | 0.65 (0.44 to 0.95) | 0.025 |

COPD, chronic obstructive pulmonary disease; CRP, C reactive protein; DAS28, Disease Activity Score for 28 joints; MHAQ, Modified Health Assessment Questionnaire; MTX, methotrexate; PsA, psoriatic arthritis; RA, rheumatoid arthritis.

While several studies have quantified the risk of SIs in patients with RA treated with biological DMARDs (bDMARDs) with IRs ranging from 2.6 to 5.6/100 person-years,[7 13 16 18] the risk of SIs in patients with PsA has been far less studied. The few observational studies assessing IRs of SIs in patients with PsA treated with biologicals have reported widespread estimates from 2.7 to 19.6/100 person-years.[8–11] The IRs found in our analyses are thus in line with these previously reported estimates.

Few studies have compared the risk of SIs between patients with RA and PsA. A recent case–control study from DANBIO, the Danish rheumatology registry, reported the risk of SIs within the first year after bDMARD initiation in bionaive RA, PsA and axial spondyloarthrtitis compared with matched population controls. The study was not specifically designed to compare the risk of SIs between patient groups, but concluded that the risk is similar.[11] However, in this study, the follow-up period was defined as 12 months regardless of drug discontinuation, and difference in drug retention between patients with RA and PsA were not accounted for. A study using administrative data found no significant difference in risk between patients with RA, PsA and/or severe psoriasis.[12] However, the PsA population was here categorised in the same cohort as patients with psoriasis.[12]

Missing data is a limitation to our analyses. Cases with missing information for disease duration had less severe disease activity, and excluding this population from the analyses may have given a falsely high-risk estimate. Also, smoking could not be adjusted for due to missingness. Another limitation is the possibility of residual confounding. Although the risk estimate was not changed by including disease activity measurements in the model (table 3, online supplemental table 4), we have to consider that disease activity in PsA was not fully captured by variables registered in NOR-DMARD. Further, we cannot exclude the possibility of misclassification of outcomes, as physicians might be more aware of infections among patients with RA than in patients with PsA, resulting in patients with RA being hospitalised for less severe infections more frequently than patients with PsA. However, our definition of SIs limits the risk of non-SIs being misclassified. Stratified analyses over co-medication indicate that differences in prednisolone use between patients with RA and PsA may partly explain the risk difference, and the effects of prednisolone should be further explored. Finally, we cannot account for initiation and discontinuation of co-medication during TNFi exposure, as only baseline co-medication data were accessible, and this limitation needs to be considered when interpreting the results.

Multi-centre high-quality observational prospective register data reflective of real-world clinical practice is a major strength to this study. The outcome (SI) was well defined using ICD-10 registered by the attending physician. Also, our patient population is defined according to international classification criteria.

In conclusion, this study found a significantly lower risk of SIs in patients with PsA than in patients with RA, during exposure to TNFi. The results need to be interpreted with caution given the many important differences between the RA and PsA population, especially with regards to the use of co-medication. Recognising the elevated risk in patients with RA supports the heightened awareness of SIs during follow-up of these patients.

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CLINICAL SCIENCE

# Analysing cord blood levels of TNF inhibitors to validate the EULAR points to consider for TNF inhibitor use during pregnancy

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## ABSTRACT

**Background** To minimise placental transfer of tumour necrosis factor inhibitors (TNFi), the European League Against Rheumatism (EULAR) created points to consider (PtC) for the use of TNFi during pregnancy. We are the first to validate the EULAR-PtC by analysing TNFi concentrations in cord blood.

**Methods** Patients were derived from the Preconceptional Counselling in Active Rheumatoid Arthritis Study. TNFi was stopped at the time points recommended by the EULAR. Maternal blood and cord blood were collected and analysed for the concentration of TNFi.

**Results** 111 patients were eligible for the analysis. Median stop time points were gestational age (GA) 37.0 weeks for certolizumab pegol, GA 25.0 weeks for etanercept, GA 19.0 weeks for adalimumab and GA 18.4 weeks for infliximab. Certolizumab pegol (n=68) was detectable in 5.9% of cord blood samples, with a median concentration of 0.3 µg/mL (IQR: 0.2–1.3) and a median cord/maternal concentration ratio of 0.010. Etanercept (n=30) was not detected in any cord blood samples. Adalimumab (n=25) was detectable in 48.0% of cord blood samples, with a median concentration of 0.5 µg/mL (IQR: 0.2–0.7) and a median concentration ratio of 0.062 (IQR: 0.018–0.15). Infliximab (n=14) was detectable in 57.1% of cord blood samples, with a median concentration of 0.4 µg/mL (IQR: 0.1–1.2) and a median concentration ratio of 0.012 (IQR: 0.006–0.081).

**Conclusion** Compliance with the EULAR-PtC results in absence or low levels of TNFi in cord blood.

## Key messages

**What is already known about this subject?**

- Tumour necrosis factor (TNF) inhibitors can be actively transported across the placenta as early as week 20 of gestation, mediated by fetal Fc receptors and dependent on TNF inhibitor structure.
- European Alliance of Associations for Rheumatology (EULAR) points to consider (PtC) recommend to stop adalimumab and infliximab at gestational age (GA) 20 weeks, etanercept at GA 30–32 weeks and conditional continuation of certolizumab pegol.
- The EULAR-PtC are based on limited evidence; only for certolizumab pegol, it has been demonstrated that cord blood concentrations are minimal when treatment is continued throughout pregnancy.

**What does this study add?**

- This study demonstrates that stopping TNF inhibitor treatment according to the EULAR-PtC results in undetectable or low levels of TNF inhibitor in cord blood.

**How might this impact on clinical practice or future developments?**

- Compliance with the EULAR-PtC results in absence or low concentration of TNF inhibitors in cord blood, indicating that the children are most likely not immunologically compromised.

## INTRODUCTION

Tumour necrosis factor inhibitors (TNFi) have become an important component of the treatment of rheumatic diseases during pregnancy.[1] A drawback of prescribing TNFi during pregnancy is active transport of these drugs across the placenta mediated by neonatal Fc receptors (FcRn).[2] Placental transfer starts around gestational week 20, and the rate of transfer increases throughout pregnancy.[2] The extend of placental transfer depends on the molecular structure of the drug. Adalimumab and infliximab are whole anti-TNF antibodies and have a strong affinity for the FcRn.[3] Etanercept is a fusion protein that comprises a TNF receptor and the Fc domain of human IgG1. Its affinity for the FcRn is lower than that of adalimumab and infliximab.[4] Certolizumab pegol is a PEGylated Fab fragment of an anti-TNF monoclonal antibody. Because certolizumab pegol lacks the Fc domain, it is not actively transported across the placenta.[5]

The European League Against Rheumatism (EULAR) created points to consider (PtC) for the use of TNFi during pregnancy.[6] These PtC recommend discontinuation of treatment at gestational age (GA) 20 weeks for adalimumab and infliximab, GA 30–32 weeks for etanercept and conditional continuation of certolizumab pegol throughout pregnancy. Until now, it is unknown whether stopping treatment at the advised GA results in the absence of TNFi in cord blood.[6]

The aim of this research is to validate the stop time points recommended by the EULAR-PtC. We hypothesise that no TNFi will be measured in cord blood when treatment was stopped at the recommended GA.

## METHODS

### Patients

Patients were derived from the Preconceptional Counselling in Active Rheumatoid Arthritis (PreCARA) cohort at Erasmus Medical Center in Rotterdam, the Netherlands (ClinicalTrials.gov registration: NCT01345071). The PreCARA cohort is an ongoing, prospective cohort study on inflammatory rheumatic diseases and pregnancy. Patients whose cord blood was collected at birth were used for the current analysis.

### PreCARA treatment protocol

Patients in the PreCARA cohort were treated according to a modified treat-to-target approach. Details on the PreCARA treatment protocol have been previously described.[1] Patients were allowed to get pregnant on the TNFi used at enrolment. TNFi were discontinued at the GAs advised by the EULAR, and a switch to certolizumab pegol and/or prednisone was considered. Certolizumab pegol was discontinued at GA 38 weeks to prevent maternal infections during delivery, based on expert opinion.[1]

### Data collection

Information on diagnosis and previous medication use was collected at the first visit. Maternal blood was collected in each trimester, at moments unrelated to the administration of TNFi. At birth, cord blood was collected by the patient's midwife or gynaecologist. Blood samples were clustered and subsequently sent to Sanquin Laboratory (Amsterdam) for analysis (online supplemental appendix).

### Statistical analysis

Descriptive statistics on clinical characteristics and TNFi use are presented as mean (SD), median (IQR) or number (%). Differences in GA at stopping TNFi treatment between patients with and without measurable TNFi levels in cord blood were assessed with the two-sample Wilcoxon rank-sum test. P values <0.05 were considered significant. Stata software V.16.0 was used for all statistical analyses.

**Table 1** Descriptive statistics of patients from PreCARA cohort that were included in the current analysis (n=111)

| Variable | Value* |
|---|---|
| Age, years | 31.2±3.9 |
| Nulliparity | 49 (44.1%) |
| Disease duration at inclusion, years | 8.0±6.5 |
| Disease activity in 3rd trimester (DAS28-CRP) | 2.2±0.8 |
| Diagnosis | |
| Rheumatoid arthritis | 53 (47.7%) |
| Spondyloarthropathies | 26 (23.4%) |
| Psoriatic arthritis | 22 (19.8%) |
| Juvenile idiopathic arthritis | 6 (5.4%) |
| Other rheumatic disorders | 4 (3.6%) |
| Medication during pregnancy, any use† | |
| Sulfasalazine | 63 (56.8%) |
| Hydroxychloroquine | 54 (48.6%) |
| Prednisone | 45 (40.5%) |
| Certolizumab pegol | 68 (61.2%) |
| Etanercept | 30 (27.0%) |
| Adalimumab | 25 (22.5%) |
| Infliximab | 14 (12.6%) |

*Values are given as mean±SD or number (%).
†Either alone or in combination with other medication. The sum of TNFi exceeds 100%, because some patients switched from etanercept, adalimumab or infliximab to certolizumab pegol during pregnancy. DAS28-CRP, Disease Activity Score 28. CRP, C-reactive protein; PreCARA, preconceptional counselling in active rheumatoid arthritis; TNFi, tumour necrosis factor inhibitor.

## RESULTS

Data from 111 patients were used for the analysis (table 1). During some pregnancies, the use of etanercept, adalimumab or infliximab was switched to certolizumab pegol. Therefore, in the cord bloods of those pregnancies, the concentration of two TNFi was to be determined, resulting in a total of 137 cord blood measurements. Most patients stopped treatment before the recommended GA (table 2). Etanercept (n=30) was stopped before GA 30 weeks by 29 (96.7%) patients, adalimumab (n=25) was stopped before GA 20 weeks by 20 (80.0%) patients and infliximab (n=14) was stopped before GA 20 weeks by 10 (71.4%) patients. For certolizumab pegol, the median GA at stopping treatment was GA 37.0 weeks (IQR: 34.1–38.1 weeks), and the median time between last dose and delivery was 15 days (IQR: 2–34 days).

Certolizumab pegol (n=68) was detected in 5.9% of cord blood samples; the median level of certolizumab pegol was 0.3 µg/mL (IQR: 0.2–1.3). The maximum concentration (2.3 µg/mL) was measured in a patient that stopped treatment at 26 days before delivery and received 200 mg every other week. The concentration ratio of cord blood to maternal blood for certolizumab pegol was 0.010 (IQR: 0.007–0.066). Etanercept was not detected in any of the cord blood samples, including the sample of one patient who stopped after GA 30 weeks (GA 36.7 weeks).

Adalimumab and infliximab were detected in 12 (48.0%) and 8 (57.1%) cord blood samples, respectively. The median cord blood concentrations were 0.5 µg/mL (IQR: 0.2–0.7) for adalimumab and 0.4 µg/mL (IQR: 0.1–1.2) for infliximab. The median concentration ratios of cord blood to maternal blood were 0.062 (IQR: 0.018–0.15) for adalimumab and 0.012 (IQR: 0.006–0.081) for infliximab. The maximum concentration for adalimumab (2.1 µg/mL) was measured in a patient who stopped treatment at GA 19.4 weeks and received 40 mg every other week. For infliximab, the maximum concentration (4.5 µg/mL) was measured in a patient who stopped treatment at GA 21.1 weeks and received 400 mg every 5 weeks (online supplemental appendix).

Differences in GA at stopping adalimumab and infliximab between patients with and without detectable TNFi in the cord blood are shown in table 3.

## DISCUSSION

In the current study, we show that stopping TNFi around the GA recommended by the EULAR-PtC results in no detectable or low levels of TNFi in the cord blood.

Most patients in our study used certolizumab pegol during pregnancy. We observed certolizumab pegol in 5.9% of the cord blood samples. In comparison, a study by Mariette *et al* observed certolizumab pegol in 20% of the umbilical cord samples.[5] The lower limit of quantification was higher in our study (0.1 µg/mL vs 0.032 µg/mL), which might explain the observed difference. Furthermore, there was one patient with a certolizumab pegol concentration of 2.3 µg/mL in our study; this was an outlier. In this particular case, placental blood sample contamination with mother's blood cannot be excluded.

The use of etanercept during pregnancy has not been investigated on a large scale before. Etanercept has a low affinity for the FcRn.[4] Our study shows that stopping treatment with etanercept before GA 30 weeks results in absence of etanercept in the cord blood. Interestingly, the patient that stopped after the recommended GA (at GA

**Table 2** TNF inhibitor (TNFi) use during pregnancy and TNFi concentrations in maternal blood and cord blood. Values are expressed as median (IQR) unless indicated otherwise

| | Certolizumab pegol (n=68) | Etanercept (n=30) | Adalimumab (n=25) | Infliximab (n=14) |
|---|---|---|---|---|
| Stop time point as recommended by EULAR-PtC, weeks | N/A | GA 30–32 | GA 20 | GA 20 |
| Gestational age (GA) at time of stopping TNFi, weeks | 37.0 (34.1–38.1) | 25.0 (17.9–28.0) | 19.0 (12.4–19.9) | 18.4 (14.0–20.1) |
| Stopped before recommended GA, n (%) | N/A | 29 (96.7%) | 20 (80.0%) | 10 (71.4%) |
| No measurable TNFi in cord blood, n (%) | 64 (94.1%) | 30 (100%) | 13 (52.0%) | 6 (42.8%) |
| Measurable TNFi in cord blood, n (%) | 4 (5.9%) | 0 (0%) | 12 (48.0%) | 8 (57.1%) |
| Maternal concentration of TNFi in the 1st trimester, µg/mL | 24.6 (19.0–31.0) | 2.1 (0.8–2.5) | 8.2 (1.5–10.0) | 14.0 (8.0–21.0) |
| Maternal concentration of TNFi in the 2nd trimester, µg/mL | 22.5 (13.0–30.72) | 1.4 (0.9–2.7) | 6.0 (4.5–7.5) | 6.4 (4.2–20.0) |
| Maternal concentration of TNFi in the 3rd trimester, µg/mL | 20.5 (13.0–29.6) | 0.2 (0.2–0.7) | 0.9 (0.1–1.4) | 1.4 (0.1–1.9) |
| Concentration of TNFi in the cord blood if measurable, µg/mL | 0.3 (0.2–1.3) | – | 0.5 (0.2–0.7) | 0.4 (0.1–1.2) |
| Concentration ratio cord blood to maternal blood* | 0.010 (0.007–0.066) | – | 0.062 (0.018–0.15) | 0.012 (0.006–0.081) |

*Concentration ratios of cord blood to maternal blood were calculated with the maternal concentrations during active use of TNFi (trimester 3 for certolizumab pegol and trimester 1 for adalimumab and infliximab).
EULAR, European League Against Rheumatism; PtC, points to consider; TNF, tumour necrosis factor .

36.7 weeks or 7 days before delivery) also had no measurable levels. This is in line with a previous study by Eliesen *et al*, which reported a low cord to maternal concentration ratio of 0.04 in a patient who used etanercept until 4 days before delivery.[7] This might be explained by the shorter half-life of etanercept (circa 3 days) compared with other TNFi (8–10 days for infliximab and 14 days for certolizumab pegol and adalimumab). Both these observations might indicate that etanercept could be used beyond GA 30–32 weeks if necessary.

We detected adalimumab and infliximab in about half of the patients' cord blood samples, however in low concentrations. A study by Julsgaard *et al* reported median concentrations of 2.5 µg/mL for adalimumab and 10.0 µg/mL for infliximab in patients who continued treatment beyond GA 30 weeks,[3] considerably higher than the respective 0.5 µg/mL and 0.4 µg/mL in patients from our study, who stopped around GA 20 weeks. These discrepancies might be the result of different indication groups included in the study of Julsgaard *et al*, which were mainly patients with inflammatory bowel diseases and have continued infliximab and adalimumab until a higher GA period during pregnancy.

The effects of low TNFi concentrations in the fetal circulation are unknown. Previous research shows that a TNFi concentration as low as 0.1 µg/mL is sufficient to bind all circulating TNF.[8] Therefore, clinical relevance cannot be excluded. Nevertheless, the concentrations are only a few percent of those found in the mothers during active use. Intrauterine exposure to TNFi can have major consequences, as it may affect the infant's immune system. Immunological changes in infants exposed to high levels of TNFi have been observed, including neutropenia, decreased $T_{reg}$ cells and B-cells with a more immature phenotype.[9] This can result in a different immune response to vaccines, resulting in reduced efficacy of vaccines in the first half year of the infant's life. In addition, the use of live attenuated vaccines in children with high serum levels of TNFi after intrauterine exposure to TNFi requires caution. These vaccines may be pathogenic in infants with a suppressed immune system. In one case, a Bacillus Calmette-Guérin (BCG) vaccination after intrauterine exposure to infliximab resulted in neonatal death after a disseminated BCG infection.[10] It can be concluded from the results of our study that, if PtC recommendations are followed, intrauterine exposure to certolizumab pegol or etanercept will not result in placental transfer and future recommendations for attenuated live vaccination could be less restrictive. If for adalimumab and infliximab minimal or absence of TNFi concentrations in cord blood are aimed, these should be withdrawn even earlier than week 20 of gestation (eg, week 15 of gestation) (online supplemental appendix). A possible consequence of TNFi in the infant's circulation is an increased risk for infections during the first months of life.[10] However, literature reports both increased and non-increased risk for infections and therefore remains inconclusive.[11 12]

Our study has several strengths. It is the first large study to evaluate the EULAR-PtC for the use of TNFi during pregnancy. All 111 patients included in the current analysis were treated at the same hospital, so differences between physicians were minimal. Patient data were retrieved directly from the patient; therefore, the risk for biases, like misclassification bias, was minimal.

A limitation of our study is that we did not measure trough and peak values of maternal TNFi concentrations. The concentration ratios we calculated are therefore less accurate than those calculated in a pharmacokinetic study. Another limitation is that the majority of patients using etanercept stopped or switched their TNFi quite earlier than the recommended stop time point of GA 32 weeks.

In conclusion, compliance with the EULAR-PtC results in undetectable levels or absence of TNFi in cord blood in most patients that use certolizumab pegol or etanercept. For adalimumab and infliximab, TNFi was detectable in cord blood in about half of the patients. The detected concentrations of TNFi in cord blood were far lower than the maternal levels during active use. The potential harmful effects of these low concentrations of TNFi in cord blood are unknown and require further investigation. If these concentrations of TNFi were

**Table 3** Stop time points of TNFi for patients with and without detectable TNFi in the cord blood

| | Stop time point if TNFi was detectable, GA, weeks | Stop time point if TNFi was undetectable, GA, weeks | P value for difference |
|---|---|---|---|
| Certolizumab pegol (n=68) | 36.9 (34.8–38.6) | 37.0 (34.1–38.1) | 0.82 |
| Etanercept* (n=30) | – | – | – |
| Adalimumab (n=25) | 19.4 (18.7–20.1) | 15.0 (4.4–18.8) | 0.08 |
| Infliximab (n=14) | 19.1 (16.7–20.3) | 13.6 (6.9–18.4) | 0.06 |

*Etanercept was not detectable in any of the cord blood samples.
GA, gestational age; TNF, tumour necrosis factor.

to be clinically relevant, stopping infliximab and adalimumab at an earlier GA than the EULAR-PtC recommend may be appropriate.

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CLINICAL SCIENCE

# Severe delayed hypersensitivity reactions to IL-1 and IL-6 inhibitors link to common HLA-DRB1*15 alleles

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## ABSTRACT

**Objectives** Drug reaction with eosinophilia and systemic symptoms (DRESS) is a severe, delayed hypersensitivity reaction (DHR). We observed DRESS to inhibitors of interleukin 1 (IL-1) or IL-6 in a small group of patients with Still's disease with atypical lung disease. We sought to characterise features of patients with Still's disease with DRESS compared with drug-tolerant Still's controls. We analysed human leucocyte antigen (HLA) alleles for association to inhibitor-related DHR, including in a small Kawasaki disease (KD) cohort.

**Methods** In a case/control study, we collected a multicentre series of patients with Still's disease with features of inhibitor-related DRESS (n=66) and drug-tolerant Still's controls (n=65). We retrospectively analysed clinical data from all Still's subjects and typed 94/131 for HLA. European Still's-DRESS cases were ancestry matched to International Childhood Arthritis Genetics Consortium paediatric Still's cases (n=550) and compared for HLA allele frequencies. HLA association also was analysed using Still's-DRESS cases (n=64) compared with drug-tolerant Still's controls (n=30). KD subjects (n=19) were similarly studied.

**Results** Still's-DRESS features included eosinophilia (89%), AST-ALT elevation (75%) and non-evanescent rash (95%; 88% involving face). Macrophage activation syndrome during treatment was frequent in Still's-DRESS (64%) versus drug-tolerant Still's (3%; $p=1.2\times10^{-14}$). We found striking enrichment for HLA-DRB1*15 haplotypes in Still's-DRESS cases versus INCHARGE Still's controls ($p=7.5\times10^{-13}$) and versus self-identified, ancestry-matched Still's controls ($p=6.3\times10^{-10}$). In the KD cohort, DRB1*15:01 was present only in those with suspected anakinra reactions.

**Conclusions** DRESS-type reactions occur among patients treated with IL-1/IL-6 inhibitors and strongly associate with common HLA-DRB1*15 haplotypes. Consideration of preprescription HLA typing and vigilance for serious reactions to these drugs are warranted.

## Key messages

**What is already known about this subject?**

- Drug reaction with eosinophilia and systemic symptoms (DRESS), a severe delayed hypersensitivity reaction (DHR), is under-recognized, especially in inflammatory conditions.
- Secondary haemophagocytic lymphohistiocytosis, indistinguishable from macrophage activation syndrome (MAS), is reported in DRESS.
- Human leucocyte antigen (HLA) associations with severe, drug-related DHR are reported and typically are stronger than HLA disease associations.

**What does this study add?**

- A subset of patients with Still's disease develop DRESS to anakinra, canakinumab, rilonacept or tocilizumab.
- MAS during treatment with inhibitors of interleukin 1 (IL)-1 or IL-6 appears to be a manifestation of this DRESS reaction.
- Diffuse lung disease occurs in some patients with Still's disease with this DRESS reaction.
- Delayed hypersensitivity reactions to inhibitors of IL-1 and IL-6 exhibit a striking association with a common HLA class II haplotype.

**How might this impact on clinical practice or future developments?**

- Our findings argue for consideration of HLA testing for prepresscription risk assessment.
- As 20% of subjects with a reaction do not carry the risk alleles and relevance in other conditions is unknown, vigilance for a DRESS-type delayed reaction is recommended during treatment with these inhibitors.

## INTRODUCTION

Adverse drug reactions are one of the leading causes of morbidity and mortality worldwide.[1] Among these reactions, severe, potentially fatal delayed hypersensitivity reactions (DHR) are under-recognized due to their complexity and variable presentation.[2–4] Particularly during treatment of inflammatory illnesses, DHR may be

misinterpreted as disease flares. The most serious types of DHR classify as severe cutaneous adverse reactions, including drug reaction with eosinophilia and systemic symptoms (DRESS). Typical features of DRESS-type DHR are latency (days to months) after drug initiation, fever, extensive rash, haematological manifestations (eosinophilia and atypical lymphocytosis), involvement of various deep organs, and often an extended time to recovery, even after the offending drug is stopped. Recognition of this serious drug reaction during complex illness is both imperative and challenging.

Increasingly, pharmacogenetic data link drug-specific reaction risk with particular human leucocyte antigen (HLA) class I and/or class II alleles. HLA associations with severe drug reactions have proven to be substantially stronger with much higher ORs and more complete penetrance than most of the well-known HLA allelic disease associations in autoimmune disorders.[1 5 6] In addition to providing clues to pathogenesis, the finding of an HLA/DHR association allows preventative HLA screening prepresscription. Some well-characterised HLA associations are specific to alleles found primarily in particular populations; others have been linked to relatively common alleles with a wide global distribution.[7] The cost/benefit ratio of HLA screening to prevent a serious drug reaction in at-risk individuals improves as the population frequency of the HLA risk allele increases.[1]

HLA molecules function to present peptides to T cells through binding to T cell surface receptors for antigen. In some severe reactions, the offending drugs have been shown to interact directly with HLA molecules, which in turn stimulate T cell responses; the drug interaction also can alter the repertoire of peptides bound to HLA.[1] Thus, HLA associations with severe DHR implicate T cells as immune effectors. This implication is corroborated by evidence from biopsies of DHR-associated skin rashes, which show infiltration of activated T cells.[8]

Systemic juvenile idiopathic arthritis (sJIA) is a chronic inflammatory disease of childhood with unknown aetiology; parenchymal lung disease is not a typical feature.[9 10] We observed DRESS among a small group of patients with sJIA who developed an unusual, non-infectious parenchymal diffuse lung disease (DLD) during treatment with inhibitors of IL-1 (anakinra, canakinumab, rilonacept) or of IL-6 (tocilizumab).[10] We hypothesised that DRESS reactions, with and without DLD, were under-recognized in sJIA and its adult counterpart, adult onset Still's disease (AOSD), which are currently considered a single disease, Still's disease, based on clinical and immunological studies.[11–17] We aimed to characterise clinical features of these drug reactions in patients with Still's disease and to assess HLA alleles as candidate inherited risk factors for DRESS to these drugs. We also hypothesised that an HLA-associated risk of delayed drug reaction might extend to other disease contexts.

## METHODS

### Subjects

The Still's disease continuum includes patients with sJIA and patients with AOSD.[9 11–17] Patients with Still's disease with probable drug reaction to anakinra, canakinumab, rilonacept and/or tocilizumab (cases) or with possible drug tolerance after exposure to the same drugs (controls) were collected from 37 centres (USA, Canada, Australia) through web-based and meeting-based solicitation. Additional Still's controls from the International Childhood Arthritis Genetics Consortium (INCHARGE) sJIA collection,[18] the largest available sJIA cohort, and the ancestry-matched, INCHARGE healthy control population were used as sources of genetic data. A small (n=19) cohort of patients with Kawasaki disease (KD) in a brief phase I/IIa trial of anakinra (NCT-02179853[19] figure 1) also provided cases and controls. In sum, we had six major groups of subjects (see table, p7, online supplemental information). For sJIA, sJIA-like and AOSD classification criteria used, see online supplemental information.

### Verification of cases (drug reactive) and controls (drug tolerant)

Clinical information required for case/control verification of the Still's disease subjects was collected by privacy-protected electronic database or by direct communication with the physician case reporter, under approved IRB protocols (see online supplemental information). Still's subjects were verified as cases (n=66, 65 DRESS plus 1 Still's with suspected delayed anakinra

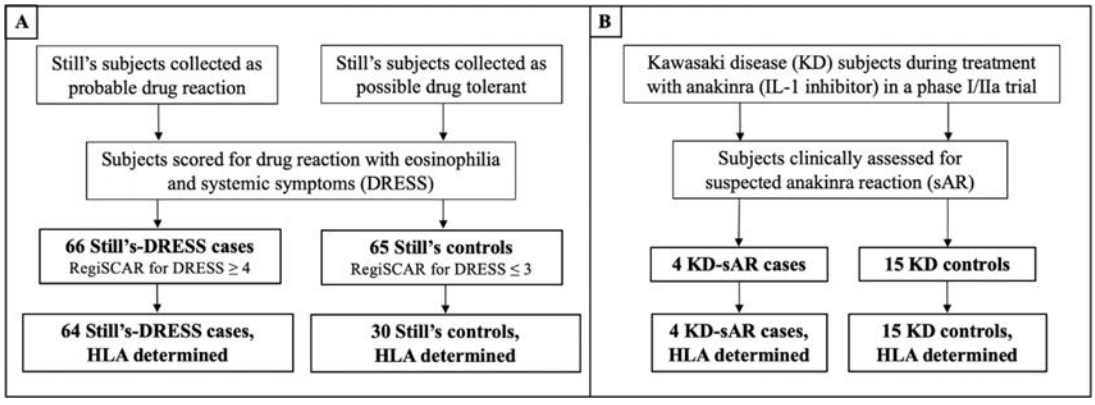



**Figure 1** Study design. Clinical information was collected on Still's disease subjects with and without clinical suspicion of drug reaction to inhibitors of interleukin (IL)-1 or IL-6 (A). Classification of patients with Still's disease was verified by RegiSCAR scoring for DRESS. Similar numbers of Still's-DRESS (n=65 + 1 suspected delayed anakinra reaction Still's; see methods) and Still's controls (n=65) subjects were enrolled for case/control comparison and do not reflect the incidence of inhibitor-triggered DRESS in Still's disease. Human leucocyte antigen (HLA) genotyping was performed on the subset of patients with available sample or sequence data. All 19 Kawasaki disease (KD) subjects were enrolled in a phase I/IIa clinical trial of anakinra in patients with KD with coronary artery abnormalities[19] (B) and were clinically scored as suspected anakinra reaction or drug tolerant; all were HLA typed. Details of scoring and HLA genotyping are provided in methods and online supplemental information. Still's disease: sJIA, systemic onset juvenile idiopathic arthritis (Still's onset <16 years) and AOSD, adult-onset Still's Disease (Still's onset ≥16 years)[9 11]; RegiSCAR, registry of experts assembled to clinically classify drug-induced severe cutaneous reactions[20]; DRESS, drug reaction with eosinophilia and systemic symptoms.

reaction) or controls (drug tolerant; n=65; *hereafter called Still's controls*), using a validated scoring system, the registry for severe cutaneous adverse reactions (RegiSCAR) for DRESS. The RegiSCAR system was validated in the setting of inflammatory diseases and uses clinical parameters allowing differentiation from active Still's disease.[9 20] See online supplemental information for RegiSCAR variables. Classification of suspected anakinra reaction (sAR) in Still's (n=1 subject) required >2 occurrences of unexplained eosinophilia (AEC ≥500) during treatment. Classification as drug tolerant (Still's controls) required inhibitor treatment duration of >1 year, RegiSCAR score of <4, and discontinuation of steroids or ≥6 weeks dosed at <0.2 mg/kg/day of prednisone equivalent; these criteria excluded those with long latency to DRESS or on sufficient steroids to blunt the reaction.

Data for full RegiSCAR scoring were unavailable for KD subjects. Classification of KD subjects as KD-sAR required eosinophilia ≥50% over pretreatment, study baseline value. Presumed drug tolerance in KD was defined as the absence of eosinophilia during anakinra exposure (9–46 days). Still's and KD subjects were verified as case or control by a board-certified allergist (vs) prior to HLA determination.

### Clinical and demographic data collection

In addition to information for case/control verification, other clinical and demographic (sex, self-identified race) information on the 131 Still's disease subjects was collected. Laboratory data collected during treatment included eosinophil count, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Eosinophilia was defined as absolute number or percent of white blood cell count above the laboratory's upper limit of normal without other cause, for example, allergic rhinoconjunctivitis in the absence of steroid treatment. AST/ALT elevation was defined as >2× the upper limit of normal more than once without infection or other non-DRESS cause, or macrophage activation syndrome (MAS). MAS was determined by the case reporter using Ravelli classification criteria.[21]

### HLA genotyping determination

As described in online supplemental information, genomic DNA was extracted from blood or tissue, and HLA genotyping was performed by one of several methods. For those with limited DNA sample or clinically typed cases, HLA genotyping was limited to class II (23% (15/64) of Still's-DRESS cases).

### Statistical analyses, including HLA association

As this is a case/control study, we used ORs, their 95% CIs and corresponding p values to summarise the association of various clinical and genetic factors of interest with DRESS.

Six major groups of subjects were studied:

1. Still's DRESS cases: *patients with Still's disease with DRESS* (n=66).
2. Still's controls: *patients with Still's disease without DRESS (drug tolerant)* (n=65).
3. INCHARGE childhood-onset Still's (sJIA) (European ancestry): *patients with Still's disease with drug exposure unknown* (n=550).
4. INCHARGE healthy controls (European ancestry): *healthy subjects* (n=3279).
5. KD-sAR cases: *patients with KD with sAR* (n=4).
6. KD controls: *patients with KD without sAR* (n=15).

Groups 3 and 4 (*INCHARGE Still's controls and healthy controls*) were constructed to rigorously ancestry match a subset of Still's-DRESS cases with European ancestry (white) for unbiased HLA analysis. To this end, two rounds of principal component analysis (PCA) were performed. These used 24 Still's-DRESS cases with whole exome sequence (WES) data, INCHARGE European Still's cases (n=773) and European healthy controls (n=6612). Genomic control inflation factors ($\lambda_{GC}$) were determined to assess robustness of matching.

*Analysis of drug exposure, demographic and clinical features*: we compared frequency of exposure to individual inhibitors or any IL-1 inhibitor between Still's-DRESS cases and Still's controls (group 1 vs group 2) using Fisher's exact test. We also compared the demographic and clinical characteristics between Still's-DRESS cases and Still's controls (group 1 vs group 2), using Fisher's exact test. We also compared age of disease onset (<2.5, 2.5–10, 10–16,>16) between Still's-DRESS cases and Still's controls (group 1 vs group 2) using proportional odds regression. These four analyses used the entire Still's case/control collection, excluding 1 or 2 subjects in some analyses of clinical features, due to missing data. In a sensitivity analysis, we compared clinical characteristics (eosinophilia, elevated LFTs and MAS) in the subgroups of Still's-DRESS cases and Still's controls with Still's onset age <16 years (subgroups of group 1 vs group 2) using Fisher's exact test.

*HLA association analysis*: the analysis of HLA allele association with DRESS to the IL-1/IL-6 inhibitors was restricted to subjects who were genotyped for HLA (subsets within groups 1 and 2, as shown in figure 1, and the INCHARGE collection). We compared HLA allele frequencies between Still's-DRESS cases with European ancestry and INCHARGE Still's controls (European ancestry patients in group 1 vs group 3) or between Still's-DRESS cases with European ancestry and INCHARGE healthy controls (European ancestry patients in group 1 vs group 4). Specifically, classical class I and class II HLA alleles were analysed by logistic regression with sex as a covariate, as described.[18] This multiallelic HLA association analysis was repeated, comparing self-identified white Still's-DRESS cases and INCHARGE Still's controls (self-identified white patients in group 1 vs group 3) and comparing self-identified white Still's-DRESS cases and INCHARGE healthy controls (self-identified white patients in group 1 vs group 4). Bonferroni corrected p value significance threshold, adjusted for multiple comparisons (254 imputed HLA alleles tested), was $p<2.0\times10^{-4}$. The identified risk allele (DRB1*15:01) was also tested for association with risk of DRESS to individual inhibitors in self-identified white Still's-DRESS cases versus INCHARGE Still's controls (self-identified white patients in group 1 vs group 3) by logistic regression with sex as a covariate. We also compared the frequency of DRB1*15:01 in self-identified white, Still's-DRESS cases and self-identified white Still's controls by Fisher's exact test (self-identified white patients in group 1 vs group 2). In a sensitivity analysis, the latter comparison was repeated using only subjects with sJIA onset age <16 years.

We also compared HLA-DRB1*11:01 frequencies between Still's-DRESS cases with European ancestry and INCHARGE Still's controls (European ancestry patients in group 1 vs group 3), between Still's-DRESS cases with European ancestry and INCHARGE healthy controls (European ancestry patients in group 1 vs group 4), between Still's-DRESS cases self-reported as white and INCHARGE Still's controls (self-identified white patients in group 1 vs group 3) and between Still's-DRESS cases self-reported as white and INCHARGE healthy controls (self-identified white patients in group 1 vs group 4). All analyses used logistic regression, adjusting for sex and the Bonferroni corrected p value significance threshold.

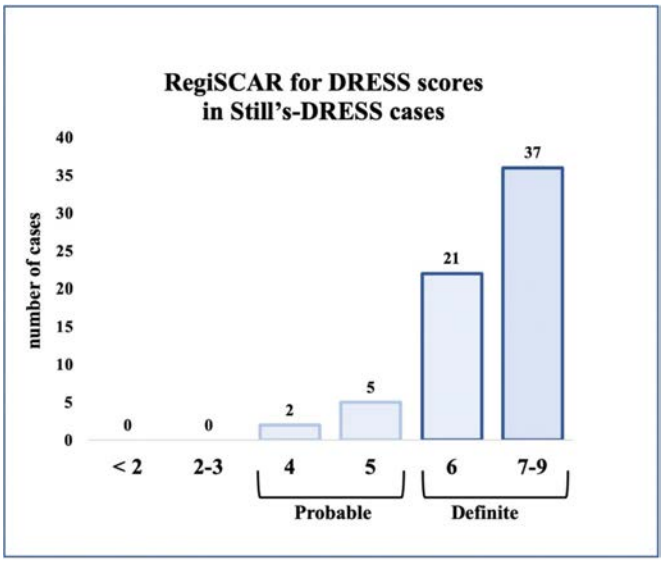



**Figure 2** Registry of experts assembled to clinically classify drug-induced severe cutaneous reactions (RegiSCAR) for drug reaction with eosinophilia and systemic symptoms (DRESS) scores in Still's-DRESS cases. Numbers of Still's-DRESS cases with RegiSCAR for DRESS scores of definite or probable are shown (n=65). The Still's case with suspected delayed anakinra reaction is not included. RegiSCAR classifies a case as definite (6–9), probable (4–5), possible (2–3) or no case (0 to negative 4).[20] For DRESS cases reacting to more than one IL-1/IL-6 inhibitor, the highest RegiSCAR value is shown. By definition, no drug-tolerant subject scored ≥4. RegiSCAR scoring elements are shown in online supplemental information.

We did not have enough non-white subjects for within ancestry comparisons. Therefore, we reported the allele frequencies in these comparisons without formal statistical analyses (eg, KD-sAR cases vs KD controls and pooled Still's-DRESS+KD sAR cases vs pooled Still's+KD sAR controls). Also note that the observed proportion of Still's-DRESS cases in Still's disease subjects or the proportion of DLD cases within Still's-DRESS cases cannot be interpreted as estimates of the prevalence rates due to the case/control study design.

Additional details on methods are in online supplemental information.

## RESULTS

### DRESS, often unrecognised, occurs in a subset of patients with Still's disease treated with IL-1 or IL-6 inhibitors

We collected cases of Still's disease subjects with probable drug hypersensitivity to IL-1 inhibitors (anakinra, canakinumab, rilonacept) or an IL-6 inhibitor (tocilizumab) and Still's disease controls with probable drug tolerance. We confirmed classification of 66 subjects as drug reactive and 65 subjects as drug tolerant, using specified criteria, including RegiSCAR/DRESS scoring (figure 1; see online supplemental materials for details of scoring).

Almost all (65/66) drug-reactive cases were classified as DRESS; the single exception was classified as sAR. The majority (89%) of DRESS patients classified as *definite* DRESS (figure 2); 7 subjects classified a *probable* DRESS and were included as cases per standard application of RegiSCAR/DRESS.[20] We observed a DRESS reaction to anakinra, canakinumab and tocilizumab used alone, indicating that each is capable of triggering DRESS (online supplemental table S1A,B); rilonacept was not used as the sole drug in any subject). 26/66 drug-reactive subjects reacted to multiple inhibitors. The frequency of drug reaction per exposed subject was not significantly enriched for IL-1 inhibitors compared with tocilizumab (anti-IL-6) or for a particular IL-1 inhibitor (online supplemental table S1C, part A). For each implicated drug, the frequency of reactions/case was comparable to the frequency of exposures/control (online supplemental table S1C, part A). These findings supported comparisons of the pooled Still's-DRESS cases to the pooled Still's controls in subsequent analyses.

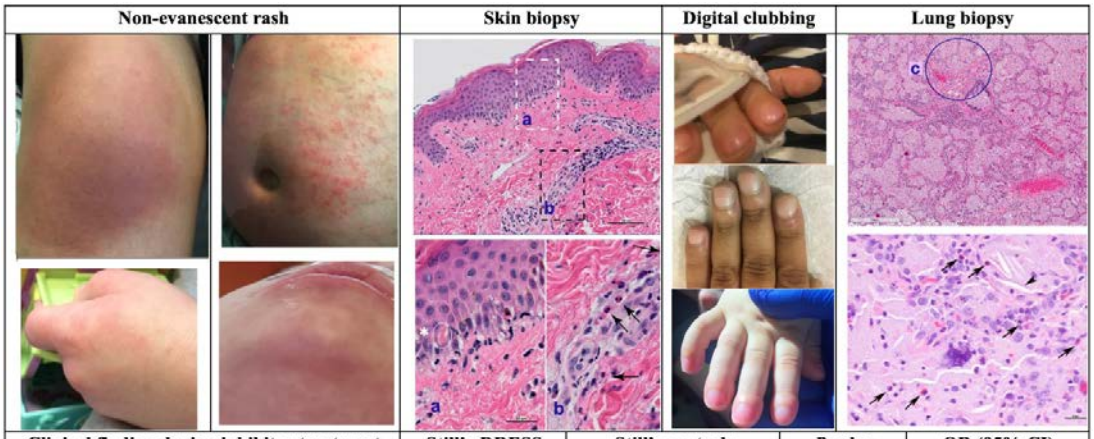
The Still's-DRESS group and the Still's control group were similar in having broad ancestral distribution (online supplemental table S1A, B), as expected in Still's disease[10]; they differed modestly in % male subjects (32% vs 51%; online supplemental table S2). Clinical features did not vary systematically based on the particular drug exposure (online supplemental table S3A) and were similar among Still's-DRESS patients across the age spectrum, with the exception of increased frequency of DLD in patients with very young onset Still's disease (online supplemental table S2).

In Still's-DRESS cases, DRESS features appeared during treatment at United States Food and Drug Administration (FDA)-approved doses for autoinflammatory diseases. Clinical DRESS differed from features of Still's flare[9] and notably included eosinophilia and non-Still's rash (figure 3). Peripheral blood eosinophilia without other cause (eg, pre-existing atopy) was observed in 57/64 (89%) cases. In >60% of cases, eosinophilia was pronounced despite concurrent steroids. Non-evanescent drug eruptions were observed in 63/66 (95%). In 42/48 (88%) providing detail, rash included facial rash and/or oedema, which are typical of DRESS.[3] Skin biopsy reports (12 cases) showed features of drug reaction/DRESS,[7] including interface dermatitis, dyskeratosis and eosinophilia. In 49/65 (75%) Still's-DRESS cases, AST-ALT elevation was noted in the absence of MAS or other explanation. MAS during inhibitor treatment, which can be a manifestation of DRESS,[2 3 22 23] was significantly more common in DRESS cases than in Still's controls ($p=1.2\times10^{-14}$). When MAS occurred during drug treatment, transient eosinophilia typically preceded this by months, consistent with evolution of DRESS-associated features.[3 4]

The drug reactions were often unrecognised, as reflected by continuation of inhibitor therapy after DRESS criteria were met. Only 17/66 (26%) patients with Still's disease with DRESS stopped IL-1/IL-6 inhibitors for ≥3 months without reintroduction. In this group, rash, eosinophilia and AST-ALT elevation resolved in all cases, consistent with resolution of DRESS. In addition, with removal of DRESS as a contributor, inflammation became easier to manage. For example, 10/17 (59%) discontinued steroids and only 2/17 cases required steroids>6 months after drug stop (median follow-up 14 months (IQR: 6–36)). By contrast, of 33 subjects who continued inhibitors after scoring as DRESS, 9 died and only 17% of survivors were off steroids, despite median follow-up of 27 months (IQR: 16–53). Restarting suspended IL-1 inhibitors was associated with fatal MAS (four of six cases) within 2 months.

### Common HLA-DRB1*15 alleles are risk factors for DHR to IL-1 and IL-6 inhibitors

To test for an HLA association with inhibitor-triggered DRESS, we studied the subset of the Still's disease subjects (n=94/131) with available HLA data. (Individual HLA data and associated clinical/demographic data on this subset are on online supplemental tables S1A, E, S3A, B.) First, PCA analyses of the 24 Still's-DRESS subjects with WES data yielded a tight cluster of every




| Clinical finding during inhibitor treatment | Still's-DRESS | Still's controls | *P* value | OR (95% CI) |
|---|---|---|---|---|
| Peripheral eosinophilia | 57/64 (89%)[1] | 1/65 (2%) | $1.6 \times 10^{-27}$ | 521 (62-4366) |
| AST-ALT elevation | 49/65 (75%)[1] | 4/65 (6%) | $8.6 \times 10^{-17}$ | 47 (15-149) |
| Macrophage activation syndrome (MAS) | 42/66 (64%) | 2/65 (3%) | $1.2 \times 10^{-14}$ | 55 (12-246) |

**Figure 3** Unusual clinical features in patients with inhibitor-treated Still's disease. Images of non-evanescent rash, typically pruritic, are shown. Upper left: on anakinra, erythema and prominent oedema affecting knee; upper right: on tocilizumab, excoriated and areas of hyperpigmentation on abdomen; lower left: on canakinumab, erythematous, oedematous rash on hand (similar rash on face and ear is not shown); lower right: on anakinra, erythema, oedema and non-herpetic vesiculation on face. Skin biopsy of drug-associated rash shows vacuolar interface dermatitis and eosinophils. Higher power images (sections a, b) show lymphocytes, vacuolation at the dermal-epidermal junction, focal dyskeratotic keratinocytes (asterisk) and perivascular eosinophils (arrows). Acute digital clubbing, often erythematous, was frequently the first indication of lung involvement in patients with DRESS and diffuse lung disease. Images of acute clubbing on tocilizumab (top), anakinra (middle), on canakinumab (bottom). Lung biopsy showing variant pulmonary alveolar proteinosis/endogenous lipoid pneumonia and arterial wall thickening (c). Higher power image (below) shows cholesterol clefts (arrowhead) and scattered eosinophils (arrows). Of 16, 8 reviewed cases showed eosinophils in many fields (see supplementary methods). Increased lung eosinophils are consistent with DRESS and also seen in various inflammatory diseases. Table: in DRESS cases, median (IQR) of peak absolute eosinophil count was 1500 /uL (980–3080) and peak eosinophil % of WBC was 18% (12–33). AST-ALT elevation was defined as aspartate aminotransferase (AST) or alanine aminotransferase (ALT) measuring >2× the upper limit of normal more than once, without alternative (eg, non-drug) explanation. The frequency of DRESS reactions did not differ significantly when combined anti-interleukin (IL)-1 inhibitors were compared with the IL-6 inhibitor (tocilizumab) or when each inhibitor was analysed separately (online supplemental table S1C). Analyses of specific clinical findings yielded similar results when patients with adult onset Still's disease were omitted (online supplemental table S5). See online supplemental information for detailed methods and additional clinical data online supplemental tables S1C, S2, S3A,B. DRESS, drug reaction with eosinophilia and systemic symptoms; MAS, macrophage activation syndrome, a form of secondary haemophagocytic lymphohistiocytosis[22 24]; p value, by Fisher's exact; OR (95% CI), OR (95% CI). [1]Eosinophil information was unavailable in two cases (n=64); AST-ALT values were unavailable in one case (n=65).

subject of white (European) ancestry (n=14) from the Still's-DRESS cohort, together with 550 INCHARGE sJIA subjects and 3279 INCHARGE healthy controls, as shown on online supplemental figure S1. Genomic control inflation factors ($\lambda_{GC}$) were 1.01–1.05, demonstrating robust matching. Comparing these groups revealed a striking enrichment for HLA-DRB1*15:01 ($p=2.7\times10^{-7}$; table 1, online supplemental table S4A, B), which is part of a common European haplotype, HLA-DRB5*01:01~DRB1*15:01~DQA1*01:02~DQB1*06:02. The strong HLA-DRB1*15:01 association was maintained when all self-identified white Still's-DRESS subjects (n=36) were compared with the European INCHARGE Still's (sJIA) cohort ($p=7.5\times10^{-13}$) (table 1, online supplemental table S4A, B). We also performed an analysis of the 36 white subjects, stratified by treatment group. The anakinra, canakinumab and rilonacept groups were each enriched for HLA-DRB1*15:01, relative to the European INCHARGE Still's (sJIA) cohort. The tocilizumab group was not adequately powered to identify an association; however, the frequency of HLA-DRB1*15:01 among tocilizumab-reactive subjects (80%) was similar to the frequencies observed in the other groups (83%–92%). The CIs overlapped with one another, so the effect sizes are statistically indiscernible online supplemental table S1C, part B. No independent HLA class I association was found (online supplemental table S4C).

As the INCHARGE collection does not include data on drug tolerance, we compared HLA frequency in the self-identified white Still's-DRESS group to self-identified white Still's drug-tolerant controls (table 1). Using HLA-DRB1* 15:01 as a haplotype proxy, the comparison (83% vs 0%) showed a highly significant enrichment in the DRESS group ($p=6.3\times10^{-10}$) with a notable effect size (OR lower bound=16.05).

Another 28 subjects with Still's-DRESS and 11 who were Still's controls had self-identified ancestry other than white. Although the sample was insufficient to perform within-group analyses, we noted a similarly striking pattern of HLA association. HLA-DRB1*15:01 was observed in 57% of non-white subjects with DRESS and 0% of drug-tolerant controls (table 1). Other alleles of the DRB1*15 family are more often present in non-white/European populations, and these appear to be associated with DRESS as well. Together, HLA-DRB1*15 alleles (specifically HLA-DRB1*15:01, *15:03, *15:06) were noted in 75% non-white subjects with Still's-DRESS compared with 18% Still's controls (table 1). Comparing the subset of all Still's DRESS subjects who could be matched for ancestry with Still's controls also showed HLA-DRB1*15:XX enrichment in the DRESS versus drug-tolerant group (82% vs 7%; figure 4). No independent HLA class I association with Still's-DRESS was observed (online supplemental table S1D, E).

When the drug-reactive and drug-tolerant cohorts (all ancestries) were analysed by drug subgroup, the carrier frequencies of HLA-DRB1*15:XX in drug-reactive cases were enriched in each subgroup and similar between groups (online supplemental table

**Table 1** HLA class II allele association with hypersensitivity to IL-1 and IL-6 inhibitors

| | | Cases | Controls | | | |
|---|---|---|---|---|---|---|
| **HLA allele** | **Ancestry** | **Still's-DRESS†** | **Still's controls** | **INCHARGE sJIA** | **P value‡** | **OR (95% CI)** |
| DRB1*15:01 | European versus European | 13/14 (93%) | | 130/550 (24%) | $2.7\times10^{-7}$ | 40.8 (5.3 to 316) |
| | Self-ID White versus European | 30/36 (83%) | | 130/550 (24%) | $7.5\times10^{-13}$ | 15.5 (6.3 to 38.1) |
| DRB1*15:01§ | Self-ID white | 30/36 (83%) | 0/19 (0%) | | $6.3\times10^{-10}$ | Inf (16.05-Inf) |
| | Self-ID non-white | 16/28 (57%) | 0/11 (0%) | | | |
| DRB1*15:XX | Self-ID non-white | 21/28 (75%) | 2/11 (18%) | | | |
| **Kawasaki disease** | | **KD-sAR** | **Drug-tolerant KD** | | | |
| DRB1*15:01 | All | 2/4 (50%) | 0/15 (0%) | | | |
| DRB1*15:XX | All | 3/4 (75%) | 2/15 (13%)¶ | | | |
| **Still's+Kawasaki disease** | | **Still's-DRESS+KD-sAR** | **Drug-tolerant Still's+KD** | | | |
| DRB1*15:01 | All | 48/68 (71%) | 0/45 (0%) | | | |
| DRB1*15:XX | All | 54/68 (79%) | 4/45 (9%) | | | |
| DQB1*06:02 | All | 47/65 (72%) | 3/45 (7%) | | | |

European: Still's-DRESS cases were ancestry matched by PCA to the INCHARGE Still's (sJIA) cohort (online supplemental figure S1).
*In analyses omitting patients with AOSD, similar results were obtained (online supplemental table S5).
†Includes one case with suspected anakinra reaction (see methods).
‡P value, top two rows are by logistic regression from multiallelic comparison to the INCHARGE cohort; only DRB1*15:01 result is shown (extended results on online supplemental tables S4A-C). Bonferroni corrected $p<2.0\times10^{-4}$. P value in third row is by Fisher's exact test, comparing Still's-DRESS to Still's controls for DRB1*15:01.
§Each HLA-DR allele group observed in self-identified white Still's DRESS subjects initially was interrogated for association; only HLA-DRB1*15 alleles showed significant association (online supplemental table S6).
¶HLA-DRB1*15:02 in two individuals, treated briefly (12d and 28d) with anakinra.
DRESS, Drug reaction with eosinophilia and systemic symptoms classified per RegiSCAR[20]; HLA-DRB1*15:XX, all HLA-DRB1*15 alleles; IL, interleukin; INCHARGE, International Childhood Arthritis Genetics Consortium[18]; Inf, infinite; KD, Kawasaki disease; OR (95%CI), odds ratio and 95% confidence interval; self-ID, self-identified; white, similar to European descent.

S1C, Part C). HLA-DRB1*15:XX was comparably enriched in DRESS subjects with and without DLD (82% vs 72%; online supplemental table S1A). Clinical features in DRESS subjects with and without the identified HLA risk alleles were similar (online supplemental table S3A).

We also examined the frequency of the sJIA-associated HLA-DRB1*11:01 allele[18] in our cohort. Unsurprisingly, frequencies of this allele in European and self-identified white Still's-DRESS cases were similar to the European INCHARGE Still's (sJIA) cases (table 2) and increased compared with INCHARGE healthy controls (tables 2 and online supplemental table 4A, B). HLA-DRB1*15:01 was not associated with Still's in the European INCHARGE cohort (table 2). HLA-DRB1*11:01 frequency did not differ significantly between white Still's-DRESS cases and Still's controls. Overall, the results were consistent with the specificity of the HLA-DRB1*11 association for sJIA (young onset Still's) and of the HLA-DRB1*15 association for DRESS in Still's disease. The effect size (OR) for the HLA-associated, inhibitor-related DRESS risk is substantially higher than for the HLA-associated Still's disease risk.

Lastly, we found that our key clinical and genetic findings persisted when the AOSD subjects were removed from the analyses (online supplemental table S5), supporting our comparison of aggregate Still's-DRESS cases to Still's disease controls.

### Common HLA-DRB1*15 alleles are also likely risk factors for sAR in KD

To determine whether HLA-linked delayed drug hypersensitivity required Still's-specific immune dysfunction, we studied a small cohort (n=19) of children with KD in a trial of 2–6 weeks of anakinra treatment.[19] Four had suspected delayed anakinra reaction (sAR; online supplemental table S6A). We observed the same striking effect; 3/4 children with sAR carried HLA-DRB1*15 alleles (HLA-DRB1*15:01 and *15:03), whereas a different HLA-DRB1*15 allele, HLA-DRB1*15:02, was observed in 2/15 apparently drug-tolerant children with KD (table 1, online supplemental table S7A). Notably, HLA-DRB1*15:01 was not observed in any drug-tolerant subject (online supplemental table S7A). No class I association was observed (table S7b).

High percentages of all DHR subjects (Still's+KD) carried DRB1*15 alleles across all ancestries (figure 4). While the HLA-DRB1*15:01~DQA1*01:02~DQB1*06:02 haplotype is in near-complete linkage disequilibrium (LD) in European populations, analysis across ancestries, in which patterns of LD differ, can help to pinpoint the associated locus. Considering the entire Still's-DRESS+KD sAR group, HLA-DRB1*15:01 was observed in 71% (46/64 Still's-DRESS subjects and 2/4 with KD-sAR) and was completely absent in drug-tolerant controls (table 1, online supplemental tables S1A, B, S7A). In contrast, HLA-DQB1*06:02 was observed in 7% of controls, in the context of different haplotypes (table 1, online supplemental tables S1A, S7A), suggesting HLA-DRB1 as the operative locus. It is important to note that HLA-DRB5*01:01, an allele of a secondary HLA-DRB locus, is found on nearly all haplotypes with HLA-DRB1*15 (online supplemental tables S1A, B, S7A). We are not able to rule it out as an effector or contributor to DHR risk.[23]

## DISCUSSION

We have uncovered strong evidence in patients with Still's disease for severe delayed hypersensitivity to anakinra, canakinumab, rilonacept (anti-IL-1) and tocilizumab (anti-IL-6). DHRs occurred with similar frequency after IL-1 or IL-6 inhibition and after any of the IL-1 inhibitors. These reactions met classification criteria for DRESS, a potentially fatal, eosinophilic systemic syndrome. DRESS can lead to organ failure and can stimulate MAS.[2 3 20 22 24] Indeed, MAS can be the presenting sign of DRESS.[22 25] MAS frequency in Still's-DRESS cases far exceeded MAS frequency in Still's controls or in published Still's disease series.[26 27] MAS as part of DRESS to inhibitors suggests a possible aetiological pathway distinct from that of Still's-associated MAS. In the relatively short-term exposure of patients with KD to anakinra, a subset of patients also developed clinical

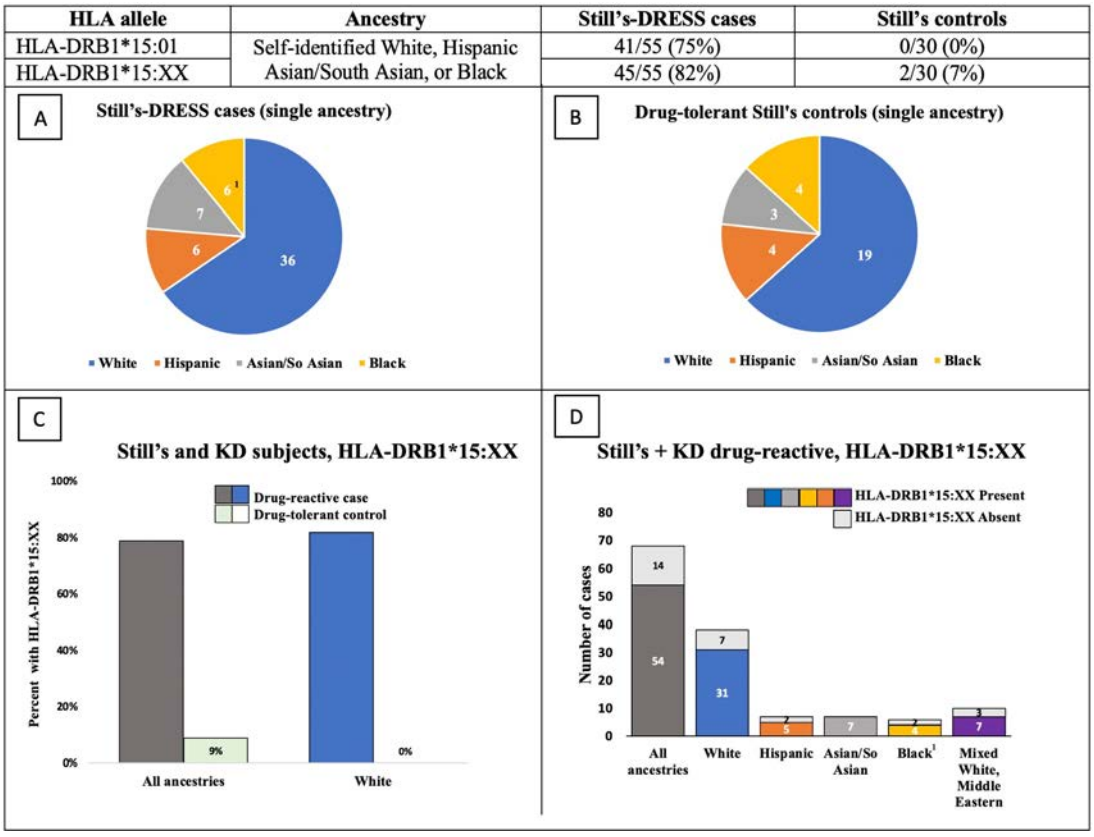



**Figure 4** HLA-DRB1*15:XX appears enriched in delayed drug reactions across ancestries. Table shows carrier frequencies of HLA-DRB1*15:01 and HLA-DRB1*15:XX in Still's-DRESS cases and Still's controls. To compare groups with balanced ancestry, 9 Still's-DRESS cases were excluded from this analysis (leaving n=55), as they could not be matched with Still's controls (n=30). (A, B) Pie charts of cases (A) and controls (B) indicate the proportions of subjects with each self-identified ancestry; absolute numbers in each group are shown. (C) Percentages are shown of delayed hypersensitivity reaction cases and drug-tolerant controls with HLA-DRB1*15:XX, in Still's+KD subjects of all ancestries and in self-identified white subjects. (D) The number of cases in Still's disease +KD subjects (Still's-DRESS and KD-sAR) with and without HLA-DRB1*15:XX in all ancestries and in each indicated ancestry group are shown. All subjects with HLA-DRB*3/4/5 information (n=34) carry both HLA-DRB1*15 and HLA-DRB5*01:01. Additional information is on online supplemental tables S1A, B and S7A. HLA-DRB1*15:XX, any HLA-DRB1*15; self-identified, self-reported ancestry; KD, Kawasaki disease, mixed white, white+non-white ancestry. [1]Includes one Still's drug-reactive case with suspected delayed anakinra reaction.

manifestations consistent with drug reaction, arguing that these DHRs can occur in conditions other than Still's disease.

Importantly, we also discovered a genetic risk factor shared across the delayed reactions to these inhibitors, analysed individually or as a group. We observed a very strong association of the HLA-DRB1*15:01 allele and the linked HLA-DRB5*01:01 in white Still's subjects. The numbers of Still's-DRESS cases, Still's controls and INCHARGE Still's (sJIA) controls allowed rigorous analysis of this ancestry group. The effect size we report is substantially greater than those seen in HLA/disease associations[28] and instead is comparable to those observed in other HLA associations with severe drug-related delayed hypersensitivity.[1 5] Other drug-related HLA associations were initially detected in sample sizes similar to the one reported here and subsequently confirmed.[29] We also detected striking penetrance of the risk allele, as evidenced by its complete absence

**Table 2** HLA-DRB1*11:01 is Still's associated in the Still's-DRESS cohort*

| HLA allele | Ancestry | Still's-DRESS | Still's controls | INCHARGE Still's (sJIA) | INCHARGE healthy controls | P value | OR (95% CI) |
|---|---|---|---|---|---|---|---|
| | European versus European | 4/14 (29%) | | 103/550 (19%) | | 0.43 | |
| DRB1*11:01 | European versus Self-ID white | 4/14 (29%) | 2/19 (10%) | | | 0.36 | |
| | European versus European | 4/14 (29%) | | | 313/3279 (10%) | 0.095 | 3.7 (1.1 to 11.9)† |
| | Self-ID white versus European | 7/35 (20%) | | 103/550 (19%) | | 0.051 | |
| DRB1*11:01 | Self-ID white versus Self-ID white | 7/35 (20%) | 2/19 (10%) | | | 0.46 | |
| | Self-ID white versus European | 7/35 (20%) | | | 313/3279 (10%) | 0.01 | 2.3 (1.0 to 5.2)† |
| DRB1*15:01 | European versus European | | | 130/550 (24%) | 822/3279 (25%) | 0.59 | |

P value, by logistic regression with sex as a covariate for INCHARGE comparisons; OR (95% CI), odds ratio and 95% confidence interval; sJIA, systemic juvenile idiopathic arthritis; DRESS, Drug reaction with eosinophilia and systemic symptoms classified per RegiSCAR[20]; INCHARGE, International Childhood Arthritis Genetics Consortium[13]; European, Still's-DRESS cases were ancestry-matched by PCA to Still's (sJIA) INCHARGE; Self-ID, self identified; white, similar to European descent.

*Additional information is provided in online supplemental tables S1A, B, S4A, B.

†Results are consistent with published data from INCHARGE consortium study of HLA association with sJIA.[18] Drug exposure in INCHARGE sJIA subjects is unknown.

in drug-tolerant controls and the highly significant p-values we report.

Although we were limited by the relative scarcity of non-white subjects in our sample, our findings also suggest that, in addition to HLA-DRB1*15:01, other alleles of the HLA-DRB1*15 family are linked to risk of inhibitor-triggered reaction in these populations. The distribution of subjects with HLA-DRB1*15:XX argues the risk applies across ancestry groups, as found in some other HLA/DHR associations.[7] Carriers of DRB1*15:01, *15:03, *15:06 alleles are common (27% (white), 15% (hispanic), 27% (black) and 16% (Asians) in US populations).[30] Our current cohort does not allow analysis of HLA-DRB1*15:02, a high frequency allele in Asian populations. Approximately 20% of the subjects with a drug reaction do not carry the risk alleles. It will be important to determine if other genetic factors confer risk, both in those with and those without the DRB1*15 risk alleles. Investigation of family history of drug reaction may be useful as regards other risk factors.

In Still's disease and KD, the drug reactions are delayed type and differ from the immediate, anaphylactic reactions to tocilizumab we observed in association with DLD in sJIA.[10] Although some Still's subjects experienced both types of drug reactions, most did not, and carriers of HLA-DRB1*15 alleles were not enriched among those with anaphylaxis to tocilizumab (online supplemental table S3A).

The HLA association we observe has some interesting features: it is restricted to HLA class II,[1 6 7] and it spans several inhibitors with different chemical structures (online supplemental figure S2). The latter raises the possibility that an excipient common to these drugs and/or a molecule increased by inhibition of the intersecting IL-1 and IL-6 pathways creates a stimulatory HLA class II molecule, which activates CD4 +T cells. Several molecular mechanisms for the modification of HLA into an immunogenic moiety in drug hypersensitivity have been identified or proposed.[7 31] A detailed picture of clinical pathogenesis remains to be elucidated and may involve a complex interplay between viruses, HLA proteins, T cells, cytokine secretion and other genetic polymorphisms.[2 7 31]

The conditions for which these inhibitors may be used are a large and expanding group.[32–34] We found scattered reports of DRESS or hypereosinophilia with rash implicating these drugs in RA, polyarthritis, undifferentiated autoinflammatory disorder, giant cell arteritis and COVID-related cytokine storm (online supplemental table S8). HLA typing was not included in these reports and will be important in future investigations. As an n of 1, our continuing case collection includes DRESS in a DRB1*15:01-positive individual with undifferentiated autoimmune disease (online supplemental table S8).

Other than a few case reports (online supplemental table S8), previous studies of IL-1 or IL-6 inhibitors do not mention DRESS. However, it is possible that the reaction was unrecognised. In a recent study of anakinra as first-line therapy for sJIA, 17% of subjects required high-dose steroids for clinical deterioration or MAS.[29] The pivotal trial of canakinumab for sJIA had a 19% non-response rate.[27] A study of tocilizumab in RA had a 15% withdrawal rate for adverse events and/or failure to respond.[34] In 24 patients with COVID-19 treated with tocilizumab, post-treatment elevation of IL-6 levels identified the 25% who died.[35] Further work is needed to determine if hypersensitivity contributes to the rates of drug failures.

There are several limitations to our study. First, our white Still's control group was small. We addressed this limitation by using the European INCHARGE Still's (sJIA) cohort as a comparator, although the drug tolerance status of these subjects is unknown. Notably, however, unidentified Still's-DRESS cases among these subjects would mean the high OR we observe is an underestimate of the true effect size. The number of Still's-DRESS cases with information for robust ancestry loci-matching with the INCHARGE controls was limited. Nonetheless, the highly significant association with HLA-DRB1*15 alleles was replicated in our total Still's-DRESS+sAR group (n=64), with and without self-identified ancestry matching, and in KD-sAR. It seems unlikely that the HLA link is indirect. Other limitations include those inherent to a retrospective observational design, such as missing data. For example, we lacked information to determine whether the DRESS subset had a higher frequency of herpes virus reactivation, particularly HHV-6, as reported in DRESS.[3 7 36] Our sample had under-representation of non-white subjects, limiting our genetic/HLA analyses. Currently, we are assembling validation cohorts for Still's-DRESS across ancestry groups.

An unanswered question is how the development of DLD in Still's disease links to immune-mediated DRESS reactions to the inhibitors.[2 3 7] The temporal correlation between increasing use of IL-1 and IL-6 inhibitors and increasing DLD in sJIA raised the question of a relationship.[37] In further support of an association, all instances of DLD during inhibitor treatment in our cohort scored as DRESS. Lung involvement occurs in DRESS to other drugs, although specific lung pathology has not been described.[38] Cases of drug-induced PAP that resolved on drug withdrawal have been reported.[39 40] It will be critical to determine if DLD in Still's improves by withdrawing the implicated inhibitor, and if there is a window of opportunity for this intervention. As young onset patients with Still's disease appear to be at greater risk for DLD with inhibitor-triggered DRESS (online supplemental table S2 and ref. 10), a possible developmental risk for DRESS-associated DLD requires further study.

Given the same HLA association in hypersensitivity cases with and without DLD, it seems unlikely that DLD is HLA-DRB1*15-associated, independent of the DRESS reaction. The possibility that the clinical features represent a new form of sJIA that is associated with the risk haplotype also seems unlikely, given the HLA-DRB1*15:01 link to anakinra reaction in KD and to DRESS in a case of undifferentiated autoinflammation. We do not know if drug tolerance develops over time, especially with concurrent immune suppression.

The HLA association we report is at least equivalent in effect to the association of HLA-B*57:01 with hypersensitivity to abacavir;[29] treatment with abacavir is contraindicated in carriers of this risk allele and in the smaller group of risk-allele negative, drug-reactive patients.[41] Similar to recent reports,[42] we observed onset of severe delayed drug reaction as early as 3 days after first exposure but also after months of treatment. Thus, our results are relevant for short-term use of the implicated inhibitors and highlight the need for continued surveillance for DHR over time. Some patients with Still's disease without the HLA risk alleles also suffered severe inhibitor-triggered DRESS reactions, including fatalities. Attention to signs of hypersensitivity to these drugs is prudent whenever they are used.

The frequency of the risk alleles across populations, the strength of the HLA association, and reaction severity, argue for prepresscription risk analysis. HLA testing is readily available and typically offered at reasonable cost. However, our data are insufficient as a basis for specific recommendations on when or if it is safe to use these inhibitors in patients with Still's disease with the reaction-associated HLA haplotypes. Further research is needed to determine underlying mechanisms, additional risk factors for

DRESS reactions to inhibitors of IL-1 and IL-6, and relevance in other conditions, particularly inflammatory diseases.

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**Correction notice** This article has been corrected since it published Online First. The twitter handle for Sampath Prahalad has been corrected.

**Twitter** Sampath Prahalad @prahaladpedrheum and Scott Canna @canna_lab

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**Contributors** Each author reviewed and approved the manuscript. EDM and VES are co-guarantors of this study, had access to the data, accept full responsibility for the work and conduct of the study and controlled the decision to publish. VES contributed to study design, collected and analysed clinical data, wrote and revised the manuscript. EDM contributed to study design, supervised collection and analysis of data, wrote and revised the manuscript. MJO contributed to study design, analysis and interpretation of HLA data, provided clinical data, genetic data and samples from NCT03510442, and wrote and revised the manuscript. JAH contributed to study design, analysis and interpretation of HLA data, and wrote and revised the manuscript. AHT, CS, JCB, SP, SC, TH, EC, OKP, AMS and IF provided data and patient samples. GM-M and MAF-V provided analysis and interpretation of HLA data. GD and ST provided images and analyses of tissue pathology. EFR, DM, JX, VM and DR analyzed sequence data. LT provided statistical analysis. JSRD and GTC provided intellectual contributions. DG provided figures.

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**Data availability statement** Data are available upon reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information. The INCHARGE consortium dataset, which was previously published (Ombrello MJ et al PNAS 2015) and used *in silico* for this study, is available through Dr Michael Ombrello.



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CLINICAL SCIENCE

# Importance of the second SARS-CoV-2 vaccination dose for achieving serological response in patients with rheumatoid arthritis and seronegative spondyloarthritis

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## ABSTRACT

**Objectives** To assess the kinetics of humoral response after the first and second dose of messenger RNA (mRNA) vaccines in patients with inflammatory joint diseases compared with healthy controls (HC). To analyse factors influencing the quantity of the immune response.

**Methods** We enrolled patients with rheumatoid arthritis (RA) and seronegative spondyloarthritis (SpA), excluding those receiving B-cell depleting therapies and assessed the humoral response to mRNA vaccines after the first and the second dose of the vaccine in terms of seroconversion rate and titre. We compared the results to a HC group and analysed the influence of therapies as well as other characteristics on the humoral response.

**Results** Samples from 53 patients with RA, 46 patients with SpA and 169 healthy participants were analysed. Seroconversion rates after the first immunisation were only 54% in patients with inflammatory arthritis compared with 98% in the HC group. However, seroconversion rates were 100% in all groups after second immunisation. Patients developed reduced antibody titres after the first vaccination compared with HC, but there was no difference after the second dose. While disease modifying anti-rheumatic drug (DMARD) monotherapy did not affect antibody levels, seroconversion rates as well as titre levels were reduced in patients receiving a combination of DMARDs compared with HC.

**Conclusions** Patients with inflammatory joint diseases under DMARD therapy show impaired humoral responses to the first vaccine dose but excellent final responses to vaccination with mRNA vaccines. Therefore, the full course of two immunisations is necessary for efficient vaccination responses in patients with inflammatory arthritis under DMARD therapy.

## Key messages

**What is already known about this subject?**

► Some patients with inflammatory arthritis treated with specific immunomodulatory drugs might be at risk for severe COVID-19 infection. Immunisation is therefore important to prevent disease. However, immunomodulatory therapies might interfere with successful immunisation. It is therefore important to develop vaccinations strategies for these patients.

**What does this study add?**

► The response of patients with inflammatory arthritis treated with immunomodulatory therapies to the first vaccination with messenger RNA vaccines is impaired compared with a healthy control (HC) group. However, after the second dose of the vaccine, patients with inflammatory arthritis mount antibody responses indistinguishable from HC.

**How might this impact on clinical practice or future developments?**

► These data suggest that the second dose of the primary vaccine series are critical for patients with inflammatory arthritis to develop a full vaccination response. Assessment of the vaccination response is not possible after the first vaccination. Moreover, non-pharmaceutical protective measures are mandatory until completion of the full vaccination schedule in these patients.



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## INTRODUCTION

Infectious diseases are a major issue in medicine, as illustrated by the dramatic events of the current COVID-19 pandemic. Of particular concern are patients with a compromised immune system, including those suffering from immune-mediated diseases. In addition to an altered immune system as part of their underlying disease, these patients are often treated with immunomodulating therapies. Vaccinations are of paramount importance to reduce the morbidity of infectious diseases. Immunocompromised patients are of particular concern with regards to vaccination responses, as their disease or treatment might interfere with vaccine efficiency, as it was shown in various instances for vaccines against influenza, tetanus toxoid or pneumococcal antigens.[1–3]

During the COVID-19 pandemic, vaccination became one of the fundamental cornerstones of the fight against this disease, and highly efficient

vaccines have been developed and licensed at phenomenal speed.[4–7] The European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) recommend vaccination in all patients with rheumatological diseases, even as there is currently insufficient data to formulate data-driven recommendations for vaccination strategies for different diseases or for patients receiving specific immunomodulatory therapies.[8 9] Therefore, data on the efficacy and safety of COVID-19 vaccines in patients with immune-mediated diseases are needed. There have been first reports on this topic, and, not surprisingly, vaccination efficiency has been demonstrated to be reduced as compared with the general population, in particular for patients receiving B-cell depleting therapies.[10 11] Methotrexate and glucocorticoids also have been shown to hamper immunogenicity of SARS-CoV-2 vaccines, with surprisingly little effects of targeted anti-cytokine treatments, often analysed only after one immunisation/one time point.[12–18] However, the kinetics of the humoral response in patients with inflammatory joint diseases treated with immunomodulatory drugs have not been analysed yet.

Here we report the response of patients with rheumatoid arthritis (RA) and seronegative spondyloarthritis (SpA) to vaccination with messenger RNA (mRNA) vaccines after the first and the second dose. We show that in patients with inflammatory joint diseases under immunomodulatory therapy, excluding those who are treated with B-cell depleting agents, vaccination responses are very efficient, with a seroconversion rate of 100% after two vaccinations, but only 50%–60% after the first vaccination. These data highlight the need for a second immunisation especially in patients with inflammatory joint diseases to obtain a sufficient serological vaccine response.

## MATERIALS AND METHODS

### Patients

Patients with clinical diagnosis of established RA or SpA (including psoriatic arthritis and peripheral and axial SpA), respectively, followed routinely at our outpatient clinic were enrolled. All patients were vaccinated twice with an mRNA vaccine. Serum samples were stored at the Biobank of the Medical University of Vienna, a centralised facility for the preparation and storage of biomaterial with certified quality management (ISO 9001:2015).[19] Antibodies against the receptor-binding domain and the nucleocapsid protein were determined.

Individuals without known inflammatory rheumatic disease and no current intake of any immunomodulatory therapy including glucocorticoids who were vaccinated twice with an mRNA vaccine served as healthy controls (HC). Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

*Anti-SARS-CoV-2 testing:* The Elecsys Anti-SARS-CoV-2 S immunoassay was used for the quantitative determination of antibodies to the receptor-binding domain of the viral spike (S) protein.[20] The quantitation range is between 0.4 and 2500.0 binding antibody units (BAU)/mL. Previous SARS-CoV-2 infection was ruled out by measuring nucleocapsid-specific antibodies with the qualitative Elecsys Anti-SARS-CoV-2 assay.[21] Both tests were performed on a cobas e801 analyzer (Roche Diagnostics, Rotkreuz, Switzerland) at the Department of Laboratory Medicine, Medical University of Vienna (certified acc. to ISO 9001:2015 and accredited acc. to ISO 15189:2012).

### Statistical analysis

Variables are depicted as medians and IQRs or means and SD (m±SD), depending on their distribution. To investigate differences in anti-SARS-CoV-2 S protein titre between rheumatic diseases and HC, either Student's t-test, Mann-Whitney-U test or one-way analysis of variance or Kruskal-Wallis test were used, depending on the distribution. Seroconversion rates were compared using $\chi^2$ and Fisher's exact test. In univariate analyses, association of anti-SARS-CoV-2 S titre with patient and disease characteristics were investigated using Spearman correlation coefficient and logistic regression analyses were implemented to assess the association of relevant variables with seroconversion rates. Cross-sectional analyses were performed in the total population, longitudinal analyses only in those with two titres available. GraphPad Prism (V.9.1.0) and IBM SPSS Statistics (V.26) were used for the statistics and graphical presentation of the data.

## RESULTS

Ninety-nine patients (53 with RA and 46 with SpA) and 169 HC were included in the study. The demographic characteristics are shown in table 1. We obtained serological responses of 72 patients and 136 HC 2–3 weeks after the first immunisation (mean after 19.6 days for patients and after 18.9 days for HC) and of 89 patients 3–6 weeks after the second immunisation (mean 29.1 days for patients and mean 24.3 days for HC); 63 patients and 145 HC had data on both time points.

### Seroconversion rates and respective antibody titres after first and second immunisation

Seroconversion rates after the first dose of an mRNA vaccine were significantly lower in our patient cohort (52.5% of the patients with RA and 54.8% of the patients with SpA) compared with the healthy control group (98%) (figure 1A). Similar results were obtained analysing patients with data on both time points (online supplemental figure 1A). Seroconversion rates were 100% in all patients after the second dose of the vaccine and in all healthy controls (figure 1A). After the first immunisation, median titre levels of antibodies directed against the spike protein of SARS-CoV-2 were significantly lower in patients with RA (median 0.61 (IQR 0–17) BAU/mL), and SpA (median 1.65 (IQR 0–55.1) BAU/mL) compared with HC (median 43.3 (IQR 14.4–191), figure 1B). However, after the second immunisation, no differences in median titre levels were observed between patients with either RA or SpA and healthy controls (median (IQR) RA 1188 (263.5–2500) BAU/mL vs SpA 1785 (410.8–2500) BAU/mL vs HC 1614 (716–2500) BAU/mL) (figure 1C).

In longitudinal analyses of patients (RA and SpA) for whom we had data both after first and second immunisation (n=63), the titres increased markedly after receiving the second vaccine dose in all patients except for one (median (IQR) change RA 1700.4 (292.8–2455.5) BAU/mL and SpA 1270 (89.6–2445.3] BAU/mL) (online supplemental figure 2).

### Factors associated with seroconversion rates and antibody-titres

Analysing factors that influenced the immunisation efficiency, we found that patients receiving disease modifying anti-rheumatic drug (DMARD) combination therapy consisting of a conventional synthetic DMARD (csDMARD) and a biological/targeted synthetic DMARD (b/tsDMARD) had significantly lower rates of seroconversion after the first immunisation compared with csDMARD or b/tsDMARD monotherapy (combination therapy (n=15), 26.7% vs csDMARD (n=28), 60.7% or b/tsDMARDs mono (n=27), 61.5 %) or healthy controls (98%; figure 2A). Again, similar results were obtained when analysing patients

**Table 1** Characteristics of patients and controls. Age, CRP and prednisolone dose are shown as mean (±SD)

| | RA (N=53) | PsA/SpA (N=46) | HC (N=169) |
|---|---|---|---|
| Age (years) | 56.15 (±11.80) | 51.33 (±12.92) | 46.18 (±12.56) |
| Female | 68% (n=36) | 48% (n=22) | 59% (n=100) |
| Male | 32% (n=17) | 52% (n=24) | 41% (n=69) |
| csDMARD | | | |
| Methotrexate | 30 | 24 | None |
| Leflunomide | 3 | 2 | None |
| Azathioprine | 2 | | None |
| Hydroxycholorquine | 4 | | None |
| Salazopyrin | 2 | 2 | None |
| TNF inhibitor | | | |
| Adalimumab | 4 | 13 | None |
| Certolizumab | | 2 | None |
| Etanercept | 2 | 3 | None |
| Golimumab | 6 | 5 | None |
| Infliximab | 2 | 2 | None |
| IL-17 inhibitor | | | |
| Secukinumab | | 5 | None |
| Ixekizumab | | 4 | None |
| IL-6 inhibitor | | | |
| Tocilizumab | 3 | | None |
| JAK inhibitor | | | |
| Baricitinib | 3 | | None |
| Upadacitinib | 1 | 2 | None |
| Filgotinib | 1 | | None |
| Apremilast | | 2 | None |
| No therapy | 2 | 2 | 169 |
| Disease duration in years | 12.53 (±10.75) | 10.26 (±8.8) | |
| Treatment duration in months | 61.25 (±65.35) | 44.79 (±49.8) | |
| Disease activity | | | |
| SJ count (28)<br>SJ=0: 63%<br>SJ ≥1: 37% | 1.24 (±2.34) | 0.64 (±1.23) | |
| TJ count (28) | 2.71 (±3.55) | 4.15 (±8.59) | |
| CRP | 0.35 mg/dL (±0.63) | 0.30 mg/dL (±0.38) | |
| Seropositive | N=24 | | |
| Prednisolone dose | | | |
| Patients without prednisolone | N=39 | N=38 | N=169 |
| Patients with daily prednisolone at 1. vaccination | N=7<br>Mean dose: 5.53 mg/day (±4.19) | N=3<br>Mean dose: 8.33 mg/day (±3.82) | None |
| Patients without prednisolone | N=38 | N=38 | N=169 |
| Patients with daily prednisolone at 2. vaccination | 7.31 mg/day (±7.73) N=8 | 8.33 mg/day (±3.81) N=3 | None |

CRP, C-reactive protein; csDMARD, conventional synthetic DMARD; HC, healthy controls; IL, interleukin; JAK, Janus kinase; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SJ, swollen joint; SpA, seronegative spondyloarthritis; TJ, tender joint; TNF, tumour necrosis factor.

with data on both time points (online supplemental figure 1B). Patients on combination therapies also developed significantly lower anti-SARS-CoV-2 S titres after the second immunisation compared with both patients on csDMARD monotherapy and HC. In contrast, there was no difference between patients receiving csDMARD or b/tsDMARD monotherapy and HC (median (IQR) combination 260.5 (96.4–1931.5) BAU/mL vs csDMARD 2062 (771–2500) BAU/mL vs b/tsDMARD 1292 (346.5–2500) BAU/mL vs HC 1614 (716–2500) BAU/mL; figure 2B).

In univariate analyses we found a moderate but significant correlation of the anti-SARS-CoV-2 S titres after the first and after the second immunisation in the patient cohort ($r=0.41$, $p<0.01$) as well as in HC ($r=0.3$, $p<0.01$). Further, we found an inverse correlation between age and anti-SARS-CoV-2 S titre after first and second vaccination in patients as well as healthy controls (online supplemental table 1). As our patient cohort is significantly older than our HC cohort, we performed age matching (±5 years) and analysed seroconversion rates and anti-SARS-CoV-2 S titres after the first immunisation as a sensitivity analysis. As in our initial analysis, we found reduced seroconversion rates in our patient cohort (54.8% (n=72) vs 95.2% in HC; n=72) and reduced anti-SARS-CoV-2 S titres (median (IQR) patient cohort compared with HC (patients 0.92 (0–28.6) BAU/mL vs HC 23.55 (6.6–132.0) BAU/mL (online supplemental figure 3A). In addition, we found no difference in age within the patient cohort between those who seroconverted after the first immunisation and those who did not (online supplemental figure 3B), suggesting that reduced vaccination responses in our patient cohort after the first immunisation is not driven by differences in age.

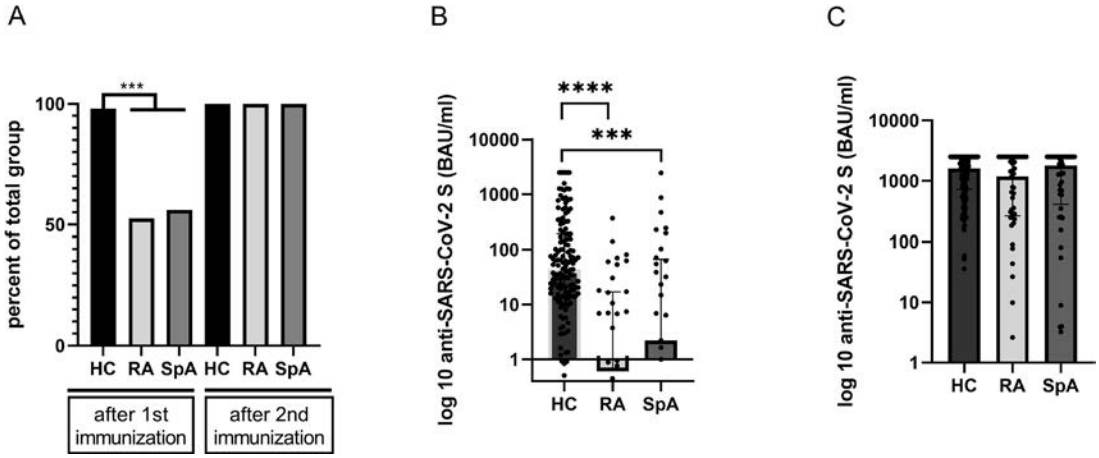


**Figure 1** (A) Seroconversion in percent was analysed in patients with rheumatoid arthritis (RA), spondyloarthritis (SpA) and healthy controls (HC) after the first and second vaccination. (B) Analysis of anti-SARS-CoV-2 viral spike (S) titres after the first vaccination in patients with RA, SpA and HC. (C) Analysis of anti-SARS-CoV-2 S titres after the second vaccination in patients with RA, SpA and HC (**p≤0.01; ***p≤0.005, ****p≤0.001). BAU, binding antibody units.

In an exploratory analysis, we analysed the effect of individual therapies on vaccination responses. When comparing individual treatments to the total HC group, we find that all regimens analysed (methotrexate (MTX) monotherapy (n=21 after first and n=15 after second immunisation), interleukin-17i monotherapy (n=3 after first and n=7 after second immunisation), tumour necrosis factor inhibitor (TNFi) monotherapy (n=19 after first, n=22 after second immunisation), TNFi +MTX (n=9 after first and n=10 after second immunisation), Janus kinase inhibitor +csDMARD) (n=3 after first and n=4 after second immunisation), showed reduced anti-SARS-CoV-2 S titres after the first immunisation. No differences were detected after the second immunisation (online supplemental figure 4A,B).

We did not detect an association between glucocorticoids or systemic inflammation, measured as C-reactive protein in serum and anti-SARS-CoV-2 S titre development both after the first or second immunisation, although the usage of glucocorticoids was negligible in our cohort (see table 1). In addition, we did not detect differences in anti-SARS-CoV-2 S titres after the first and after the second immunisation between patients with active arthritis (swollen joint count (SJC) >0) and patients with no active arthritis (SJC=0) (online supplemental figure 5A,B). In univariate logistic regression models OR predicting seroconversion rates after first immunisation were significant for combination therapy (OR 0.24, 95% CI 0.06 to 0.93). No significant effect of glucocorticoids, age or gender could be identified (see figure 2C, online supplemental table 2). Furthermore, we have analysed the role of age, gender, therapy and glucocorticoids in a multivariate regression model, with similar results (online supplemental figure 6).

We detected no change in disease activity after immunisation that required modification of the DMARD therapy. One patient, however, developed a swollen wrist after the first immunisation that was treated with a short course of glucocorticoids. Analysing adverse events, we found increased incidences of local reactions, fatigue and myalgia, but decreased incidences of fever, nausea, shivering and sweating in our patient cohort compared with HC (figure 3).

## DISCUSSION

Our study on the kinetics of the humoral response in patients with inflammatory arthritis reveals that while the immunisation efficiency after two doses of an mRNA vaccine is comparable to

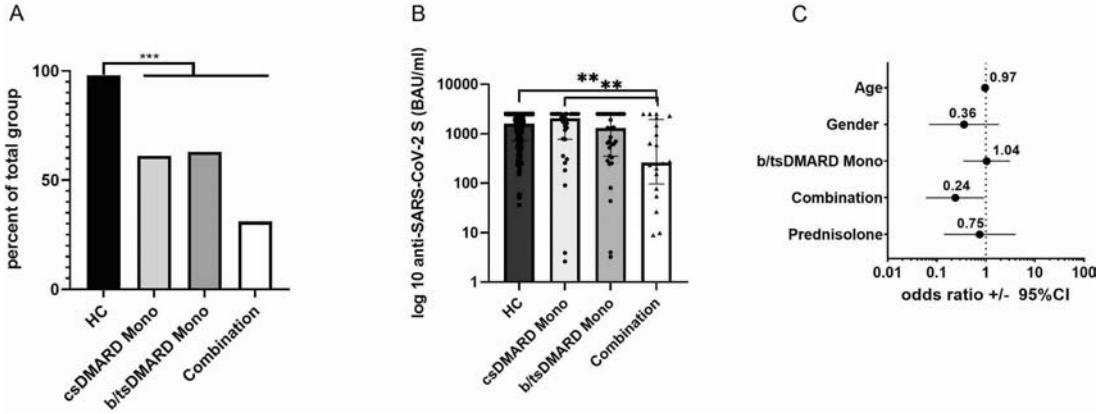


**Figure 2** (A) Seroconversion rates after the first vaccination in patients treated with csDMARD or b/tsDMARD or a combination of csDMARD and b/tsDMARD. (B) Analysis of anti-SARS-CoV-2 S titres in patients treated with csDMARD or b/tsDMARD or a combination of a csDMARD and a b/tsDMARD. (C) ORs of univariate logistic regression assessing seroconversion in patients with RA and SpA after first immunisation (csDMARD used as reference category). (**p≤0.01; ***p≤0.005). BAU, binding antibody units; b/tsDMARD, biological/targeted synthetic DMARD; csDMARD, conventional synthetic DMARD; DMARD, disease-modifying anti-rheumatic drug; HC, healthy controls.

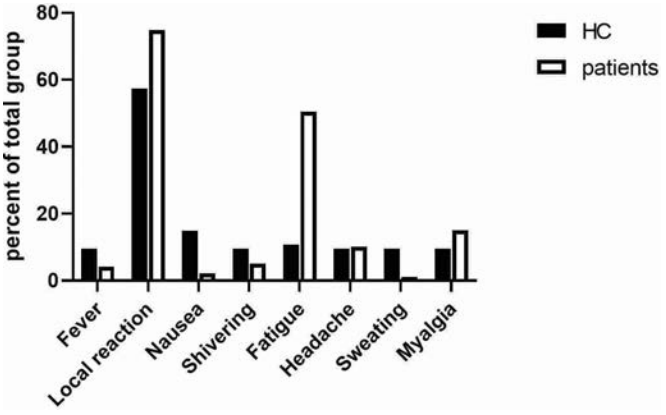


**Figure 3** Adverse events after immunisation in healthy controls (HC) and the patient cohort.

HC, the response to only a single dose of the vaccine is significantly reduced. While it is unclear what titre is necessary to fully protect people from COVID-19 disease, it is obvious that any measurable humoral immune response against anti-SARS-CoV-2 S protein is a prerequisite for a protective vaccination response. While we did not measure neutralising antibodies in our study, there is increasing evidence that titres of antibodies directed against the S protein measured in our study are a very good approximation of vaccine efficiency,[22] and as it has been shown earlier, that titres and neutralising antibodies are highly correlated.[10]

The current study suggests that most patients with inflammatory joint diseases need both vaccinations to develop a substantial antibody response. It is therefore important for the management of patients with inflammatory joint diseases that non-pharmaceutical protective measures are mandatory until completion of the full vaccination schedule. Seroconversion rates after the first vaccination of our patients with arthritis are significantly below those of healthy controls, which in our hands as well as in previous reports were beyond 90% in the age groups comparable to our cohort.[23] It is noteworthy that seroconversion rates in this patient group after the first vaccination are even smaller than in patients with cancer, which were reported to have a 83% response rate after the first vaccination.[24] We need to highlight, that evaluation of the vaccination response in patients with inflammatory arthritis should be performed after the second vaccine dose, as seroconversion rates and titres after the first immunisation are low and do not predict successful immunisation after the full course of two doses.

It is reassuring, however, that after full immunisation with two doses of an mRNA vaccine, the seroconversion rate in our patient group was 100%. Overall, our data suggests that the response of patients with rheumatic diseases to single-dose vaccines needs to be evaluated before it can safely be recommended, and initial analyses indeed suggest reduced efficacy in patients with rheumatic diseases.[25] It is important to keep in mind that we excluded patients receiving B-cell depleting therapies from our study, who were reported to have significantly reduced seroconversion rates even after full immunisation.[10 13] Other studies have reported reduced immunogenicity of SARS-CoV-2 vaccines in patients on MTX,[16] which might be explained by the different age of the patient groups, especially since we could demonstrate a significant inverse relation between vaccination efficiency and age in our arthritis cohort, which is younger than those reported previously, or the time point of the analysis of the serological response.[26] In our cohort, we found that only patients treated with a combination of DMARDs develop reduced titres after completing the full vaccination course, but patients on csDMARD monotherapy show responses indistinguishable from healthy control. These data would argue against withholding csDMARDs after vaccinations, as currently suggested by the ACR.[27] It will be important to collect data for meta-analyses, which will then be able to address more in-depths questions in the future.

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# Risk and prognosis of SARS-CoV-2 infection and vaccination against SARS-CoV-2 in rheumatic and musculoskeletal diseases: a systematic literature review to inform EULAR recommendations

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## ABSTRACT

**Objectives** Perform a systematic literature review (SLR) on risk and prognosis of SARS-CoV-2 infection and vaccination against SARS-CoV-2 in patients with rheumatic and musculoskeletal diseases (RMDs).

**Methods** Literature was searched up to 31 May 2021, including (randomised) controlled trials and observational studies with patients with RMD. Pending quality assessment, data extraction was performed and risk of bias (RoB) was assessed. Quality assessment required provision of (1) an appropriate COVID-19 case definition, and (2a) a base incidence (for incidence data) or (2b) a comparator, >10 cases with the outcome and risk estimates minimally adjusted for age, sex and comorbidities (for risk factor data).

**Results** Of 5165 records, 208 were included, of which 90 passed quality assessment and data were extracted for incidence (n=42), risk factor (n=42) or vaccination (n=14). Most studies had unclear/high RoB. Generally, patients with RMDs do not face more risk of contracting SARS-CoV-2 (n=26 studies) or worse prognosis of COVID-19 (n=14) than individuals without RMDs. No consistent differences in risk of developing (severe) COVID-19 were found between different RMDs (n=19). Disease activity is associated with worse COVID-19 prognosis (n=2), possibly explaining the increased risk seen for glucocorticoid use (n=13). Rituximab is associated with worse COVID-19 prognosis (n=7) and possibly Janus kinase inhibitors (n=3). Vaccination is generally immunogenic, though antibody responses are lower than in controls. Vaccine immunogenicity is negatively associated with older age, rituximab and mycophenolate.

**Conclusion** This SLR informed the July 2021 update of the European Alliance of Associations for Rheumatology recommendations for the management of RMDs in the context of SARS-CoV-2.

## INTRODUCTION

In April 2020, the European Alliance of Associations for Rheumatology (EULAR) commissioned provisional recommendations for the management of patients with rheumatic and musculoskeletal diseases (RMDs) in the context of SARS-CoV-2, the virus, causing the disease COVID-19, which has gripped the world since December 2019.[1] In the absence of an evidence base to inform those recommendations, those statements were based largely on expert opinion. However, the number of publications in this field has grown exponentially since then. In light of the newly accrued data with the opportunity to provide evidence-based guidance, it was therefore time to update the April 2020 recommendations. This paper presents the systematic literature review (SLR) on risk and prognosis of SARS-CoV-2 infection and vaccination against SARS-CoV-2 in patients with RMDs that accompanies the July 2021 update of the recommendations.

## METHODS

### Research questions

This SLR was used to inform the EULAR task force for the July 2021 update of the recommendations for the management of RMDs in the context of SARS-CoV-2. The task force outlined the scope of the literature search by defining five research questions according to the Participants, Interventions, Comparators, Outcomes format (see online supplemental material)[2]:

1. Do patients with RMDs face more risk of contracting SARS-CoV-2?
2. Do patients with RMDs have a worse prognosis when contracting SARS-CoV-2?
3. In patients with RMDs who contract SARS-CoV-2, is antirheumatic medication associated with a worse outcome?
4. Should patients with RMDs who contract SARS-CoV-2 continue their drug treatment?
5. What evidence informs the use of vaccination against SARS-CoV-2 in patients with RMDs?

Effects of the SARS-CoV-2 pandemic on the referral and monitoring of patients with RMDs (eg, (postponement of) regular blood monitoring and face-to-face consultation) were not included as a separate research question, as this will be investigated by a separate EULAR task force.

### Literature search

A systematic search was conducted in PubMed/MEDLINE, Embase, Cochrane CENTRAL and the WHO COVID-19 databases up to 31 May 2021 by an experienced librarian (JWS). Additionally, conference abstracts of the EULAR 2021 annual conference were screened. No language restrictions were applied. Papers only published on a preprint server were excluded, unless they provided evidence on vaccination (in order not to miss relevant studies on this novel subject). The search strategy can be found in the online supplemental material.

The review focused on available evidence specifically in patients with RMDs and was not intended to summarise evidence for the prevention, diagnosis, treatment or prognosis of SARS-CoV-2 infection in the general population. While the EULAR recommendations will focus primarily on the management of patients with autoimmune inflammatory rheumatic diseases, studies including patients with other types of ('non-inflammatory') RMD were not excluded. Studies including participants with non-RMD diagnoses were only eligible if the results were presented separately for participants with RMDs or if ≥75% of the study population had an RMD.

Studies with a comparator were viewed higher in the hierarchy of evidence, though studies without a comparator were not a priori excluded. All outcomes relevant for the research questions were extracted without specific hierarchy.

Eligible study types were (randomised and non-randomised) controlled trials (RCT/CCT) and observational studies (cohort, case–control, cross-sectional; prospective or retrospective, including registries). The following hierarchy of study design was adopted: RCT/CCT, prospective observational longitudinal cohort study, retrospective observational longitudinal cohort study, case–control study, cross-sectional study.

Studies were excluded when the number of participants was lower than 75 (arbitrary cut-off), with the exception of studies on vaccination against SARS-CoV-2. Studies that were not published as a full-text manuscript were only eligible if the authors provided sufficient data to extract information on the population, intervention, comparator and study outcomes.

### Study selection, data extraction and risk of bias (RoB) assessment

Two reviewers (FPBK and AA/AN) independently screened titles and abstracts, and thereafter the full-text for eligibility. The same reviewers performed a quality assessment of included studies, based on predefined criteria set by the steering group as minimal requirements to justify data extraction. All studies were required to have an appropriate case definition of COVID-19, defined as a positive SARS-CoV-2 PCR+ test, serological antibody response, typical imaging abnormalities on X-ray or CT, physician diagnosis, International Classification of Diseases, 10th Revision (ICD)-10 diagnostic code or fulfilment of WHO diagnostic criteria set. Studies with data on incidence of COVID-19 in a RMD population or prevalence of RMDs in a COVID-19 population were required to report a base incidence of the outcome in the base population (ie, population from which the study was sampled) to be able to compare the reported and the base incidence. Studies with data on risk factors for development or worse prognosis of COVID-19 were required to (1) include a comparator, (2) have at least 10 cases with the outcome and (3) provide risk estimates at least adjusted for age, sex and comorbidities.

Data from eligible studies were extracted by one reviewer (FPBK) and verified by a second reviewer (AA/AN) using a standardised data-extraction form.

RoB of all studies was assessed in duplicate by junior (AA/AN/FPBK) and senior (PMM/RBML/VN-C) reviewers using an appropriate tool depending on the study type: Newcastle-Ottawa Scale was used for longitudinal observational cohort and case–control studies,[3] and the AXIS tool was used for cross-sectional studies.[4] For the final RoB judgement, an additional weighting was applied, in which studies were *not* rated low RoB when (1) possible selection bias had not been recognised and somehow adjusted for; (2) selection bias was irreparable by design (eg, voluntary enrolment of SARS-CoV-2-positive cases); or (3) ascertainment of cases, exposure or outcome was uncertain.

For study selection, quality assessment, data extraction and RoB assessment, disagreements were discussed until consensus was reached, and a third reviewer (PMM/RBML/VN-C) was involved whenever necessary.

## RESULTS

Of 5165 records (after deduplication), 501 were selected for full-text review and 208 articles were included (see flowchart in online supplemental figure 1). Of these, 90 articles passed quality assessment and were eligible for data extraction of incidence data (n=42), risk factor data (n=42) or vaccination data (n=14). The most important reasons for a negative quality assessment were lack of a base incidence, having no comparator or presentation of risk estimates with no minimal adjustment for age, sex and comorbidities (see online supplemental tables 1 and 2) for an overview of studies that did not pass quality assessment). The detailed RoB assessment is provided in online supplemental tables 3–5.

### Incidence of (severe) COVID-19 in patients with RMDs

#### Incidence of COVID-19

In total, 26 studies reported on the incidence of COVID-19 in patients with RMDs (online supplemental table 6). Most (n=17) were cross-sectional studies; 8 were retrospective; and 1 was a prospective study. The number of patients varied from 25[5] to 39 835[6] patients with RMD with 1[7]–199[8] COVID-19 cases. All but two studies were performed in the first wave of the pandemic.[9 10] Most studies included multiple inflammatory RMDs (n=13) or any type of RMD (n=5). COVID-19 diagnosis was defined as PCR+ (n=18), a combination of laboratory testing, imaging or symptoms (n=6) or through diagnostic criteria (n=2). RoB was high (n=15) or unclear (n=10) in most studies. The reported incidence of COVID-19 in patients with RMDs varied substantially (0.16%–0.36%), with a similar variation in the base population. Compared with the general population, most studies reported an equal incidence (n=19); six reported a higher incidence (n=5 with patients with various RMDs, n=1 with patient with systemic lupus erythematosus (SLE)) and one a lower incidence. Three studies assessed age-adjusted and sex-adjusted incidence rates,[11–13] of which one was at low RoB, reporting an equal incidence of COVID-19 in patients with RMD and the general population.

#### Incidence of severe COVID-19

Eleven studies investigated the incidence of COVID-19-related hospitalisation (table 1). All were retrospective studies, from the first wave of the pandemic. Study size varied from 8[14] to 110 567[15] patients with RMD with 1[16]–581[15] hospitalisations. Four studies had a high or unclear RoB, while three were at low RoB. The reported hospitalisation rate in patients with RMDs varied substantially (0.11%–44%), as did the hospitalisation rate in the general population. Compared with the general population, six studies found a higher hospitalisation rate, while four studies reported an equal and one a lower incidence of hospitalisation. Only three studies (low RoB) investigated age-adjusted and sex-adjusted hospitalisation rates[11 15 17]; among these, Bower *et al* found that the increased risk of hospitalisation for COVID-19 was comparable to the increased risk of all-cause hospitalisation in patients with RMD.[15]

Six studies, five of which were retrospective and all were conducted during the first wave of the pandemic, assessed the

**Table 1** Studies with data on the incidence of severe COVID-19 in patients with RMDs compared with persons without RMDs

| First author | Country | Cohort | Study period | Study type | Setting | Study population, recruitment | Case definition RMD | Case definition COVID-19 | Source population of base incidence | Total (N) | Incidence in patients with RMD | Base incidence | Incidence in patients with RMD versus base population (higher, equal, lower) | RoB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (A) Outcome: Hospitalisation for COVID-19 | | | | | | | | | | | | | | |
| Bachiller-Corral[72] | Spain | – | 1 March–30 April 2020 | Retrospective | Secondary care | Patients with inflammatory RMD in hospital area of care, hospital records | Physician diagnosis | PCR+ or typical imaging | Non-RMD population in hospital area of care | 4592 | 41/4592 (0.89%) (all), 16/1708 (0.94%) (RA), 3/862 (0.35%) (SpA), 4/515 (0.78%) (PsA), 4/254 (1.57%) (SLE), 4/175 (2.29%) (SjS), 3/165 (1.82%) (vasculitis), 1/88 (1.14%) (IM), 6/474 (1.27%) (PMR) | 2274/488 153 (0.47%) | Higher for all (OR 1.91, 95% CI 1.41 to 2.61), RA (OR 2.01, 95% CI 1.23 to 3.28), SLE (OR 3.38, 95% CI 1.28 to 8.95), SjS (OR 4.90, 95% CI 1.86 to 12.94), vasculitis (OR 3.90, 95% CI 1.27 to 11.99), PMR (OR 2.71, 95% CI 1.23 to 6.02); equal for SpA (OR 0.74, 95% CI 0.24 to 2.31), PsA (OR 1.66, 95% CI 0.63 to 4.43), IM (OR 2.43, 95% CI 0.35 to 17.13) | |
| Bjornsson[11] | Iceland | ICEBIO | Until 3 June 2020 | Retrospective, registry, matched | Population-based | tsDMARD/bDMARD-treated patients with RMD and MTX-treated patients with RMD; data from national registries | Physician diagnosis | PCR+ | General population registries, matched (age, sex, location) | 39 961 | 3/9 (33%)(tsDMARD/bDMARD), 1/5 (20%) (MTX) | 3/84 (3.6%)(tsDMARD/bDMARD controls), 13/134 (9.7%) (MTX controls) | Higher for tsDMARD/bDMARD (RR 9.33, 95% CI 2.20 to 39.6) and MTX (RR 6.22, 95% CI 1.19 to 32.46) | |
| Bower[15] | Sweden | – | May–September 2020 | Retrospective, registry, matched | Population-based | Patients with inflammatory arthritis, data from national registries | Physician diagnosis | ICD-10 | General population registries, matched (age, sex, location) | 110 567 | 581/110 567 (0.5%) (all), 379/53 455 (0.7%) (RA), 202/57 112 (0.4%) (other IJD) | 1443/484 277 (0.3%) (all controls), 784/484 277 (0.4%) (RA controls), 659/484 277 (0.3%) (other IJD controls) | Higher for all (fully adjusted HR 1.32, 95% CI 1.19 to 1.46), RA (HR 1.40, 95% CI 1.22 to 1.60), other IJD (HR 1.20, 95% CI 1.02 to 1.41) Comparable to increased all-cause hospitalisation risk | |
| Comarmond[14] | France | – | 4–20 May 2020 | Retrospective | Secondary care | Patients with TAK or GCA, followed up at outpatient clinic | Physician diagnosis | PCR+ or typical CT imaging or serology+ | Modelled risk in country | 148 | 3/8 (37.5%) | 9.6% (age 70–79), 21% (age>80) | Higher | |
| Cordtz[17] | Denmark | DANBIO | 1 March–12 August 2020 | Retrospective, registry | Population-based | tsDMARD/bDMARD-treated patients with RA, SpA, CTD or vasculitis; data from national registries | Physician diagnosis | ICD-10 | General population registries | 58 052 | 69/58 052 (age-adjusted and sex-adjusted IR per 1000 py 1.73, 95% CI 1.34 to 2.23) | 2536/4.5 mln (age- and sex-adjusted IR per 1000 py 1.26, 95% CI 1.21 to 1.31) | Higher (HR 1.46, 95% CI 1.15 to 1.86) | |
| Fernandez-Gutierrez[26] | Spain | – | 1 March–15 Apr 2020 | Retrospective | Secondary care | Patients with Inflammatory RMD with COVID-19; all patients followed up at outpatient clinic from March 2019 to March 2020 | ICD-10 | PCR+or physician diagnosis | General population in region | 3951 (5896 patient-months) | 54/3,951 (1.36%; cumulative incidence 15 per 1000 patients; IR 9.15 (7–11.9) per 1000 patient-months) | 1059/325 900 (cumulative incidence 3.2 per 1000 persons; IR 4.6 (3.4–6.1) per 1000 person-months) | Higher (p<0.001) | |

Continued

**Table 1** Continued

| First author | Country | Cohort | Study period | Study type | Setting | Study population, recruitment | Case definition RMD | Case definition COVID-19 | Source population of base incidence | Total (N) | Incidence in patients with RMD | Base incidence | Incidence in patients with RMD versus base population (higher, equal, lower) | RoB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Flood[73] | Ireland | – | Until 3 June 2020 | Retrospective | Secondary care | Patients with RMD, all patients followed up at outpatient clinic | Physician diagnosis | PCR+ or physician diagnosis | General population in city | 7500 | 15% (Inflammatory RMD) | 13% | Equal | |
| Jovani[74] | Spain | – | Until 2 May 2020 | Retrospective | Secondary care | tsDMARD/bDMARD-treated patients with RMD, followed up at outpatient clinic | Physician diagnosis | PCR+ | General population in city | 1037 | 3/1037 (0.29%) | 306/274 122 (0.11%) | Equal (OR 2.61, 95% CI 0.84 to 8.16) | |
| Ramirez[16] (*COVID-19 in*) | Italy | – | 17 April to 27 April 2020 | Retrospective | Secondary care | Patients with SLE, all patients followed up at three outpatient clinics | Physician diagnosis | Self-reported PCR+ | General population in region | 417 | 1/417 (0.24%) | 42 889 hospitalised (0.43%) | Equal | |
| Salvarani[5] (*Susceptibility and*) | Italy | – | Until 24 April 2020 | Retrospective | Secondary care | tsDMARD/bDMARD-treated patients with RMD, treated since December 2019 (pharmacy data) | Physician diagnosis | PCR+ | General population in region | 1195 | 4/9 (44.4%) | 1342/3746 (35.8%) | Equal (p=0.73) | |
| Santos[75] (*Biological agents*) | Spain | – | NR | Retrospective | Secondary care | bDMARD-treated patients with RMD, treated between December 2019 and December 2020 (hospital records) | Physician diagnosis | PCR+ | General population in region | 820 | 4/820 (0.48%) | 4464 hospitalised (3.6%) | Lower | |
| (B) Outcome: COVID-19-related death | | | | | | | | | | | | | | |
| Aries[18] | Germany | Hamburg COVID-19 registry | Until 9 June 2020 | Cross-sectional, registry | Secondary care | DMARD-treated patients with RMD with COVID-19, cases reported by rheumatologists | Physician diagnosis | Symptoms and PCR+ or IgG+ | General population in region | 11 771 | 0/30 (0%) | 226/5120 (4.4%) | Lower | |
| Bower[15] | Sweden | – | May–September 2020 | Retrospective, registry, matched | Population-based | Patients with inflammatory arthritis, data from national registries | Physician diagnosis | ICD-10 | General population registries, matched (age, sex, location) | 110 567 | 161/110 567 (0.1%) (all), 134/53 455 (0.3%) (RA), 27/57 112 (0.05%) (other IJD) | 338/484 277 (0.07%) (all controls), 245/484 277 (0.11%) (RA controls), 93/484 277 (0.04%) (other IJD controls) | Higher for RA (fully adjusted HR 1.27, 95% CI 1.02 to 1.59)<br>Equal for all (fully adjusted HR 1.18, 95% CI 0.97 to 1.44), other IJD (0.83, 95% CI 0.54 to 1.28)<br>Comparable to increased all-cause mortality risk and not different from 2015 to 2019 | |
| Cleaton[76] | UK | – | 1 Febuary–1 May 2020 | Retrospective | Secondary care | Patients with RMD, followed up at outpatient clinic | Physician diagnosis | PCR+ | General population in region | 10 387 | 12/10 387 (0.12%) | 4131/7 415 149 (0.12%) | Equal | |
| Comarmond[14] | France | – | 4 May–20 May 2020 | Retrospective | Secondary care | Patients with TAK or GCA, followed up at outpatient clinic | Physician diagnosis | PCR+ or typical CT imaging or serology+ | Modelled risk in country | 148 | 1/8 (12.5%) | 2.2% (age 70–79), 8% (age >80) | Lower | |

Continued

**Table 1** Continued

| First author | Country | Cohort | Study period | Study type | Setting | Study population, recruitment | Case definition RMD | Case definition COVID-19 | Source population of base incidence | Total (N) | Incidence in patients with RMD | Base incidence | Incidence in patients with RMD versus base population (higher, equal, lower) | RoB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FAI Consortium[19] | France | French COVID-19 registry | Until 18 May 2020 | Retrospective, matched (age, sex, comorbidities) | Secondary care | Patients with inflammatory RMD and COVID-19; cases reported by rheumatologists | Physician diagnosis | PCR+ or serology+ or typical CT imaging or highly suspected based on clinical symptoms | Patients with COVID-19 from the Lille University Hospital COVID-19 Research Network | 694 | 58/256 hospitalised subgroup (22.6%); death rate in matched subgroup (n=175) 25.1% (95% CI 18.7% to 31.6%) | Death rate 18.9% (95% CI 13.1% to 24.7%) | Equal (OR 1.45, 95% CI 0.87 to 2.42) | |
| Salvarani[5] (*Susceptibility and*) | Italy | – | Until 24 April 2020 | Retrospective | Secondary care | tsDMARD/bDMARD-treated patients with RMD, treated since Dec 2019 (pharmacy data) | Physician diagnosis | PCR+ | General population in region | 1195 | 1/9 (11.1%) | 383/3746 (10.2%) | Equal (p=1.0) | |
| (C) Outcome: Other (ICU or 'severe COVID-19') | | | | | | | | | | | | | | |
| Aries[18] | Germany | Hamburg COVID-19 registry | Until 9 June 2020 | Cross-sectional, registry | Secondary care | DMARD-treated patients with RMD with COVID-19, cases reported by rheumatologists | Physician diagnosis | Symptoms and PCR+ or IgG+ | General population in region | 11 771 | ICU admission 3/30 (10%) | 227/5120 (4.4%) | Lower | |
| Bower[15] | Sweden | – | May–September 2020 | Retrospective, registry, matched | Population-based | Patients with inflammatory arthritis, data from national registries | Physician diagnosis | ICD-10 | General population registries, matched (age, sex, location) | 110 567 | ICU admission 45/110 567 (0.04%) (all), 31/53 455 (0.06%) (RA), 14/57 112 (0.6%) (other IJD) | 162/484 277 (0.01%) (all controls), 79/484 277 (0.02%) (RA controls), 83/484 277 (0.03%) (other IJD controls) | Equal for all (fully adjusted HR 1.18, 95% CI 0.97 to 1.44), RA (1.53, 95% CI 0.98 to 2.40), other IJD (0.83, 95% CI 0.54 to 1.28) | |
| Cordtz[17] | Denmark | DANBIO | 1 March–12 August 2020 | Retrospective, registry | Population-based | tsDMARD/bDMARD-treated patients with RA, SpA, CTD or vasculitis, data from national registries | Physician diagnosis | ICD-10 | General population registries | 29 440 | 'Severe COVID-19'* 22/47 (age-djusted and sex-adjusted IR per 1000 py 35.2, 95% CI 20.8 to 59.4) | 945/2536 (age-adjusted and sex-adjusted IR per 1000 py 32.7, 95% CI 30.7 to 34.9) | Higher (HR 1.43, 95% CI 0.80 to 2.53) | |

*Severe COVID-19 was only assessed in patients with RA and defined as need for mechanical ventilation (procedure code), ICD-10 code of acute respiratory distress syndrome due to COVID-19 or death following COVID-19. Colours denote overall RoB assessment of each study (green denotes low RoB; orange denotes unclear RoB; and red denotes high RoB).

bDMARD, biological disease-modifying antirheumatic drug; CTD, connective tissue disease; DMARD, disease-modifying antirheumatic drug; FAI, Filière des Maladies Autoimmunes et Autoinflammatoires Rares; GCA, giant cell arteritis; ICD-10, International Classification of Diseases, 10th Revision; ICU, intensive care unit; IJD, inflammatory joint disease; IM, inflammatory myopathy; IR, incidence rate; mln, million; MTX, methotrexate; NR, not reported; PMR, polymyalgia rheumatica; PsA, psoriatic arthritis; py, person years; RA, rheumatoid arthritis; RMD, rheumatic and musculoskeletal disease; RoB, risk of bias; SjS, Sjögren syndrome; SLE, systemic lupus erythematosus; SpA, spondyloarthritis; TAK, Takayasu arteritis; tsDMARD, targeted synthetic disease-modifying antirheumatic drug.

incidence of COVID-19-related death, including 8[14]–110 567[15] patients with RMD with 0[18]–161[15] deaths (table 1). Reported mortality rates in patients with RMDs varied considerably (0%–22.6%), with similar variation observed in the general population. Studies demonstrated an equal (n=4) or lower (n=2) risk of COVID-19-related death in patients with RMD compared with the general population. Two studies with age-matched and sex-matched analyses reported an equal incidence rate, of which one was at low RoB.[15 19] Of note, although Bower *et al* did report an increased risk of COVID-19-related death in the rheumatoid arthritis (RA) subgroup, they also demonstrated that this increased risk was comparable to the increased all-cause mortality risk in patients with RA and that the increased mortality risk in 2020 in patients with RA was not different from that in 2015 to 2019.

Finally, two studies reported on the risk of intensive care unit (ICU) admission and found an equal[15] or lower[18] risk of ICU admission for COVID-19 in patients with RMD compared with the general population (table 1). A large Danish registry study (low RoB) found that the risk of severe COVID-19 (a composite outcome including several COVID-19 complications) was higher in patients with RA compared with the general population, although the reported (non-significant) risk estimate did not seem to have a clinically relevant impact on a population-level (table 1).[17]

### Prevalence of RMDs in patients with COVID-19

Five studies (high RoB) investigated the prevalence of different RMDs in a COVID-19 population. Most reported an equal prevalence of RMDs compared with the general population, though some found a higher prevalence (online supplemental table 7).

## Risk factors for developing (severe) COVID-19

### Demographics

In total, 13 studies investigated the association between a variety of demographic factors and different COVID-19-related outcomes (online supplemental table 8). Generally, these studies found that evidence for well-known risk factors for developing (severe) COVID-19 in the general population, such as increased age, male gender and high body mass index (BMI), also applied to patients with RMDs. One USA-based study reported that the risk of hospitalisation, COVID-19-related death and severe COVID-19 is elevated in people from Afro-American, Latin–American, Asian or other/mixed race compared with people from the white race.[20]

### Comorbidities

The risk of various common comorbidities for developing (severe) COVID-19 in patients with RMDs was investigated in 14 studies (online supplemental table 9). Associations are similar to those known from the general population, such as cardiovascular disease, diabetes mellitus, chronic lung disease and chronic kidney disease.

### RMD type

In total, 19 studies assessed the association between type of RMD and the risk of contracting SARS-CoV-2 (n=4), COVID-19-related hospitalisation (n=9), COVID-19-related death (n=7) and severe COVID-19 (n=7) (online supplemental table 10). A wide range of RMD types and comparisons were studied. Most studies were at unclear or high RoB. The majority did not adequately adjust for important confounders, such as antirheumatic medication or disease activity. Overall, no consistent difference in risk between different RMDs was found. Some studies reported a signal for an increased risk of hospitalisation in patients with autoinflammatory diseases or systemic autoimmune diseases, and for developing 'severe COVID-19' in patients with connective tissue disease (CTD), compared with patients with inflammatory arthritis. However, these results were not consistent across all studies that compared these patient groups.

## Risk associated with antirheumatic medication and disease activity

A total of 26 studies assessed the association between a variety of antirheumatic medication and the risk of contracting SARS-CoV-2 (n=4), COVID-19-related hospitalisation (n=13), COVID-19-related death (n=9) and severe COVID-19 (n=10) (online supplemental table 11).

### Disease activity

Two studies, both from the Global Rheumatology Alliance (GRA)-COVID-19 registry, reported moderate or high disease activity as a risk factor for COVID-19-related death in patients with RMD (OR 1.87, 95% CI 1.27 to 2.77)[21] and for severe COVID-19 in patients with SLE (OR 2.24, 1.46–3.43),[22] even after extensive adjustment including the use of antirheumatic medication.

### Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs were not associated with the risk of contracting SARS-CoV-2 (n=2, 1 low RoB),[12 23] COVID-19-related hospitalisation (n=1)[24] or COVID-19-related death (n=2, 1 low RoB).[23 25]

### Glucocorticoids

Glucocorticoid use was associated with an increased risk of COVID-19 hospitalisation in seven studies (one low RoB), although not all analyses reached statistical significance.[17 19 24 26–29] Two studies showed that this increased risk was particularly present in those using a daily dosage of 10 mg or more.[24 27] Similar results were found in studies assessing the association between glucocorticoid use and COVID-19-related death (n=2)[21 30] or severe COVID-19 (n=5).[19 22 31–33] Again, a dose–response effect was found.[21 22 31] Strangfeld *et al* performed subgroup analyses of patients with inflammatory arthritis and CTD/vasculitis separately, and reported that the increased risk of COVID-19-related death associated with glucocorticoid use remained only in the CTD/vasculitis subgroup.[21] A post hoc analysis of the same study, using data from the GRA-COVID-19 registry, strongly suggested that the association with glucocorticoids mainly results from confounding by disease activity.

### Conventional synthetic disease-modifying antirheumatic drugs (csDMARDs)

Antimalarial drugs were not associated with the risk of contracting SARS-CoV-2 (n=2),[34 35] COVID-19-related death (n=4)[21 36–38] or severe COVID-19 (n=3).[31 38 39] Five studies (one low RoB) also found no association with COVID-19-related hospitalisation,[17 27 36 38 39] though a small study by Haberman *et al* reported an increased risk.[29]

Single studies investigated the risk associated with the use of various other csDMARDs, including methotrexate (COVID-19 hospitalisation, no association; n=2),[29 36] sulfasalazine (COVID-19-related death, higher risk (OR 3.6, 95% CI 1.66 to 7.78); n=1)[21] and leflunomide (COVID-19-related death, no association; n=1).[21]

### Biological disease-modifying antirheumatic drug (bDMARDs)/targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs)

Tumour necrosis factor alpha inhibitors (TNFis) were not associated with COVID-19-related hospitalisation in four studies (two low RoBs),[15 17 26 29] while two studies suggested a 'protective' effect.[19 27] TNFi use was not associated with COVID-19-related death (n=2, 1 low RoB)[15 21] or severe COVID-19 (n=1, low RoB).[15]

One study suggested that rituximab was associated with an increased risk of contracting SARS-CoV-2,[40] though two other studies (one low RoB) did not confirm this association.[15 27] Multiple studies found a higher risk of COVID-19-related death (n=4, 1 low RoB)[15 21 30 40] and severe COVID-19 (n=4),[19 31 40 41] although not all analyses reached statistical significance. Several of these studies are separate analyses of (parts of) the GRA-COVID-19 registry.

Fewer studies investigated Janus kinase inhibitors (JAKis), of which most found a higher risk of COVID-19-related hospitalisation (n=2, 1 low RoB),[15 29] COVID-19-related death (n=1, low RoB)[15] and severe COVID-19 (n=1).[41] Strangfeld *et al* reported no association between JAKi use and COVID-19-related death.[21]

Single studies investigated other bDMARDs/tsDMARDs, including abatacept, belimumab, interleukin-6 inhibitors (IL-6i), IL-17i and IL-23i, but no association was observed with any of the COVID-19 outcomes.

Studies (n=3, 1 low RoB)[15 26 29] found no association with COVID-19-related hospitalisation, COVID-19-related death (n=1, low RoB)[15] or severe COVID-19 (n=2, 1 low RoB)[15] for any bDMARD users versus non-bDMARD/tsDMARD users.

### Immunosuppressive medication

Few studies investigated the risk associated with use of immunosuppressive medication. One study found a higher risk of COVID-19-related death in users of immunosuppressive medication (a heterogeneous group composed of azathioprine, cyclosporine, cyclophosphamide, mycophenolate or tacrolimus users), compared with methotrexate users (OR 2.22, 95% CI 1.43 to 3.46).[21] One study also reported a higher risk of severe COVID-19 in mycophenolate mofetil users (OR 6.60, 95% CI 1.47 to 29.62),[19] while another found no association with this outcome in users of immunosuppressive medication.[31] These studies were all conducted in the GRA-COVID-19 registry.

## Vaccination against SARS-CoV-2

In total, 14 articles, two of which were preprints, with data on vaccination against SARS-CoV-2 in patients with RMDs, were identified (online supplemental table 12).

### Efficacy

Nine out of 14 studies reported on the efficacy of vaccination against SARS-CoV-2, measured as (presence or level of) antibody response (online supplemental table 12). Four studies had a prospective design; three were cross-sectional; and one was retrospective. The studies consisted of patients with (inflammatory) RMDs (n=5) or patients with various chronic inflammatory/autoimmune diseases including RMDs (n=3). Five studies also included a healthy control group. The number of patients with RMD ranged from 68[42] to 807.[43] All participants received an mRNA vaccine. Responsiveness was measured after the second dose in most studies (n=6). RoB was high (n=6) or unclear (n=2) in most studies.

The percentage of cases with a detectable antibody response ranged from 62% to 100% (median 88%, n=8 studies), while this was 96%–100% (median 100%, n=5 studies) in controls. Five studies measured the level of antibody response, all demonstrating lower IgG antibody titres or neutralising titres in cases versus controls.

One study assessed T-cell response using flow cytometry in a subset of participants, reporting a significant increase in spike-specific B cells, T-follicular helper cells, activated CD4+ T cells and HLA-DR+CD8+ T cells in cases and controls, though activated CD8+ T cells and granzyme-B-producing CD8+ T cells were only induced in patients with RMD not using methotrexate and healthy controls.[44]

Factors that were negatively associated with antibody response in more than one study were increased age (3/4 studies) and use of rituximab or anti-CD20 (6/6), mycophenolate (4/4) and glucocorticoids (3/3). Two studies showed that a longer interval between vaccination and rituximab infusion was associated with a positive antibody response.[43 45] Ruddy *et al* detailed that 86% of negative responders on glucocorticoids concurrently used rituximab or mycophenolate.[46] Less convincing results were seen for methotrexate (negative association in 2/5 studies), abatacept (2/3) and JAKi (1/2). Use of anticytokine therapy was not, or even positively, associated with antibody response. Furer *et al* (low RoB) found detectable antibodies in 86% of cases versus 100% of controls, lower antibody titres in cases, and a negative association with vaccine responsiveness for increased age, rituximab, mycophenolate, glucocorticoids and abatacept (but not methotrexate or JAKi).[43]

In total, 19/2989 (0.6%, n=5 studies) patients with RMD developed postvaccination COVID-19.[43 46–49] One study reported a post-vaccination COVID-19 case in a control subject (1/807, 0.1%).[43]

### Safety

Ten studies (one low RoB) reported safety data (online supplemental table 12). In all but one study, all patients received an mRNA vaccine. Generally, vaccination was well tolerated. Reported adverse events, though common, were mild and similar in type and severity/seriousness between patients with RMD and controls. Most reported were local symptoms, such as pain at the injection site, and less frequently systemic symptoms such as fatigue, myalgia and fever.

Three studies found no postvaccination disease flare of the underlying RMD in 868 patients with RMD,[42 43 50] while a report from the EULAR COVAX registry describes a disease flare in 73 out of 1375 (5%) patients, of whom 17 experienced a severe flare (mean±SD) 5±5 days postvaccination.[48]

No RMD-specific factors (eg, disease type or medication) were consistently associated with the development of adverse events

## Other outcomes

One USA-based, prospective study assessed the association of SARS-CoV-2 infection with development of a disease flare in Latin-American patients with RMDs, reporting an increased risk (OR 4.57, 95% CI 1.2 to 17.4).[9]

One US-based, retrospective study in the TriNetX database compared outcomes of matched patients with inflammatory RMDs and COVID-19 in the early (January–April 2020) and late (April–July 2020) phases of the pandemic.[51] The study showed that patients with COVID-19 in the late cohort fared better than those in the early cohort, based on lower risk of COVID-19-related hospitalisation (RR 0.71, 95% CI 0.67 to

0.76), ICU admission (RR 0.56, 95% CI 0.47 to 0.65), mechanical ventilation (RR 0.39, 95% CI 0.31 to 0.49), death (RR 0.48, 95% CI 0.39 to 0.60) and severe COVID-19 (composite of ICU admission, mechanical ventilation and death; RR 0.51, 95% CI 0.45 to 0.58). Results from several sensitivity analyses were similar. The results of this study are confirmed in studies from the GRA-COVID-19 database, where adjustment for time period was also significant.[22]

### Post hoc data

As this SLR covers a highly dynamic field in which new studies emerge on a weekly basis, particularly regarding vaccination against SARS-CoV-2, during the review process of the manuscript, a partial literature search update was done for vaccination studies only, in order to provide a more up-to-date overview of these data. Importantly, these data were not available for the task force at the time of deciding on the recommendations. We searched PubMed up to 11 October 2021 using previously described search terms (see online supplemental material), with the addition of specific terms for vaccination. The search retrieved 189 new hits, of which 23 were eligible (online supplemental table 13). Three reports concerned different outcomes and/or follow-up moments of a study already included in the main search,[52–54] and two reports concerned different outcomes and/or follow-up moments of the same study.[55 56] RoB was not assessed for this post hoc analysis.

Twelve studies, primarily concerning mRNA vaccines, provided efficacy data. Most studies confirmed a lower seroconversion rate or antibody titre in patients with RMD.[57–62] One large study by Boekel *et al* showed that after double exposure (ie, first dose after previous SARS-CoV-2 infection or second dose of a two-dose vaccination scheme), seroconversion rates became similar in cases and controls, except among those treated with anti-CD20 therapies.[57] Seven studies confirmed the negative association between anti-CD20 therapy and antibody response,[52 53 55 57 58 60 63] though studies assessing T-cell response (all based on interferon-γ release assays) showed signs of a present T-cell response, independent of antibody response.[58 63] Other antirheumatic medications reported to be associated with impaired antibody response include methotrexate (3/3 studies), mycophenolate (3/3 studies) and glucocorticoid use (3/6 studies). One study reported lower immunogenicity of the Ad26.COV2.S vaccine (Johnson & Johnson) compared with mRNA vaccines,[52] but other studies did not report differences between vaccine types. It should be noted that such analyses are hampered by low patient numbers. One small study reported a beneficial effect of withholding mycophenolate in the perivaccination period on antibody response, but at the cost of a disease flare in 2/24 patients.[53]

Seventeen studies assessed vaccine safety, but no new safety signals were reported. Nine studies assessed postvaccination RMD disease flares, which occurred in 0.6%–15.0% of patients, were generally mild to moderate and not leading to treatment changes (except in one study on patients with SLE)[64] and resolved quickly.[54 59 61 64–69] Disease flare within 6–12 months prior to vaccination appeared a risk factor for postvaccination flare.[54 64] Two case studies described characteristics and outcomes of 26 patients with RMD with SARS-CoV-2 infection after complete vaccination.[70 71] The most commonly used antirheumatic medication among these patients were glucocorticoids (n=8, 31%), methotrexate (n=6, 23%), rituximab (n=6, 23%) and mycophenolate (n=5, 19%). Three of the four patients who died were on rituximab. We did not find studies investigating the yield of an additional vaccine dose after an initial primary vaccine series in patients with RMD.

## DISCUSSION

Current literature provides no evidence that patients with RMDs face more risk of contracting SARS-CoV-2 than individuals without RMDs. While some studies suggest a higher rate of COVID-19-related hospitalisation in patients with RMDs compared with the general population, there is no evidence that patients with RMDs suffer from higher rates of COVID-19-related mortality or ICU admission. This apparent contradiction may be explained by other factors that influence hospitalisation than COVID-19 severity, such as concern of a worse prognosis by the treating physician and consequently a lower threshold for hospital admission. A large Swedish registry study, judged as being at low RoB, provided convincing evidence for this conclusion by demonstrating that the increased risk of hospitalisation and mortality observed in patients with RMD, particularly patients with RA, during the COVID-19 pandemic was similar to the increase reported in previous years.[15] Notably, results of a Danish registry study, which seem to point towards a higher incidence of severe COVID-19 in patients with RA, may be explained by the same mechanism as the Swedish study, but this was not investigated by the authors.[17] Still, if true, the impact of the reported risk estimate from that study is not clinically relevant at the population level.

Several risk factors for developing (severe) COVID-19 in patients with RMDs were assessed in this systematic review. Generally, demographic risk factors (increased age, male gender and high BMI) and comorbidities (cardiovascular disease, diabetes mellitus, chronic lung disease and chronic kidney disease), known to be associated with a worse prognosis of COVID-19 in the general population, are also applicable to patients with RMDs. Few studies investigated the role of ethnicity, but they found that patients with RMD from most non-white ethnicities, compared with individuals from the white race, likely suffer from a worse prognosis. No consistent difference in risk of developing (severe) COVID-19 was found between different RMDs. While single studies reported a worse prognosis in patients with RA compared with non-RA controls as well as in patients with autoinflammatory or systemic autoimmune diseases or CTD compared with those with inflammatory arthritis, these results were not consistent across all studies. In addition, adequate adjustment for factors known to affect prognosis, such as RMD medication and disease activity, was rarely assessed. Only few studies assessed disease activity as a risk factor for worse COVID-19 prognosis, but studies that did so found compelling evidence that moderate or high disease activity is a negative prognostic factor, even after extensive adjustment for RMD medication, including glucocorticoid use. At the start of the pandemic, a potentially negative effect of NSAIDs and a potentially positive effect of antimalarial drugs in COVID-19 were widely discussed, also outside the rheumatology field, but we did not find an increased or decreased risk of developing (severe) COVID-19 related to either type of medication. Similarly, potential positive effects of IL-6i or TNFi were not evident from the literature. On the other hand, current literature provides evidence for concerns regarding a few other drugs. This particularly pertains to rituximab, the use of which seems to be associated with an increased risk of COVID-19-related complications and death. While glucocorticoid users, in particular those receiving a daily dose above 10 mg of prednisone or equivalent, seem to be at an increased risk of hospitalisation, COVID-19-related complications and death,

there is evidence that this may be largely due to confounding by disease activity. Some studies also provide a signal for worse prognosis of COVID-19 in patients on JAKi. However, in many countries, these drugs are prescribed in patients who have failed (multiple) other therapies, and therefore, patients on JAKi generally suffer from more severe disease, providing ample room for confounding by indication as an alternative explanation for the observed increased risk, which may be too large to adjust for, even in well-designed observational studies. No other consistent associations between various RMD medication and developing (severe) COVID-19 were found in the current literature.

The first studies assessing efficacy and safety of vaccination against SARS-CoV-2 have been published, with many more expected to come since vaccination in many Western countries has taken flight. Current data show that, in general, SARS-CoV-2 vaccines are immunogenic in patients with RMDs, although the antibody response is lower compared with healthy controls. Still, the reported number of postvaccination COVID-19 cases in patients with RMDs remains low, and no information is available on the severity of these cases. Particularly older patients, as well as rituximab and mycophenolate users, appear to be at risk of lower antibody response. The (negative) effect of methotrexate on antibody response is uncertain. Patients on anticytokine therapy do not seem to exhibit lower antibody responses. Notably, the relation between measured antibody response and immune protection of the vaccines is unknown, and the extent and impact of T-cell response to SARS-CoV-2 vaccination remain unclear, as it was only reported in a subgroup of patients from one study. Adverse event profiles were comparable to the general population regarding the type and severity/seriousness. There was no literature to inform risk–benefit ratios of additional dose after an initial primary vaccine series in (subgroups of) patients with RMDs. None of the studies investigated the usefulness of stopping or postponing (specific) RMD medication in light of vaccination, although two studies showed that in patients in whom a longer period between rituximab infusion and vaccination existed, the antibody response was higher.[43 45]

Since the end of 2019, a large number of publications on COVID-19 have appeared. However, as is often the case, quantity is not necessarily a measure for quality. This becomes clear from the large number of included studies that were not considered eligible for data extraction after quality assessment and from the judgement of high RoB among those that passed the quality filter. A critical caveat relevant for (cohort) studies on COVID-19 in patients with RMDs is 'selection bias', which, even in well-established registries or large cohorts with extensive correction for confounders, can hardly be eliminated and may lead to spurious associations, particularly in studies with voluntary enrolment of COVID-19 cases. Studies at lowest risk of selection bias, and therefore most informative in this context, are population-based studies using, for example, national registries in which all patients from a country are included irrespective of patient characteristics. Examples of such studies are those from Bower *et al* and Cordtz *et al*.[15 17] Another problem in many studies is 'confounding by indication' stemming from selective testing for SARS-CoV-2, particularly at the beginning of the pandemic, when testing was not yet widely available.

When interpreting the data presented in this review, it is important to take into account that almost all studies were done during the first wave of the pandemic. This has some advantages for data interpretation, such as the presence of a lower number of different strains and therefore more homogeneous SARS-CoV-2 infection, and less confounding by indication by suspected risk factors of which at the time knowledge about their association with prognosis was lacking. However, this was also the time at which, for example, SARS-CoV-2 testing was not done ubiquitously, introducing bias as discussed previously. The association between risk factors discussed earlier or efficacy of vaccination in different strains of SARS-CoV-2 is unknown. Furthermore, patients included in studies at a later stage of the pandemic appear to have a better prognosis than those included in the beginning, so it may be true that in general, the studies from the first months of the pandemic paint a more negative picture than is currently justifiable.

In conclusion, this SLR presents an overview of currently available literature on risk and prognosis of SARS-CoV-2 infection and vaccination against SARS-CoV-2 in patients with RMDs, and provided evidence to inform the EULAR task force and formulate the July 2021 update of the recommendations for the management of RMDs in the context of SARS-CoV-2.

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EPIDEMIOLOGICAL SCIENCE

# Influenza outcomes in patients with inflammatory joint diseases and DMARDs: how do they compare to those of COVID-19?

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## ABSTRACT

**Objectives** To estimate absolute and relative risks for seasonal influenza outcomes in patients with inflammatory joint diseases (IJDs) and disease-modifying antirheumatic drugs (DMARDs). To contextualise recent findings on corresponding COVID-19 risks.

**Methods** Using Swedish nationwide registers for this cohort study, we followed 116 989 patients with IJD and matched population comparators across four influenza seasons (2015–2019). We quantified absolute risks of hospitalisation and death due to influenza, and compared IJD to comparators via Cox regression. We identified 71 556 patients with IJD on active treatment with conventional synthetic DMARDs and biological disease-modifying antirheumatic drugs (bDMARDs)/targeted synthetic disease-modifying antirheumatic drug (tsDMARDs) at the start of each influenza season, estimated risks for the same outcomes and compared these risks across DMARDs via Cox regression.

**Results** Per season, average risks for hospitalisation listing influenza were 0.25% in IJD and 0.1% in the general population, corresponding to a crude HR of 2.38 (95% CI 2.21 to 2.56) that decreased to 1.44 (95% CI 1.33 to 1.56) following adjustments for comorbidities. For death listing influenza, the corresponding numbers were 0.015% and 0.006% (HR=2.63, 95% CI 1.93 to 3.58, and HR=1.46, 95% CI 1.07 to 2.01). Absolute risks for influenza outcomes were half (hospitalisation) and one-tenth (death) of those for COVID-19, but relative estimates comparing IJD to the general population were similar.

**Conclusions** In absolute terms, COVID-19 in IJD outnumbers that of average seasonal influenza, but IJD entails a 50%–100% increase in risk for hospitalisation and death for both types of infections, which is largely dependent on associated comorbidities. Overall, bDMARDs/tsDMARDs do not seem to confer additional risk for hospitalisation or death related to seasonal influenza.

## Key messages

**What is already known about this subject?**

► Patients with rheumatoid arthritis (RA) or other inflammatory joint diseases (IJDs) are at increased risk of infections. Historical studies indicate that this applies also to seasonal influenza. Risks in contemporary patients with IJDs and with modern antirheumatic therapies remain unclear.

**What does this study add?**

► Patients with RA and other IJDs are at increased risk of hospitalisations and death related to seasonal influenza. Much though not all of these increases can be explained by contextual factors rather than the rheumatic disease diagnosis as such. Taken together, biological disease-modifying antirheumatic drugs (DMARDs)/targeted synthetic DMARDs do not confer additional risks beyond conventional synthetic DMARDs. These patterns of relative risks are largely similar to those recently observed for COVID-19, although the absolute risks with the latter are much higher.

**How might this impact on clinical practice or future developments?**

► Our results indicate the need and potential to further optimise risk mitigation measures against common and epidemic infections such as seasonal influenza in patients with IJDs, but also suggest that common antirheumatic therapies are not strong drivers of this increase.

## INTRODUCTION

Rheumatoid arthritis (RA) and biological disease-modifying antirheumatic drugs (bDMARDs) or targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs) have been linked with increased risks of infections, which in turn constitute one of the reasons behind the increased morbidity and preterm mortality in RA and other inflammatory joint diseases (IJDs).[1 2] Some of these infection risks are relatively specific to infectious agent and clinical context (eg, tuberculosis in patients treated with tumour necrosis factor inhibitors (TNFis). Others are less specific with respect to infectious agent and causative context, and rather arise against a background of suboptimally controlled rheumatic disease activity, comorbid conditions associated with RA and a certain level of perturbated immune competence, be it from oral glucocorticoids or from bDMARDs/tsDMARDs. It is clear, however, that 'infection risk' is a broad entity, that risks may not be directly translatable across infectious agents, and thus that a complete understanding of infection risks in IJD and with disease-modifying



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antirheumatic drugs (DMARDs) calls for studies of specific types of infections.

Seasonal influenza is known to lead to significant morbidity and mortality in the general population.[3] Despite the notion that RA, IJD and DMARDs are associated with increased infection risks, that the absolute risk of contracting seasonal influenza in many countries is several times higher than the risks of less prevalent infections such as tuberculosis and other opportunistic infections that have received much (more) attention, and that risk mitigation measures for seasonal influenza, such as annual vaccination, are indeed available,[4 5] surprisingly little is known on influenza outcomes in patients with RA or other IJDs, and in relation to DMARDs as currently used in clinical practice. Cross-sectional self-report studies have indicated an increased occurrence of influenza-like illnesses in patients with RA.[6–8] A claims-data based study from the USA reported increased risks of influenza-related complications in patients with RA, but little impact of bDMARDs, but these studies were all based on data from more than 10 years ago.[9]

For many common infections such as seasonal influenza, clinical risks lie not so much with the infection per se as with its severity and outcome, suggesting that further studies of influenza outcomes are needed. In IJD, this is particularly important since common DMARDs may both reduce effectiveness of vaccines against seasonal influenza[10] (the main intended effect of which is to prevent serious disease rather than infection per se) and impair host immune competence of relevance for the clinical severity of seasonal influenza infection.

Recently, we presented absolute and relative risks of hospitalisation and death following COVID-19 in patients with RA or other IJD and in relation to the general population, and could demonstrate that patients with RA and other IJD are at increased risks of hospitalisation and death following COVID-19, but also that most of these increased risks appear attributable to comorbid conditions associated with IJD rather than to the IJD disease or its DMARD treatment per se.[11] A full interpretation of these risks and the impact of COVID-19 on the IJD population call for contextualisation with risks of other prevalent infections, such as seasonal influenza during the past years.

In this study, we therefore aimed to estimate absolute and relative risks of hospitalisation and death following seasonal influenza in patients with RA, other IJD and with specific DMARDs in Sweden. The second aim was to put risks with seasonal influenza next to those we recently presented for COVID-19.

## METHODS

### Study population and period

We used an existing multiregister linkage of IJD (Anti-Rheumatic Treatments In Sweden ('ARTIS')), described elsewhere,[11 12] to identify our study population, exposures, outcomes and covariates (see online supplemental tables S1–S3 for details). To enable comparison with our recent COVID-19 study, we used similar methodological approaches and definitions.[11]

We first identified all adult individuals with IJD in Sweden alive at the beginning of at least one of four consecutive influenza seasons (15 September–15 May the following year, as defined by the Swedish National Board of Health and Welfare) 2015/2016, 2016/2017, 2017/2018 and 2018/2019. IJD was identified using the International Classification of Diseases 10th Revision, 10th Revision (ICD-10) codes for RA, psoriatic arthritis, ankylosing spondylitis, other spondyloarthropathies and juvenile idiopathic arthritis via the National Patient Register (NPR) using previously devised algorithms (online supplemental table S2).[11] Each unique individual was matched on year of birth, sex and region of domicile to five randomly selected population comparator subjects from the Swedish Population Register.

### Treatment exposures

DMARD treatment status of the patients with IJD at the start of each influenza season was identified using the closest ongoing treatment on or before 15 September per season recorded in the Swedish Rheumatology Quality Register and the Prescribed Drug Register. These were categorised into the following exposures: conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) and bDMARDs/tsDMARDs. The latter was further divided into TNFi, abatacept, tocilizumab, rituximab and janus kinase inhibitors.

### Outcomes, follow-up and covariates

We defined the following outcomes: (1) hospitalisation listing influenza (main and contributory diagnoses based on data from the NPR, and with influenza defined as ICD-10=J09 J11), and (2) death from influenza (main and contributory causes based on data from the Cause of Death register). Hospitalisation and deaths listing any cause were also identified to contextualise the influenza-specific outcomes. We followed individuals from the start of the influenza season to the first recorded event of interest (ie, multiple events within each influenza season were not allowed), or censoring at death, migration from Sweden or end of the influenza season.

Information on age, sex, region, socioeconomic factors (education, civil status and country of birth), influenza hospitalisation during the previous year and comorbidities (history of cancer, diabetes, heart failure, ischaemic heart disease, lung disease, stroke, surgery, venous thrombotic event and kidney failure) at the start of each influenza season was obtained from the NPR and the Prescribed Drug Register (online supplemental table S3).

### Statistical methods

Absolute risks of outcomes of interest were presented as percentages and calculated as the number of events divided by the number of individuals at risk; this was averaged across seasons for influenza outcomes.

To assess whether the risk of our outcomes was elevated in IJD versus the general population, we estimated crude rates per week (number of events per person−time at risk) for each outcome for each influenza season. We estimated HRs comparing patients with IJD to comparators via Cox proportional hazards models adjusted for influenza season, and with age, sex and region accommodated by the matched design, and further covariate adjustment for socioeconomic factors and comorbidities.

In order to determine the role of DMARDs on the risk of influenza outcomes, we estimated absolute risks and HRs comparing bDMARDs/tsDMARDs to csDMARDs via Cox proportional hazards models. Robust cluster SEs were used in order to account for the fact that one individual could contribute data from more than one influenza season. Cox regressions models were adjusted in the same way as described earlier, plus adjustments for disease duration, Disease Activity Score on 28 joints (DAS28), number of previous bDMARDs/tsDMARDs and concomitant steroid use. No imputation of missing data was performed; sensitivity analyses were performed to determine the effect of missing DAS28 values included in the DMARD treatment analyses (see online supplemental table S10 for details). Comparative analyses were not performed where the number of events in one group was less than five.

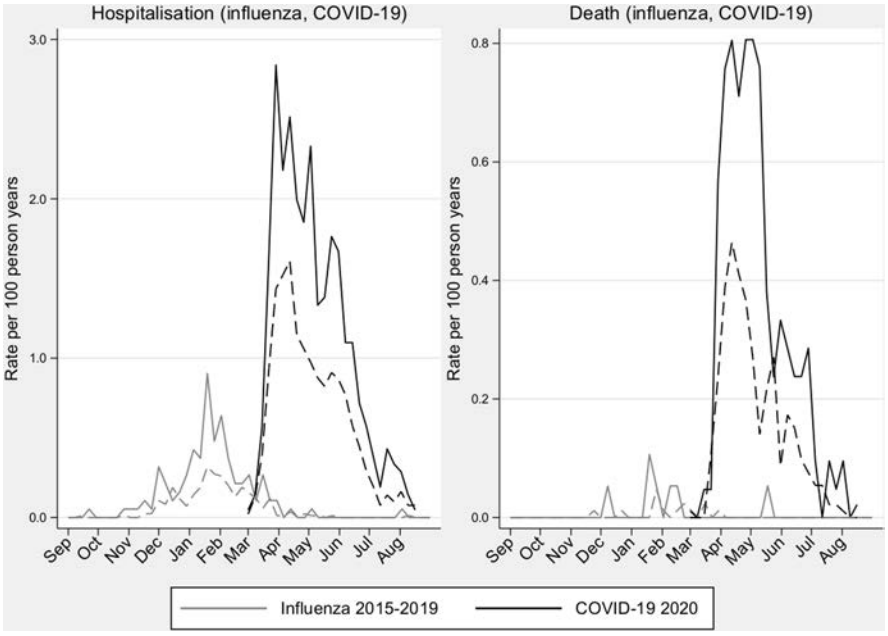


**Figure 1** Weekly rate per 100 person-years of hospitalisation and death listing influenza during seasons 2015/2016–2018/2019 and COVID-19 during 2020, for individuals with inflammatory joint diseases (solid lines) and population comparators (dashed lines) in Sweden.

## RESULTS

### Role of IJD during the influenza seasons 2015–2019

We identified 116 989 unique patients with IJD contributing data to at least one of the four influenza seasons (99 175, 102 811, 106 360 and 109 465 patients during the influenza seasons 2015–2016, 2016–2017, 2017–2018 and 2018–2019, respectively). Descriptive statistics can be found in online supplemental tables S4–S6.

Crude absolute rates (see figure 1) showed an increased rate of hospitalisation listing influenza and death due to influenza for patients with IJD versus the general population. The average absolute risk of influenza outcomes per season in patients with IJD was approximately three times that seen in the general population, although all risks were low (table 1, left panel; for hospitalisation listing influenza: 0.25% and 0.1% for IJD and comparators, respectively (one additional hospitalisation for influenza for every 666 patients with IJD); for death listing influenza: 0.015% and 0.006% (one additional death from influenza for every 11 111 patients with IJD), respectively).

Prior to adjustment for comorbidities and socioeconomic factors, HRs comparing IJD to the general population for hospitalisation listing influenza and death due to influenza were 2.38 (95% CI 2.21 to 2.56) and 2.63 (95% CI 1.93 to 3.58), respectively (table 1, left panel). Following adjustment, the HRs decreased to 1.44 (95% CI 1.33 to 1.56) for hospitalisation listing influenza and 1.46 (95% CI 1.07 to 2.01) for death due to influenza; similar reductions after adjustment were seen for hospitalisation and deaths due to any cause (online supplemental table S7). Using an alternative definition of death due to influenza, defined as any death occurring 30 days after a hospitalisation listing influenza, we obtained similar results (online supplemental table S8).

When considering patients with RA separately (whose mean age was higher than that of other IJDs), absolute risks were slightly higher than for all IJDs, but the pattern of crude and adjusted relative risks (HRs) remained similar (table 1, left panel).

### Role of DMARDs during the influenza seasons 2015–2019

Comparing b/tsDMARDs to csDMARDs, we found a 32% increased rate of hospitalisation listing influenza (adjusted HR=1.32, 95% CI 1.06 to 1.64) but no statistically significantly different rates for death from influenza. A minor but statistically significant higher rate of all-cause hospitalisation was found in bDMARDs/tsDMARDs compared with csDMARDs (online supplemental table S9; adjusted HR=1.08, 95% CI 1.05 to 1.12), but no increased risk for death due to any cause. With respect to specific bDMARDs/tsDMARDs, we noted increased HRs for hospitalisation listing influenza for abatacept and for rituximab (table 2).

### Contextualising risks with COVID-19

The right panels in tables 1 and 2 and figure 1 display corresponding risk estimates from our study on COVID-19.[11] The pattern of HRs and the impact of adjustment were largely similar for seasonal influenza, although the decline in disease-specific HRs (here: hospitalisation and death specifically from influenza) following adjustment was somewhat less pronounced for seasonal influenza than for COVID-19. The crude rates for hospitalisation and death presented in figure 1 were much higher for COVID-19 outcomes than for influenza outcomes. Results for hospitalisation listing any cause and death due to any cause can be found in online supplemental table S7 (n.b., the study period was 8 months for seasonal influenza vs 6 months for COVID-19, and they further spanned different calendar months).

## DISCUSSION

In this study, one of the few to date that have investigated the pattern of absolute and relative risks for hospitalisation and deaths associated with seasonal influenza in patients with IJD and with currently available DMARDs as used in clinical practice, we noted that the absolute risk of each of the four outcomes under study was higher in IJD (in RA in particular) compared with the general population, but also that a large part (though not all) of these increases could be explained

**Table 1** Number of events, absolute risk, excess risk and HRs estimated from Cox proportional hazards models comparing patients with IJDs to matched comparators for outcomes hospitalisation listing influenza (during 2015/2016–2018/2019 influenza seasons) or COVID-19 (during the first wave of COVID-19 2020), and death listing influenza (influenza seasons 2015–2019) or COVID-19 (during the first wave of COVID-19 2020)

| Influenza, seasons 2015–2019 | | | | | | COVID-19 2020* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Outcome | Events (n) (average risk, %) in IJD cohort | Events (n) (average risk, %) in the general population | Crude excess risk per 100 patients | HR 1† | HR 2‡ | Outcome | Risk (%) in IJD cohort | Risk (%) in the general population | Crude excess risk per 100 patients | HR 1† | HR 2‡ |
| **All IJD** | | | | | | **All IJD** | | | | | |
| Hospitalisation listing influenza | 1066 (0.25) | 2011 (0.1) | 0.15 | 2.38 (2.21 to 2.56) | 1.44 (1.33 to 1.56) | Hospitalisation listing COVID-19 | (0.5) | (0.3) | 0.2 | 1.77 (1.61 to 1.95) | 1.32 (1.19 to 1.46) |
| Death listing influenza | 64 (0.015) | 109 (0.006) | 0.009 | 2.63 (1.93 to 3.58) | 1.46 (1.07 to 2.01) | Death listing COVID-19 | (0.10) | (0.07) | 0.03 | 2.09 (1.73 to 2.52) | 1.18 (0.97 to 1.44) |
| **RA** | | | | | | **RA** | | | | | |
| Hospitalisation listing influenza | 743 (0.35) | 1312 (0.15) | 0.2 | 2.41 (2.21 to 2.64) | 1.47 (1.34 to 1.62) | Hospitalisation listing COVID-19 | (0.7) | (0.4) | 0.3 | 2.02 (1.78 to 2.28) | 1.40 (1.23 to 1.60) |
| Death listing influenza | 50 (0.02) | 78 (0.009) | 0.011 | 2.73 (1.91 to 3.90) | 1.56 (1.08 to 2.27) | Death listing COVID-19 | (0.30) | (0.11) | 0.19 | 2.28 (1.85 to 2.81) | 1.27 (1.02 to 1.59) |

*COVID-19 data taken from Bower *et al*,[11] from Cox models fitted identically to those of ‡. Note that season lengths differ for COVID-19, and for influenza, the former was from March to September (6 months) 2020, whereas the latter was from September to May (8 months) each year 2015–2019.

†HR 1 accounts for age, sex and region via the matching (and for influenza season via adjustment for influenza analyses) .

‡HR 2 additionally adjusts for socioeconomic factors (education, civil status and country of birth), influenza hospitalisation in the previous year (in influenza analyses) and comorbidities (history of the following diseases: cancer, diabetes, heart failure, ischaemic heart disease, lung disease, stroke, surgery, venous thrombotic event and kidney failure).

IJD, inflammatory joint disease; RA, rheumatoid arthritis.

**Table 2** HRs comparing the rates of hospitalisation listing influenza and death listing influenza (during 2015/2016–2018/2019 influenza seasons) in patients with inflammatory joint diseases receiving csDMARDs to patients receiving bDMARDs/tsDMARDs in Sweden

| Outcome | Cohort | Events (n) | Absolute risk (%) | HR 1 (95% CI)* | HR 2 (95% CI)† | HR 2 COVID-19 (95% CI)‡ |
|---|---|---|---|---|---|---|
| Hospitalisation | csDMARD | 327 | 0.3 | 1 (ref) | 1 (ref) | 1 (ref) |
| | TNFi | 110 | 0.2 | 0.54 (0.43 to 0.66) | 1.18 (0.92 to 1.52) | 1.05 (0.67 to 1.64) |
| | Abatacept | 25 | 0.6 | 2.05 (1.33 to 3.16) | 2.01 (1.26 to 3.19) | 0.49 (0.15 to 1.59) |
| | Tocilizumab | 9 | 0.2 | 0.74 (0.38 to 1.43) | 1.28 (0.65 to 2.51) | – |
| | Rituximab | 42 | 0.5 | 1.83 (1.33 to 2.52) | 1.49 (1.04 to 2.14) | 1.03 (0.58 to 1.81) |
| | All bDMARDs/tsDMARDs combined§ | 191 | 0.2 | 0.75 (0.62 to 0.89) | 1.32 (1.06 to 1.64) | 1.08 (0.73 to 1.58) |
| Death | csDMARD | 21 | 0.02 | 1 (ref) | 1 (ref) | 1 (ref) |
| | TNFi | 3 | 0.004 | – | – | 1.03 (0.40 to 2.61) |
| | Abatacept | 1 | 0.02 | – | – | – |
| | Tocilizumab | 0 | 0 | – | – | – |
| | Rituximab | 1 | 0.01 | – | – | 3.20 (1.19 to 8.57) |
| | All bDMARDs/tsDMARDs combined§ | 5 | 0.006 | 0.30 (0.11 to 0.81) | 0.65 (0.04 to 12.00) | 1.26 (0.60 to 2.64) |

Note: Results only presented where treatment cohorts have five or more events.
Previously published corresponding HRs for COVID-19 are in the rightmost column.
*Adjusted for influenza season; age, sex and region accounted for via matching.
†Additionally adjusted for disease duration, Disease Activity Score on 28 joints, number of previous bDMARDs/tsDMARDs and concomitant steroid use, socioeconomic factors (education, civil status and country of birth), influenza hospitalisation in the previous year and comorbidities (history of the following diseases: cancer, diabetes, heart failure, ischaemic heart disease, lung disease, stroke, surgery, venous thrombotic event and kidney failure).
‡Taken from the COVID analyses presented in Bower *et al*,[11] adjusted for the same factors as †, but via inverse probability treatment weighting via propensity score estimation.
§Includes Janus kinase inhibitors.
bDMARD, biological disease-modifying antirheumatic drug; csDMARD, conventional synthetic disease-modifying antirheumatic drug; ref, reference; TNFi, tumour necrosis factor inhibitor; tsDMARD, targeted synthetic disease-modifying antirheumatic drug.

by socioeconomy and associated comorbidities. Further, and for the same outcomes, we noted no major differences in risk with bDMARDs/tsDMARDs compared with csDMARDs, although we noted signals (for the outcome hospitalisation listing influenza) with abatacept and rituximab. When put next to our previously published data on risks and relative risks with COVID-19, we noted that whereas the absolute risks of influenza outcomes were around half (hospitalisation) and one-tenth (death) those for COVID-19, the pattern of relative risks for influenza-specific outcomes and for COVID-19-specific outcomes comparing IJD to the general population was qualitatively quite similar.

As mentioned, there is a scarcity of data on risks, relative risks and risk determinants for outcomes of seasonal influenza infections in patients with IJD and with DMARDs. Historically, rheumatic disease has been identified as a risk factor for influenza hospitalisation in the elderly.[4 5] Our results suggest that despite marked improvements in the general disease status of patients with IJD during the past decades, the relative risk increase (a 50%–100% increase compared with the general population) remains.

We noted a strong effect on our HRs of adjustment for comorbidities and other contextual factors. Although little studied previously, this is in keeping with observations from at least one previous study.[9] While the increased risks signal a need for clinical vigilance or preventive measures, the marked attenuation of the strength of the association following adjustments suggests that much of the increase is related to the clinical context rather than the rheumatic disease diagnosis itself, although our results formally do not rule out any level of risk increase in individuals with IJD in remission but otherwise at full health.

With respect to DMARDs, we noted no clear overall difference in influenza outcomes with bDMARDs/tsDMARDs versus csDMARDs, but signals for certain bDMARDs (and too small numbers to make explicit comparisons with tsDMARDs). In the few previous studies, one similarly reported little effect of bDMARDs.[9] While seemingly in keeping, our results are not comparable as that study focused on risks of influenza rather than risks of adverse influenza outcomes, and (using data until 2007) effectively only studied TNFis. By contrast, a small Dutch questionnaire-based study and a small Italian study (both also based on data from more than 10 years ago) indicated no higher and an increased prevalence, respectively, of influenza-like illness in those patients treated with TNFis.[6 7]

Despite the fact that the vulnerable population is similar for COVID-19 and seasonal influenza, direct comparisons of absolute risks in COVID-19 versus seasonal influenza are not straightforward since they hit during different(-ly long) seasons, and since the underlying rates for our outcomes (death and hospitalisation for all causes) also have a seasonal variation. Relative risks, however, should be more directly comparable as they accommodate this seasonal effect. Further, it is important to remember that during our study period, SARS-CoV-2 was a new virus, for which no herd immunity, specific treatment or vaccine existed. For COVID-19, our results thus reflect effects of the virus per se, social distancing, absence of immunity (whether from previous infection or from vaccination) and a largely trial-and-error based treatment of severe COVID-19. By contrast, for influenza, our results are reflective of the impact of seasonal influenza per se,

plus the seasonal public health influenza campaigns and the effects of immunity from previous epidemics or vaccination, and antiviral treatment for severe cases of influenza.

Our study has limitations. We studied outcomes of seasonal influenza but did not have individual-level data to assess risks of acquiring influenza infection in the first place, or individual-level information on host protection against severe influenza outcomes, either through previous influenza infection or from vaccination. In Sweden, the yearly influenza vaccine is recommended for all individuals above 65 years of age and for individuals with certain comorbidities (but not specifically IJD, although Swedish rheumatologists likely encourage their patients to get vaccinated). The proportion vaccinated in the IJD cohort could thus be expected to be somewhat higher than that in the general population, which would make our observed increased risk in IJD an underestimate for what would be observed in a completely unvaccinated population. However, a sensitivity analysis restricted to only those aged 65 years and over showed almost identical results (results not shown), suggesting that differences in vaccination rate have not had a major impact on our data. We also cannot infer whether or to what extent RA, other IJDs or DMARDs increase the susceptibility to influenza infection, only that patients with RA and other IJDs are at increased risk of unfavourable outcomes once infected. Further, since influenza may progress to pneumonia, death (and to some extent also hospitalisation) may be recorded as being due to pneumonia rather than to influenza. This may lead to an underestimation of the influenza-specific outcomes, but we believe this will impact for the IJD and the population comparator subjects equally. Similarly, our influenza definition may be subject to misclassification since we did not have access to data which could confirm the physician-assigned diagnosis. Treatment switches during follow-up are clinically relevant, but were not accounted for in this study to align with the approach taken in our COVID-19 study. However, since <1.1% of patients changed treatment cohort during each influenza season, we do not expect this to measurably alter the results. Although we accommodated many comorbid and contextual factors, our adjusted HRs may contain residual or unmeasured confounding, including (for the drug-specific comparisons, eg, for rituximab) residual confounding by indication.

To conclude, in absolute terms, IJD is a risk factor for hospitalisation and death following seasonal influenza, but the impact of COVID-19 on patients with IJD outnumbers that of seasonal influenza. On the other hand, and compared with the general population, IJD is a risk factor of similar (relative) strength for seasonal influenza as previously observed for COVID-19. In both instances, much of this increase can be explained by other factors suggesting that merely having IJD is in itself not a strong risk factor (although having its comorbid consequences may increase risk). Overall, bDMARD/tsDMARD treatment does not seem to markedly increase risk of adverse influenza outcomes, but signals for abatacept and rituximab call for replication. Our results underscore the continued need to optimise risk-mitigation measures against epidemic infections beyond COVID-19 in the rheumatic disease population.

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# Spectrum of short-term inflammatory musculoskeletal manifestations after COVID-19 vaccine administration: a report of 66 cases

In the past months, mass vaccination represented the turning point of the global battle against the COVID-19 pandemic, an unprecedented challenge for physicians, healthcare professionals, health systems and pharmaceutical companies. More than 6 billion doses of vaccine have been administered to date, covering nearly 50% of the world's population. Although the vaccination campaign is still thwarted by spread of fake news disseminated by a ubiquitous antivaxxer movement, accumulating real-life data[1] confirm the favourable safety profile already demonstrated in phase III clinical trials.[2]

Despite the lack of a steady literature evidence,[3] the potential role of vaccines in promoting autoimmunity continues to intrigue many researchers. The theoretical basis of this association relies on the possible molecular mimicry between macromolecular components of the vaccine and specific human proteins and the exuberant immune response elicited by adjuvants contained in vaccines.[4]

Adverse events (AEs) associated with COVID-19 vaccines are usually mild and mainly restricted to injection site reactions. Interestingly, among systemic AEs, arthralgia is one of the most common.[2] To the best of our knowledge, only isolated cases[5] of arthritis developed after COVID-19 vaccine administration has been described; however, in a recently published survey including 1377 participants with rheumatic diseases, 11% of the respondents reported flare requiring treatment following injection of mRNA-based vaccines.[6]

The 'COVID-19 and Autoimmune Systemic Diseases' is a collaborative network of Italian rheumatologists, equally distributed across the country, spontaneously born in response to the COVID-19 pandemic with the aim to contribute to the advancing knowledge about COVID-19 and rheumatic diseases, by providing real-life data obtained from participating centres. To date, more than 60 rheumatologists from 40 different rheumatology clinics are affiliated to the study group.

In December 2020, we published a web-based survey form and invited all members of the study group to inform cases of inflammatory musculoskeletal manifestations (eg, synovitis, tenosynovitis, enthesitis, inflammatory spinal pain or girdles pain/stiffness with serological evidence of inflammation) with onset within 4 weeks from the administration of the first or second dose of one of the COVID-19 vaccines approved in Italy (BNT162b2, mRNA-1273, AZD1222 and Ad26.COV2.S), prospectively encountered during routine clinical practice since the beginning of the vaccination campaign, in January 2021, and up to August 31, 2021. Exclusion criteria were a history of any inflammatory rheumatic disease, isolated arthralgia/myalgia without clear evidence of inflammation, or vague and/or non-specific musculoskeletal complaints. Written informed consent was obtained from all patients.

By using this approach, we built a case series comprising 66 individual patients reported by 16 different rheumatology centres; most of them (59%) received the BNT162b2 vaccine. The average delay between the day of the 'trigger' injection (44.4% coinciding with the first dose) and arthritis onset was 11–13 days.

Stratification according to the predominant pattern of involvement at presentation (table 1) revealed that girdles pain/stiffness with acute-phase reactant elevation resembling polymyalgia rheumatica (PMR-like) was the most common (41%) clinical picture followed by oligoarthritis (32%) and polyarthritis (27%). Polyarticular and PMR-like cases were mainly symmetric (83% and 89%, respectively); involvement of small joints and tenosynovitis (39%) were significantly more frequent in polyarthritic forms (61% and 39%, respectively), while enthesitis was more common in oligoarthritic presentation (14%). Of note, two patients (one in the polyarticular group and one in the oligoarticular group, respectively) had also inflammatory back pain with evidence of active sacroiliitis and/or spondylitis on MRI. Detection of autoantibodies in sera was an uncommon finding;

**Table 1** Clinical features of the patients stratified according to the pattern of presentation

| | Polyarthritis (n=18) | Oligoarthritis (n=21) | PMR-like (n=27) | P value |
|---|---|---|---|---|
| Age (years) | 54±16 | 64±15 | 67±10 | **0.006** |
| Female gender, n (%) | 10 (55.6) | 16 (76.2) | 17 (63.0) | 0.696 |
| Past COVID-19, n (%) | 2 (11.1) | 1 (4.8) | 0 (0.0) | 0.211 |
| Specific vaccine administered | | | | |
| BNT162b2, n (%) | 9 (50.0) | 12 (57.1) | 18 (66.7) | 0.363 |
| mRNA-1273, n (%) | 0 (0.0) | 1 (4.8) | 2 (7.4) | 0.535 |
| AZD1222, n (%) | 9 (50.0) | 7 (33.3) | 7 (25.9) | 0.197 |
| Ad26.COV2.S, n (%) | 0 (0.0) | 1 (4.8) | 0 (0.0) | 0.295 |
| Vaccine-related adverse events | | | | |
| None, n (%) | 3 (16.7) | 9 (42.9) | 11 (40.7) | 0.195 |
| Pain at the injection site, n (%) | 12 (66.7) | 10 (47.6) | 13 (48.1) | 0.440 |
| Fever, n (%) | 5 (27.8) | 1 (4.8) | 3 (11.1) | 0.112 |
| Headache, n (%) | 2 (11.1) | 2 (9.5) | 2 (7.4) | 0.869 |
| Fatigue, n (%) | 6 (33.3) | 3 (14.3) | 1 (3.7) | **0.023** |
| Rheumatic manifestation onset after first dose, n (%) | 11 (61.1) | 7 (33.3) | 12 (44.4) | 0.322 |
| Delay between vaccine administration and rheumatic manifestation onset (days) | 12±9 | 11±7 | 13±7 | 0.450 |
| Rheumatic manifestations | | | | |
| Symmetrical involvement, n (%) | 15 (83.3) | 9 (42.9) | 24 (88.9) | **0.001** |
| Involvement of small joints, n (%) | 11 (61.1) | 4 (19.0) | 2 (7.4) | **<0.001** |
| Tenosynovitis, n (%) | 7 (38.9) | 4 (19.0) | 2 (7.4) | **0.029** |
| Enthesitis, n (%) | 0 (0.0) | 3 (14.3) | 0 (0.0) | **0.023** |
| Bursitis, n (%) | 1 (5.6) | 1 (4.8) | 0 (0.0) | 0.456 |
| Inflammatory back pain with MRI evidence of sacroiliitis or spondylitis, n (%) | 1 (5.6) | 1 (4.8) | 0 (0.0) | 0.456 |
| Fatigue, n (%) | 4 (22.2) | 3 (14.3) | 8 (29.6) | 0.613 |
| Laboratory features | | | | |
| ESR (mm/hour) | 51±34 | 36±25 | 45±28 | 0.108 |
| CRP (mg/dL) | 2.13 (1.25–5.20) | 1.90 (0.50–3.61) | 2.13 (1.25–5.20) | 0.121 |
| RF positive, n (%)* | 1 (6.3) | 0 (0.0) | 0 (0.0) | 0.255 |
| ACPA positive, n (%)* | 1 (6.3) | 0 (0.0) | 0 (0.0) | 0.255 |
| RF and ACPA positive, n (%)* | 1 (6.3) | 0 (0.0) | 0 (0.0) | 0.255 |
| ANA positive, n (%)† | 2 (15.4) | 3 (17.6) | 0 (0.0) | 0.061 |
| Treatment | | | | |
| NSAIDs, n (%) | 6 (33.3) | 11 (52.4) | 9 (33.3) | 0.507 |
| Paracetamol or opioids, n (%) | 5 (27.8) | 3 (14.3) | 6 (22.2) | 0.669 |
| Glucocorticoids, n (%) | 9 (50.0) | 13 (61.9) | 21 (77.8) | 0.113 |
| Methotrexate, n (%) | 4 (22.2) | 5 (23.8) | 3 (11.1) | 0.490 |
| Sulfasalazine, n (%) | 1 (5.6) | 0 (0.0) | 0 (0.0) | 0.523 |
| Follow-up duration (weeks) | 6 (2–8) | 4 (3–8) | 2 (1–5) | 0.209 |
| Outcome | | | | |
| Active disease, n (%) | 12 (66.7) | 9 (42.9) | 6 (22.2) | **0.007** |
| Remission, n (%) | 6 (33.3) | 10 (47.6) | 20 (74.1) | **0.014** |
| N/A, n (%) | 0 (0.0) | 2 (9.5) | 1 (3.7) | 0.296 |

Data are expressed as mean±SD or median (25th–75th percentiles), as appropriate. P values refer to one-way analysis of variance or Kruskal-Wallis H test for continuous or categorical variables, respectively (in bold p values < 0.05).
*Information for RF/ACPA status available for 56 patients.
†Information for ANA status available for 48 patients.
ACPA, anticitrullinated protein antibody; ANA, antinuclear antibody; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; N/A, not available; NSAID, non-steroidal anti-inflammatory drug; PMR, polymyalgia rheumatica; RF, rheumatoid factor.

HLA-B27 status was obtained in only 21 (31.8%) patients, of which one in the polyarthritis subgroup tested positive.

Most patients were treated with glucocorticoids (50%–78%), non-steroidal anti-inflammatory drugs (33%–52%) or analgesics (14%–28%), while disease-modifying antirheumatic drugs were used in five (28%) patients with polyarthritis, five (24%) patients with oligoarthritis and only three (11%) patients with PMR-like presentation.

Despite the limitation of a very short follow-up, the clinical course seemed excellent in patients with PMR-like onset with 74% achieving full remission of symptoms after 2 weeks; on the other hand, 67% of patients with polyarthritis had active disease after an average follow-up of 6 weeks.

In conclusion, despite the fact that a clear cause–effect relationship is far to be ascertained, our data suggest that inflammatory musculoskeletal symptoms may occasionally develop in close temporal association with COVID-19 vaccine administration. However, even assuming a direct causal relationship, we feel that the overall safety of COVID-19 vaccines remains unaffected, and the benefits of vaccination largely outweigh the minimal risks associated with such uncommon inflammatory complications, probably reflecting a transient reactogenic response to the vaccine rather than a structured, chronic inflammatory joint disease.

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## Flares of mixed cryoglobulinaemia vasculitis after vaccination against SARS-CoV-2

Studies on the safety and immunogenicity of SARS-CoV-2 vaccination in patients with inflammatory rheumatic diseases

**Table 1** Demographic, clinical and immunological features of patients who had flare of stable MC vasculitis bona fide caused by vaccination against SARS-CoV-2

| Patient | Age (years)/ sex | MC type | SVR (months) | Last active symptoms and RTX before vaccination (months) | | Vaccine | Symptoms after first dose | Symptoms after second dose | Cryocrit, % | | SARS-CoV-2 Antibody titre (Binding Antibody Units;/ mL) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Symptoms | RTX | | | | Prevaccination | Flare | |
| 1 | 70/male | EMC | N/A | P (40) | N/T | AstraZeneca | Diffuse P (day 3) | Second dose refused | 1 | 6 | N/A |
| 2 | 41/female | EMC | N/A | P (20) | 20 | Pfizer | None | Diffuse P (day 1) | 0 | 0 | 900 |
| 3 | 76/female | EMC | N/A | P (27) | N/T | Pfizer | None | Diffuse P (day 5) | 0 | 0 | 2961 |
| 4 | 57/female | HCV-MC | 67 | PN (42) | N/T | Pfizer | None | Moderate P, PN (day 10) | Traces | Traces | 694 |
| 5 | 66/female | HCV-MC | 62 | P, PN (48) | N/T | Pfizer | None | Moderate P, PN (day 7) | Traces | 0 | 3115 |
| 6 | 63/female | HCV-MC | 30 | P, PN (26) | N/T | Pfizer | None | Moderate P (day 7) | 0 | 10 | 2430 |

EMC, essential mixed cryoglobulinaemia; HCV-MC, hepatitis C virus-related mixed cryoglobulinaemia; MC, mixed cryoglobulinaemia; N/A, not applicable; N/T, never treated; P, purpura; PN, peripheral neuropathy; RTX, rituximab therapy; SVR, sustained virological response after antiviral therapy.

have so far not included mixed cryoglobulinaemia (MC) vasculitis.[1–3] We report a prospective observational multicentre study on this disorder.

Participants were followed at four tertiary referral centres and were instructed to promptly inform the attending physicians about unusual events felt as possibly related to vaccination. Seventy-one patients were recruited: they had infection-cured hepatitis C virus (HCV)-related MC, either uncomplicated (HCV-MC, n=50) or complicated by low-grade non-Hodgkin's lymphoma (MC-NHL, n=8), or essential MC (EMC, n=13). The characteristics of the patients, exclusion criteria and definition of bona fide vaccination-related flare are described in online supplemental methods.

Overall, 9 of 71 (12.7%) patients had postvaccination MC vasculitis flare. However, 8 of 71 patients had experienced within 12 months before vaccination spontaneous flares, where 7 cases required rituximab and 3 of them (37.5%) had postvaccination flare (see online supplemental information). Thus, to exclude the confounding effects of high proneness to spontaneous flare as the facilitator and of rituximab as the preventor, we further restricted the evaluation of postvaccination flare rate to 63 patients off-therapy and without spontaneous flares for 20–48 months before vaccination (see online supplemental information). In none of them rituximab was postponed in view of vaccination.

Six of the 63 patients (9.5%) with stable MC had bona fide vaccination-related flares (table 1). Flares were more frequent in patients with EMC (3 of 8, 37%) than with HCV-cured HCV-MC or MC-NHL (3 of 55, 5.4%) (p=0.023). Flares were characterised by purpura, new onset in one case, which subsided within 1–2 weeks; in three cases the purpura was so diffuse (online supplemental figure 1) that one patient defined it as 'never experienced before' and another refused the second dose. Two patients also had flare of peripheral neuropathy that had remained stable for several months. Cryoglobulins (online supplemental figure 2A) increased in 2 of 6 patients with and in 0 of 25 patients without flare tested (p=0.032).

Anti-SARS-CoV-2 IgG responses were measured 8–14 days after the second dose of vaccine in 50 patients. Five of 43 (11.6%) rituximab-free and 5 of 7 (71%) rituximab-treated patients (p=0.002) proved seronegative (<7 binding antibody units /mL) (online supplemental figure 2B). Seronegativity was more frequent (p=0.04) among patients with EMC (2 of 5) than with HCV-MC (1 of 33) (online supplemental figure 2C), suggesting lower immune dysregulation in HCV-MC due to reversion of B cell abnormalities after clearance of infection.[4] Among rituximab-treated patients, seronegativity correlated with B cell count <5 cells/µL (online supplemental table 2). No correlations were found between seronegativity and vasculitis flare or cryocrit level (online supplemental figure 2D,E).

Concerning possible mechanism(s) of post-vaccination flare, it is interesting that pathogenic rheumatoid factor-specific B cells expanded in MC are unresponsive to the stimulation of the B cell receptor and of toll-like receptors (TLR) 7 and 9, but can be activated by the simultaneous engagement of these receptors[5]; thus, vaccination-induced immune complexes acting as autoantigen for rheumatoid factor-specific B cells and vaccine nucleic acids acting as TLR 7/9 ligands could work together in activating pathogenic B cells in vivo.

The overall rate of postvaccination flare observed in patients with MC is similar to that reported in other autoimmune rheumatic diseases[1–3]; importantly, flares did not endanger patients and subsided spontaneously. This reassures the safety of SARS-CoV-2 vaccination in patients with MC.

While in other inflammatory rheumatic diseases lack of immunogenicity of the SARS-CoV-2 vaccine was mostly attributed to immunosuppression especially with rituximab,[1 2] the 11.6% seronegativity rate in treatment-free patients with MC suggests that disease-related factors may impair vaccine immunogenicity in this disorder. Two patients contracted mild COVID-19, one (rituximab-treated, seronegative) 3 weeks after and one (rituximab-untreated, seropositive) 17 weeks after the second dose of vaccine (see online supplemental information). Our observations encourage administering vaccine booster[6] to patients with MC and postponing vaccination of rituximab-treated patients after B cell repopulation.

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# Safety and disease flare of autoimmune inflammatory rheumatic diseases: a large real-world survey on inactivated COVID-19 vaccines

COVID-19 vaccines are of great importance in reducing SARS-CoV-2 infection and severe cases. Patients with autoimmune inflammatory rheumatic diseases (AIIRDs) have been strongly recommended to be vaccinated according to the novel guidance because they are more vulnerable to SARS-CoV-2 infection.[1] However, patients with AIIRDs were largely excluded from vaccine trials, leading to very limited data on the safety of COVID-19 vaccines. Notably, previous studies mainly focused on mRNA and adenovirus vector vaccines; however, little is known about inactivated COVID-19 vaccines that also have been authorised by WHO and widely used in several most populated countries, for instance, China, Brazil, Turkey and Indonesia.[2] A large randomised clinical trial consisting of 40 382 participants has demonstrated two inactivated COVID-19 vaccines significantly reduced the risk of symptomatic COVID-19.[3]

We conducted a real-world survey to evaluate the safety profiles and disease flare in patients with AIIRDs who received any dose of inactivated COVID-19 vaccines in China. From 1 Aug 2021 to 15 Oct 2021, eligible participants completed a predefined 25-question-based questionnaire by invitation on social media or visiting the outpatient department. There was no restriction on the time interval from vaccination to completing the survey.

In total, 1507 adults patients with AIIRDs who received inactivated COVID-19 vaccine participated in this study (flow diagram in online supplemental figure 1). The median age of participants was 39 (IQR 31–51) years. There were 1166 (77.4%) female patients and 209 (13.9%) patients with self-identified allergic history. Systemic lupus erythematosus (SLE) (614, 40.7%) was the most common AIIRD among participants, followed by rheumatoid arthritis (RA) (434, 28.8%), Behcet's disease (BD, 122, 8.1%), psoriatic arthritis/psoriasis (PsA/PsO) (76, 5.0%), primary Sjogren's syndrome (74, 4.9%) and ankylosing spondylitis (44, 2.9%) (online supplemental figure 2).

Among all participants, 450/1507 (29.9%) participants experienced adverse events (AEs) after vaccination (table 1). Local AEs, such as pain, redness or swelling at injection site, were reported to occur in 287 (19.0%) participants. Meanwhile, 260 (17.3%) patients reported systemic AEs after vaccination. Fatigue or sleepless (123, 8.2%) was the most reported systemic AE, followed by headache (82, 5.4%) and skin rash (55, 3.6%). The median time from vaccination shot to onset of AEs was 2 days. Most AEs were mild to moderate and self-limiting. Overall, 28 (1.9%) patients self-reported severe AEs. There were only three patients who were hospitalised due to serious diarrhoea, headache and cough. No one reported AE of interests or fatal AE, including myocarditis, idiopathic thrombocytopenic purpura, anaphylactic shock or death.

Flare of existing AIIRDs was reported by 158 (10.5%) participants, with requirement of treatment escalation in 53 (3.5%)

**Table 1** Safety and flare data of AIIRDs after receiving inactivated COVID-19 vaccines

| Variables | All AIIRDs | SLE | RA | BD | PsA/PsO | pSS |
|---|---|---|---|---|---|---|
| Participants (n) | 1507 | 614 | 434 | 122 | 76 | 74 |
| Female (n, %) | 1166 (77.4%) | 572 (93.2%) | 342 (78.8%) | 63 (51.6%) | 34 (44.7%) | 69 (93.2%) |
| Age (median, years) | 39 (31, 51) | 33 (27, 40) | 50 (39, 60) | 37 (30, 45) | 46 (36, 58) | 48 (39, 59) |
| Disease duration (median, years) | 5 (2, 10) | 5 (3, 10) | 4 (2, 10) | 6 (3, 10) | 10 (3, 20) | 3 (2, 5) |
| Allergic history (n, %)* | 209 (13.9%) | 127 (20.7%) | 36 (8.3%) | 21 (17.2%) | 4 (5.3%) | 6 (8.1%) |
| Complete two-dose vaccine (n, %) | 1197 (79.4%) | 436 (71.0%) | 407 (93.8%) | 87 (71.3%) | 63 (82.9%) | 62 (83.8%) |
| Inactivated vaccine band (n, %) | | | | | | |
| Sinopharm | 607 (40.3%) | 272 (44.3%) | 156 (35.9%) | 59 (48.4%) | 25 (32.9%) | 26 (35.1%) |
| Sinovac | 874 (58.0%) | 340 (55.4%) | 268 (61.8%) | 62 (50.8%) | 50 (65.8%) | 47 (63.5%) |
| Others/uncertain band | 26 (1.7%) | 2 (0.3%) | 10 (2.3%) | 1 (0.8%) | 1 (1.3%) | 1 (1.4%) |
| AEs (n, %) | 450 (29.9%) | 232 (37.8%) | 106 (24.4%) | 34 (27.9%) | 14 (18.4%) | 24 (32.4%) |
| Local (n, %) | 287 (19.0%) | 160 (26.1%) | 65 (15.0%) | 19 (15.6%) | 7 (9.2%) | 13 (17,6%) |
| Systemic (n, %) | 260 (17.3%) | 120 (19.5%) | 66 (15, 2%) | 28 (23.0%) | 8 (10.5%) | 15 (20.3%) |
| Rash | 55 | 28 | 13 | 9 | 2 | 3 |
| Fever/chills | 43 | 19 | 10 | 4 | 2 | 1 |
| Headache | 82 | 40 | 21 | 11 | 2 | 4 |
| Fatigue/sleepless | 123 | 57 | 31 | 14 | 2 | 7 |
| Nausea/vomiting | 26 | 15 | 10 | 3 | 0 | 1 |
| Diarrhoea | 10 | 7 | 0 | 1 | 1 | 1 |
| Others | 32 | 11 | 9 | 6 | 1 | 2 |
| Side effects after first vaccine (n, %) | 321/1507 (21.3%) | 179/614 (29.2%) | 69/434 (15.9%) | 32/122 (26.2%) | 10/76 (13.2%) | 18/74 (24.3%)‡ |
| Timing of onset, days (median) | 2 (1, 3) | 1 (1, 3) | 2 (1, 3) | 2 (1, 2) | 1 (1, 2) | 2 (1, 2) |
| Side effects after second vaccine (n, %) | 140/1210 (11.8%) | 68/436 (15.6%) | 44/302 (14.6%) | 9/87 (10.3%) | 5/63 (7.9%) | 4/62 (6.5%)‡ |
| Timing of onset, days (median) | 2 (1, 5) | 1 (1, 7) | 1 (1, 5) | 2 (1, 3) | 1 (1, 2) | 2 (1, 7) |
| Self-reported severe AE (n, %) | 28 (1.9%) | 11 (1.8%) | 4 (0.9%) | 8 (6.6%) | 0 (0%) | 1 (1.4%) |
| Fatal AE of interest (n, %)† | 0 | 0 | 0 | 0 | 0 | 0 |
| Self-reported flare after vaccine (n, %) | 158 (10.5%) | 65 (10.6%) | 41 (9.4%) | 14 (11.5%) | 3 (3.9%) | 5 (6.8%) |
| Flare required treatment escalation (n, %) | 53 (3.5%) | 19 (3.1%) | 11 (2.5%) | 7 (5.7%) | 1 (1.3%) | 2 (2.7%) |

*This question was described as 'Have you ever been allergic to any food, drug or environmental substance etc before?'.
†Means anaphylactic shock, myocarditis, idiopathic thrombocytopenic purpura and death.
‡Three participants were not fully clear about that the side effects occured.after first or second vaccination.
AE, adverse event; AIIRDs, autoimmune inflammatory rheumatic diseases; BD, Behcet's disease; PsA/PsO, psoriatic arthritis/psoriasis; pSS, primary Sjogren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

patients. Joint pain (61/158, 38.6%) and swelling (31/158, 19.6%) were the most common manifestations of disease flare, followed by skin rash (27/158, 17.1%), morning stiffness (20/158, 12.7%) and febrile recurrence (14/158, 8.9%). Interestingly, the frequencies of AE and flare of AIIRDs were generally lower in inflammatory arthritis patients (RA or PsA/PsO) than those in patients with systemic AIIRDs (eg, SLE and BD) (online supplemental figure 3). Multivariable logistic analyses demonstrated that elderly, allergic history was the risk factor for disease flare of their underlying AIIRDs, while stable disease of AIIRDs was the negative predictor for self-reported disease flare only (online supplemental table 1).

Our data confirmed the safety profiles, and for the first time demonstrated the disease flare after inactivated COVID-19 vaccination in patients with AIIRDs. Overall, 29.9% of participants experienced AEs after vaccination and no fatal AEs occurred, indicating the well tolerability of inactivated COVID-19 vaccines in AIIRDs population. Importantly, our results aligned with a large real-world study supported by European League against Rheumatism(EULAR) COVID-19 database (83% mRNA vaccines), whose vaccine-related AEs were observed in 31% of patients.[4] Considering the possibility of over-activating immune system by adjuvanted vaccines, the stability of AIIRDs after vaccinations has been a principal concern. In this study, we found that although 1 in 10 reported a flare of disease after inactivated COVID-19 vaccination, fewer than 1 in 25 required treatment escalation. No episode of severe flare needing emergent hospitalisation was reported. Furthermore, we found elderly patients and those with allergic history were more likely to have disease flare after vaccinations. These call for important clinical needs for early warning of flare and close monitoring after vaccination. The incidence of AEs and AIIRD flares was generally comparable among all COVID-19 vaccines.[4–6] These may provide evidence for rheumatologists in critical discussion on vaccine acceptance.

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# Higher serum levels of short-chain fatty acids are associated with non-progression to arthritis in individuals at increased risk of RA

Transition from the autoimmune to the clinical phase of rheumatoid arthritis (RA) is a critical step that is yet insufficiently understood. Identification of factors that facilitate the progression from this prodromal RA at-risk state to clinical RA may open new possibilities for preventive interventions. In this context, nutritional factors may be critical. Short-chain fatty acids (SCFAs) are intestinal microbial metabolites that result from nutritional fibre digestion and exert immune regulatory properties.[1] SCFAs have shown to effectively inhibit the onset of experimental arthritis.[2] Furthermore, serum butyrate levels decrease shortly before the onset of arthritis.[2] Whether SCFA levels may play a role in the transition from the autoimmune to the clinical phase of RA in humans, however, has not been studied to date.

To address this concept, we measured serum SCFA levels in a prospective cohort of 82 individuals with an increased risk to develop RA.[3] At inclusion, these individuals were positive for anti-citrullinated protein antibodies (ACPA) and had musculoskeletal pain but no clinical signs of arthritis (joint swelling). Baseline characteristics are shown in online supplemental table 1. Following a median follow-up of 72 months, 39 patients (48%) had developed clinical arthritis after a median of 6 months. Baseline serum samples were analysed for SCFA concentrations as previously described.[4] At-risk individuals not progressing to arthritis had significantly higher mean baseline serum levels of total SCFA (ie, the sum of acetate, butyrate, propionate or pentanoate), butyrate and acetate as compared by t-test to individuals who progressed to arthritis (figure 1). In contrast, levels of propionate and pentanoate did not significantly differ (figure 1). Univariable Cox regression analyses revealed significant association between lower total SCFA levels and progression to arthritis ($p=0.029$), while for the individual SCFA, we found significant associations concerning butyrate ($p=0.038$) and acetate ($p=0.039$) levels, but not regarding pentanoate or propionate (online supplemental table 2). Statistical significance remained after adjusting for age, sex, symptom duration, rheumatoid factor status, ACPA levels and CRP levels (total SCFA $p=0.030$; butyrate $p=0.009$ and acetate $p=0.045$, online supplemental table 2).

Butyrate levels inversely correlated with serum IgA-ACPA levels ($r=-0.23$, $p=0.039$), but not with IgG-ACPA or IgM-ACPA. No other SCFAs were significantly correlated with any ACPA subtype.

These data suggest that SCFA, in particular butyrate and acetate, influences the risk for the transition from the autoimmune to the clinical phase of RA. Although most p values would not remain significant after correction for multiple testing, the data are in line with previous findings in animal models[2] and thus confirm our prespecified hypothesis. As SCFAs are produced by intestinal microbiota on fermentation of dietary fibres, our findings strengthen the concept that nutritional factors could influence the onset of RA. SCFAs are critical for the barrier function

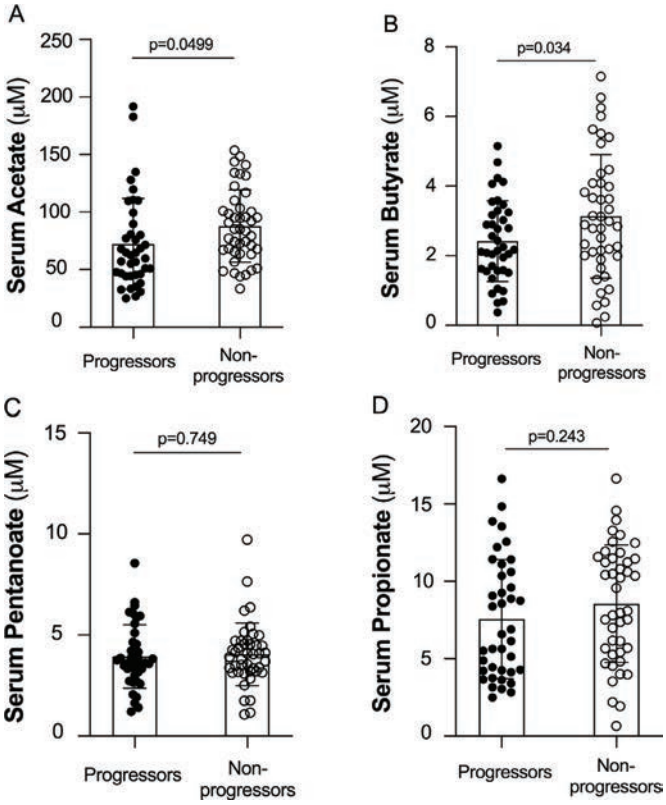


**Figure 1** Baseline serum samples from rheumatoid arthritis at-risk individuals (ACPA+; musculoskeletal pain+) progressing (n=39) or not progressing (n=43) to arthritis in a prospective observational cohort study[3] were analysed for (A) acetate, (B) butyrate, (C) pentanoate and (D) propionate levels. Bars represent means and error bars represent SD. ACPA, anti-citrullinated protein antibodies.

of the intestinal epithelium and thereby influences the migration of cells from the gut to the joints.[2] Increasing SCFA levels by direct supplementation, fiber-rich diet or faecal transplantation to restore early dysbiosis thus represent potential strategies to inhibit the onset of arthritis.[4–6] In this context, high-fibre diet has already shown to increase SCFA levels and decrease inflammatory burden in patients with established RA[4] but has not been applied in a preventive setting. These data suggest that a state of high SCFA concentrations, which can be reached by dietary interventions such as high-fibre diet, may go along with a lower risk to progress to clinical arthritis in individuals with a high risk to develop RA.

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**Ethics approval** We declare that this study was approved by Swedish Ethical Review Authority (2020-06162) and was conducted in accordance with the ethical standards set forth in the Declaration of Helsinki and its subsequent amendments. All study subjects provided written informed consent before inclusion in the TIRx cohort.

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# When extended genetics rescues diagnosis: a patient with CANDLE-like phenotype and de novo mutation in the *SAMD9L* gene

We read with interest Rusmini *et al* report[1] that discussed the application of next-generation sequencing (NGS) in the diagnosis of systemic auto inflammatory diseases (SAID) in 2016. By developing an NGS panel of 10 SAID-associated genes on 50 patients with a known Sanger-identified variant, a third of them were found to carry one or more additional possible effective variants in at least one other gene. Nevertheless, their phenotypic contribution was doubtful, representing the most challenging issue for the use of NGS panels in the daily clinical practice. Herein, we report a striking illustration of the value of extended NGS in patients with unexpected phenotype. We describe the case of a child with prominent inflammatory and cutaneous phenotype, in whom the first NGS panel's results hypothesised a diagnosis that is analogous to a chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature syndrome (CANDLE-like). Despite the identification of a single heterozygous variant in the *PSMB8* gene, a second underlying pathogenic variant was suspected given that a genetic digenism is usually frequent in proteasome-associated auto-inflammatory syndromes. But there was more that met the eye: as the patient did not present typical features of CANDLE, genetic investigation was pursued with a whole-exome sequencing revealing a de novo frameshift mutation in the Sterile Alpha Motif Domain–containing protein 9-Like (*SAMD9L*) gene and leading to the diagnosis of SAMD9L-associated autoinflammatory disease (SAMD9L-SAAD).

The girl was born to unrelated Caucasian parents. From birth, she presented with urticaria that covered her face and limbs (figure 1A). The rash became ecchymotic, also extending to her palms and soles. Since the age of 3 months old, she developed unexplained recurrent fever (2 days long, two times a month) and a painful peripheral oedema without real signs of arthritis. Moreover, blood workup showed continuous and moderate inflammation during and in-between outbreaks (C reactive protein 57 mg/L, serum amyloid A 93 mg/L, erythrocyte sedimentation rate 34 mm/hour) with high cytokines levels. Interferon signature, performed three times, showed moderate positivity only once: 13.7 (N<2.3). Corticosteroid treatment was efficient but resulted in failure to thrive. Finally, a diagnosis of SAID was suggested. The patient received a 3 mg/Kg daily dose of anakinra with partial efficacy (figure 1B). Skin biopsy showed inflammatory infiltrate in the whole dermis. The presence of cells with both histiocytic and myeloperoxidase positive phenotype was compatible with the diagnosis of CANDLE. Initial genetic investigations through NGS of a 55 auto-inflammatory genes panel did not detect any pathogenic variant, mostly in the *NLRP3*-associated autoinflammatory syndrome and the mevalonate kinase deficiency responsible genes, but it showed one heterozygous p.(Thr74Ser) variant[2] in the *PMSB8* gene that was confirmed by Sanger sequencing.

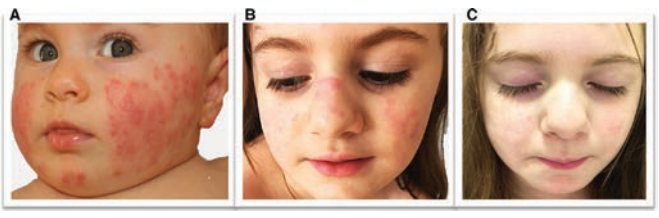


**Figure 1** Disease skin involvement over time and treatments. (A): At 9 months old, before treatments. (B): At 6 years old, 4 years after IL-1 inhibitor start. (C): At 7 years old, 1 year after treatment switch from IL-1 inhibitor to JAK 1/2 inhibitor.

CANDLE syndrome belongs to a group of rare monogenic autoinflammatory diseases also called interferonopathies. They originate from aberrant interferon production and signalling. The prototypical CANDLE syndrome is linked to recessive mutations in the immunoproteasome subunits genes, that is, subunit beta type 8 (PSMB8), affecting the clearance of damaged proteins along the proteasome.[3]

Despite the fact that the patient had prominent inflammatory and cutaneous phenotype, the constellation of criteria was incomplete and atypical for CANDLE syndrome. First, the examination of her skin by an experienced dermatologist did not show any signs of lipodystrophy. Then, other factors marked some differences, such as the absence of hepatomegaly and cytopenia as well as the mild interferon signature. Genetic analysis was, therefore, pursued with a whole-exome sequencing revealing a de novo frameshift variant p.(Ile876Leufs*15) in the *SAMD9L* gene. Treatment switch with Janus Kinase (JAK) inhibitor (baricitinib) finally enabled a clear skin improvement (figure 1C).

SAMD9L-SAAD is a new entity previously described in eight patients whose clinical features overlap with CANDLE[4 5] (see online supplemental file 1). The overall phenotype of patients is very severe as three out of eight patients deceased at an early age and three other one of them underwent bone marrow transplantation for severe cytopenia or interstitial lung disease (ILD). Our patient (and seemingly the other one carrying the c.2626del/p.(Ile876Leufs*15) mutation[4]) had a milder phenotype than the seven others. So far, at 7 years old, she does not present more than a systemic autoinflammatory phenotype with panniculitis. Indeed, the cerebral CT scan was found normal with no ganglia basa calcifications. Moreover, the pulmonary function test and pulmonary CT scan did not suspect interstitial lung disease. Finally, the immunological workup showed a normal B cells subpopulation (CD19+ cells count: 466/mm$^3$). Ataxia-pancytopenia syndrome was excluded due to the absence of clinical ataxia and cytopenia. Bone marrow karyotype did not evidence a monosomy 7.

Our report underlines the interest of pursuing the investigations when both the phenotype and the genotype of a patient does not fall into the framework of what has been previously known. A recent research conducted and published by de Jesus *et al* in *The Journal of Clinical Investigation* expands the diagnostic armamentarium that supports the challenging evaluation of patients with undifferentiated autoinflammatory diseases.[4] It substantiates the value of genetic investigations via NGS in patients with unexpected phenotype, interferon signature or IL-18 levels. Extended NGS rescued the diagnosis in seven patients with SAMD9L-associated autoinflammatory disease previously diagnosed with CANDLE. SAMD9L-SAAD is a new entity that is worth being differentiated from CANDLE as it may predispose to early and severe complications that need to be prevented.

From a treatment perspective, patients with SAMD9L-SAAD may respond to JAK-inhibiting therapies despite their mild

interferon signature.[6] The functional consequences of *SAMD9L* gene mutations need to be clarified to optimise a targeted therapy.

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# Challenges in optimising patient participation in research: do patients participating in meetings represent the actual patient population with Behçet's syndrome?

The importance of patient involvement in healthcare research is increasingly emphasised. Patients participate as research partners in designing studies and development of management recommendations, measurement tools and outcome measures.[1] Both Outcome Measures in Rheumatology (OMERACT) and the British Medical Journal encourage patient involvement in various aspects of research.[2 3] However, ensuring representation of the general patient population by specific patient groups may be challenging for multisystem diseases with heterogeneous phenotype. This is important for ensuring a successful structural involvement of patients in research, without over/underrepresentation of certain groups based on demographic or clinical features. Behçet's syndrome (BS) is a multisystem variable vessel vasculitis that shows substantial heterogeneity in clinical findings and disease course.

We aimed to evaluate whether patients with BS participating in a patient convention represent the actual patient population attending the clinic. A questionnaire was applied to 104 patients with BS (Meeting group) attending the patient convention, which was held during the Cerrahpasa Behçet's Disease Symposium in Istanbul in February 2020. Patients had been invited to the convention through posters, advertisement on our website and social media. The questionnaire was carried out with a keypad provided to the patients and consisted of 21 items such as age, gender, education level, working status, duration of illness, BS symptoms and treatment. The same questionnaire was also applied to 100 consecutive patients (Clinic group) who attended our rheumatology outpatient clinic for their routine controls. Three patients from the Clinic group were excluded due to incomplete data on their questionnaires. Both groups self-reported their disease manifestations and medications. The questionnaire was prepared in lay language and included explanatory pictures of BS manifestations and medications. $\chi^2$ test was used to compare the groups.

Table 1 shows the demographic and disease characteristics of the patient groups. There were three overlapping patients. The groups were similar in terms of sex. There were more men in both groups, probably reflecting the more severe disease course among men in BS. In the Meeting group, the number of patients who were >40 years of age and had a disease duration of >20 years were significantly higher. Although not significant, there were more patients who had a university education in the Meeting group. This may be associated with a higher level of health literacy in the Meeting group and we think it would be

**Table 1** Demographics, clinical characteristics and treatments

| Characteristics | Meeting group (n=104) (n/N, %) | Clinic group (n=97) (n, %) | P |
|---|---|---|---|
| Sex | | | |
| Female | 43/97 (41) | 35 (36) | 0.30 |
| Age (years) | | | |
| <20 | 1/95 (1) | 3 (3) | 0.026* |
| 20–30 | 6/95 (5) | 15 (15) | |
| 30–40 | 27/95 (25) | 36 (37) | |
| >40 | 61/95 (59) | 43 (45) | |
| Education level | | | |
| Primary school | 39/101(38) | 41 (42) | 0.142 |
| Secondary school | 15/101 (16) | 12 (13) | |
| High school | 23/101 (22) | 31 (32) | |
| University | 24/101 (24) | 12 (13) | |
| Patients with a job | 49/101 (49) | 55 (57) | 0.26 |
| Disease duration (years) | | | |
| <5 | 13/98 (13) | 27 (28) | 0.03* |
| 5–10 | 12/98 (12) | 18 (19) | |
| 10–20 | 31/98 (32) | 32 (33) | |
| Over 20 | 42/98 (43) | 20 (20) | |
| Patients with regular follow-up | 90/101 (89) | 86 (89) | NS |
| Only mucocutaneous involvement | 11/99 (11) | 14 (14) | 0.53 |
| Patients with any major organ involvement | 88/99 (89) | 83 (86) | 0.53 |
| BS manifestations | | | |
| Oral aphthous ulcers | 88/97 (91) | 94 (97) | 0.13 |
| Genital ulcers | 86/104 (83) | 68 (70) | 0.045 |
| Erythema nodosum | 77/103 (75) | 47 (48) | 0.0003 |
| Papulopustular lesions | 69/103 (67) | 75 (77) | 0.09 |
| Arthritis | 78/102 (77) | 46(47) | <0.0001 |
| Eye involvement | 51/103 (50) | 53 (55) | 0.48 |
| Vascular involvement | 42/98 (43) | 25 (26) | 0.036 |
| CNS involvement | 14/103 (14) | 2 (2) | 0.016 |
| GI involvement | 14/97 (14) | 6 (6) | 0.10 |
| Treatment | | | |
| Prednisolone | | | |
| Still using | 30/104 (29) | 34 (35) | 0.37 |
| Ever used | 88/104 (85) | 72 (74) | 0.08 |
| Colchicine | | | |
| Still using | 43/100 (43) | 46 (47) | 0.57 |
| Ever used | 86/100 (86) | 74 (76) | 0.10 |
| AZA | | | |
| Still using | 45/100 (45) | 41 (42) | 0.77 |
| Ever used | 81/100 (81) | 74 (76) | 0.49 |
| CYC | | | |
| Still using | 1/96 (1) | 0 (0) | NS |
| Ever used | 16/96 (17) | 7 (7) | 0.048 |
| bDMARDs | | | |
| Still using | 20/101 (20) | 26 (27) | 0.31 |
| Ever used | 28/101 (28) | 32 (33) | 0.44 |

*Adjusted p values by Bonferroni correction were <0.001.
AZA, azathioprine; bDMARDs, biologic disease-modifying anti-rheumatic drugs; BS, Behçet's syndrome; CNS, central nervous system; CYC, cyclophosphamide; GI, gastrointestinal; NS, not significant.

interesting to assess this in a larger group of patients. There were fewer patients who were employed in the Meeting group, but the difference was not significant. Central nervous system involvement, vascular involvement, genital ulcers, erythema nodosum and arthritis were significantly more common in patients in the Meeting group compared with those in the Clinic group. The frequency of eye involvement, gastrointestinal involvement and papulopustular lesions was similar in the two groups. Cyclophosphamide use was significantly more common in the Meeting group compared with the Clinic group. Overall, patients in the Meeting group had more severe disease compared with the Clinic group. Patients with all types of involvement were adequately represented in the Meeting group.

Patients' participation in healthcare research helps better reflection of patients' needs and difficulties.[4] However, for each medical condition, patients' needs and difficulties may be different according to their disease activity, severity and organs that are involved. The European Alliance of Associations for Rheumatology have developed recommendations for the inclusion of patient representatives in scientific projects. Good communication skills, motivation and constructive assertiveness were recommended to be taken into account when selecting patient research partners and it was recommended to include at least two patient research partners in each project. We additionally suggest ensuring adequate representation of the spectrum of patient population for multisystem heterogeneous conditions. We think this is important for improving the quality of management recommendations, measurement tools and outcome measures, especially when voting is the definitive step in the development of these.

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# Can sexual dimorphism in rheumatoid arthritis be attributed to the different abundance of *Gardnerella*?

Rheumatoid arthritis (RA) is a common chronic inflammatory joint disease that can cause recurrent attacks of joint pain and swelling. Progression of RA eventually causes joint destruction, ankylosis and deformity, which are associated with significant increases in socioeconomic costs and burdens.[1 2] Epidemiological studies conducted worldwide have shown that women are more likely than men of the same age to develop RA, and that the prevalence increases with age. More importantly, relevant literature indicates that the prevalence of RA continues to increase among women.[3 4] In addition, female patients experience more severe joint dysfunction compared with male patients.[4] Emerging data clearly demonstrate that women with RA have a significantly worse functional status as measured by instruments such as the 28-joint Disease Activity Score (DAS28), Health Assessment Questionnaire and visual analogue scale.[4 5] Consequently, an understanding of the causes of this sexual dimorphism may provide new insights and guidance for the development of interventions intended to reduce the incidence of RA.

Previously, this sexual dimorphism of RA has been explained in terms of genes located on the X and Y chromosomes, sex hormones and specific physiological and psychosocial conditions.[4] However, the above perspectives were accompanied by corresponding limitations. Notably, most of the relevant literature tended to elaborate from the perspective of sex hormones. Those studies focused on the effects of sex hormones on the immune system and enabled a detailed understanding of the target immune cells and immune-related genes regulated by sex hormones. Similarly, the causes of this sexual dimorphism remain equivocal.

Recent work by Kishikawa *et al* is expected to elucidate this phenomenon of RA. The researchers performed a genome-wide association study (GWAS) to analyse the role of the gut microbiome in Japanese patients with RA.[6] In this study, faecal samples were subjected to whole-genome shotgun sequencing. Through case–control phylogenetic association tests conducted using a generalised linear regression model, nine strains present at significantly higher levels of abundance in patients with RA were finally identified, namely *Prevotella denticola*, *Gardnerella*, *Gardnerella vaginalis*, *Porphyromonas somerae*, *Prevotella marshii*, *Prevotella disiens*, *Bacteroides sartorii*, *Prevotella corporis* and *Prevotella amnii*.[6] Moreover, the authors clearly demonstrated correlations of multiple *Prevotella* spp beyond *P. copri* with the aetiology of RA. Furthermore, a gene related to oxidative stress (*R6FCZ7*) was more abundantly expressed in patients with RA than in healthy subjects. Moreover, by comparing the results of a RA microbial GWAS with those of a RA GWAS, the authors determined a population-specific biological pathway linking the metagenome and host genome. These results newly revealed a relationship between the gut microbiome, host genome and RA pathology, and thus represent a new reference that better clarifies the aetiology of RA.[6] Certainly, this study is expected to explain the sexual dimorphism of RA because of the nine strains mentioned by Kishikawa *et al*, *Gardnerella*, or *G. vaginalis*, is a clinical indicator of vaginitis in female patients. This gram-negative bacillus is isolated from female vaginal secretions and is the most frequent causative organism of vaginitis.[7 8] Consequently, might we also interpret the differential treatment of RA according to sex from the perspective of the microbiome?

We propose the following scientific hypothesis or conjecture: *Gardnerella* or *G. vaginalis* may be associated with sexual dimorphism in RA. To our knowledge, however, no previous study has clarified this phenomenon of sexual dimorphism directly from the perspective of the microbiome. Therefore, we considered whether the mechanism of bacterial vaginitis, which is closely related to *Gardnerella*, might provide some clues. Fortunately, a new review of *Gardnerella* and vaginal health revealed that *Gardnerella* spp are related to female vaginal infection and also appear in other types of infection.[8] More notably, these other types of infections include conditions such as acute hip arthritis, hip swelling, disc space infection, discitis, spinal osteomyelitis, joint infection and reactive arthritis.[8] Although these phenomena were described only in certain clinical cases, the data were sufficient to suggest an association of *Gardnerella* with severe RA symptoms in female patients. However, we did not know whether the above relationship was direct or indirect. The relevant literature mentioned a mutually beneficial relationship between *Gardnerella* and *Prevotella bivia*.[9] More precisely, specific amino acids produced by *Gardnerella* could be used by *P. bivia*, thus enhancing the growth of the latter species.[9] This led to the bold speculation of an unknown homoplastic link between certain strains of *Gardnerella* and *Prevotella*. In other words, an interaction of *Gardnerella* with *Prevotella* may contribute to the onset of RA. The different concentrations of *Gardnerella* in male and female humans would eventually lead to sexual dimorphism in RA. In this regard, a single strain of *Gardnerella* could be isolated, cultured and transplanted into germ-free mice to verify the existence of a direct relationship with RA. *Prevotella* should also be isolated, cultured and transplanted into germ-free mice together with *Gardnerella*. A single homologous *Prevotella* strain should also be transplanted into germ-free mice as an experimental control. Such an experiment may roughly prove that *Gardnerella* directly influences the onset of RA by interacting with *Prevotella* spp.

In addition, relevant reports have mentioned that sex hormone levels may support the expansion of some selected microbial lineages via a positive feedback mechanism, which would contribute to the sexual dimorphism phenomenon observed in autoimmune diseases.[10 11] In other words, changes in the compositions of some microbial lineages may be affected by sex hormone levels, and in turn, regulation of the composition of some microbial lineages may contribute to changes in sex hormone levels. Again, this expands our speculation that *Gardnerella* may also indirectly lead to sexual dimorphism in RA by influencing the levels of sex hormones.

Regardless, more specific scientific research regarding this issue is needed. Although some of the aforementioned conjectures or speculations may fail to provide further experimental verification, we still hope to discuss our ideas through this short article and hope that our colleagues in the field of RA will pay slightly more attention to *Gardnerella*.

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# Response to: 'Can sexual dimorphism in rheumatoid arthritis be attributed to the different abundance of *Gardnerella*?' by Liu *et al*

We thank Liu *et al* for their interest in our paper and for providing their thoughts through correspondence.[1] We agree that gut microbiome studies are promising to identify novel insights into the sexual dimorphism in rheumatoid arthritis (RA). We reported that the genus of *Gardnerella* and the species of *Gardnerella vaginalis* increased in the gut microbiome of patients with RA.[2] *G. vaginalis* is known as a representative causal bacterium of vaginosis. In our study, *G. vaginalis* was detected in both male and female samples, and there were no significant gender differences in their relative abundance ($P$=0.41). Thus, the increment of *G. vaginalis* was less likely to be contamination from the female genital organ in perineum. We further performed case-control association tests of *G. vaginalis* stratified by gender with age, sequencing groups and the top two principal components as covariates (figure 1A). We found that the effect size was larger in female samples (beta=1.14, $P=4.1\times10^{-4}$) than in male samples (beta=0.596, $P$=0.22). This result suggests that the increment of *Gardnerella* in the gut microbiome of patients with RA is specific to the female samples.

*Gardnerella* has been detected not only in vaginitis but also in a variety of infections, such as hip arthritis and joint infections.[3] However, their biological and pathological roles in gut microbiome have been elusive. *G. vaginalis* was reported to have a symbiotic positive relationship with *Prevotella bivia*.[4] Considering the association of *Prevotella* with RA aetiology,[5–7] Liu *et al* proposed that *G. vaginalis* affects RA aetiology through the symbiotic proliferation of *Prevotella*. In our study, there was no significant positive correlation between the relative abundance of the genus *Prevotella* and that of *G. vaginalis* ($r$=0.054, $P$=0.47; figure 1B). However, when we focused on the total abundance of the five *Prevotella* species with significant RA-control discrepancy (i.e., *P. denticola*, *P. marshii*, *P. disiens*, *P. corporis* and *P. amnii*), significant positive correlation was found ($r$=0.20, $P$=0.024). Among the five RA-associated species, *P. amnii* had nominally significant positive correlation ($r$=0.19, $P$=0.035) with *G. vaginalis*, while the others did not ($P$>0.062). There was no significant correlation between *P. bivia* and *G. vaginalis* either ($P$=0.84). These results demonstrated that *Gardnerella*

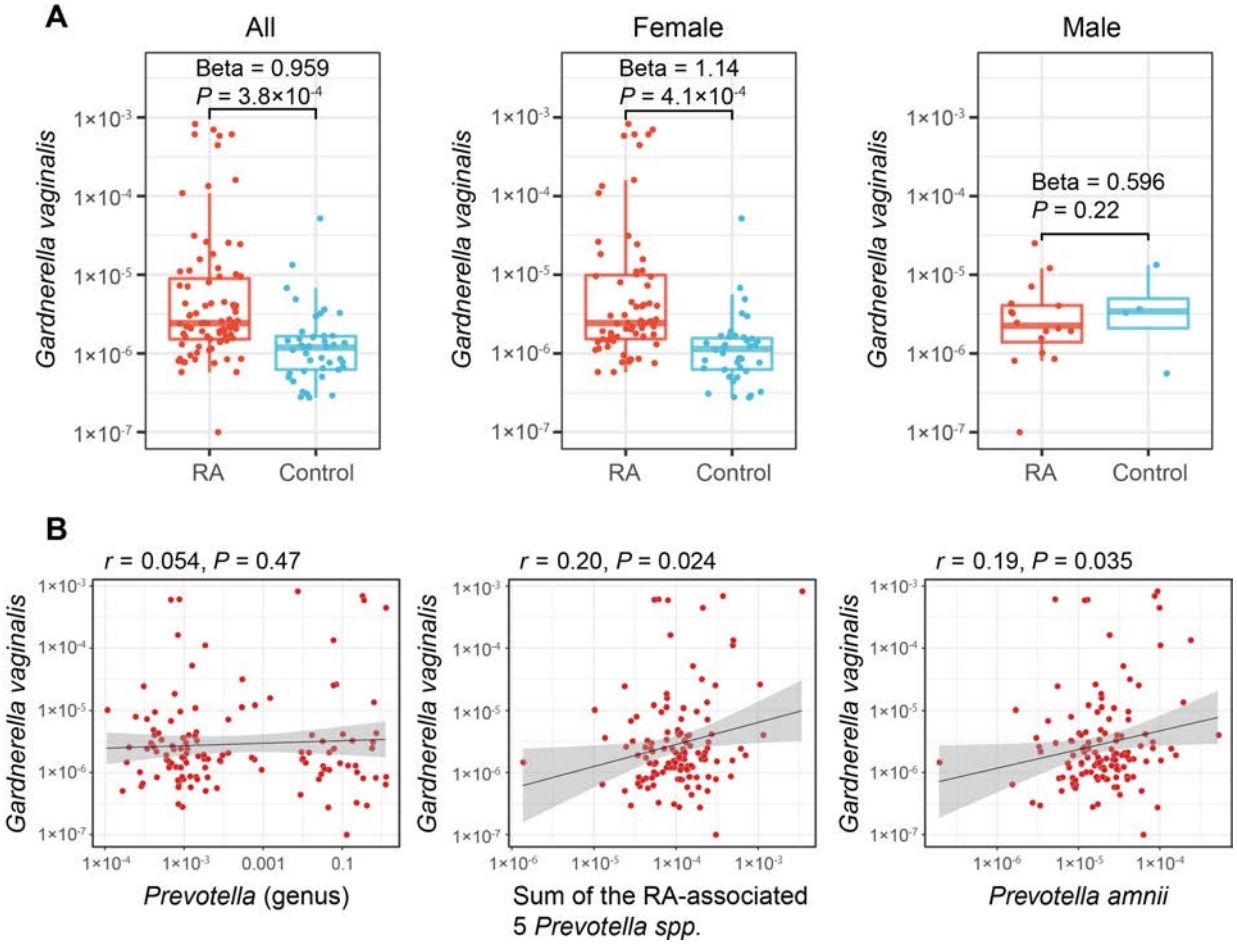


**Figure 1** Characteristics of the relative abundance of *Gardnerella vaginalis* in RA samples. (A) Boxplots of the relative abundance of *G. vaginalis* in RA and control samples. The *y*-axes indicate the relative abundance of *G. vaginalis* in a logarithmic scale. The left, centre and right boxplots are for all samples, only female samples and only male samples, respectively. The lower and upper hinges of the boxes indicate the first and third quartiles, respectively. The horizontal lines within the boxes indicate median levels. (B) Correlation of the relative abundance of *G. vaginalis* with that of *Prevotella* spp. The *x*-axes of the left, centre and right figures indicate the relative abundance of the genus *Prevotella*, the total abundance of the five *Prevotella* species with significant RA-control discrepancy (i.e., *P. denticola*, *P. marshii*, *P. disiens*, *P. corporis* and *P. amnii*) and the relative abundance of *P. amnii* in a logarithmic scale, respectively. The *y*-axes indicate the relative abundance of *G. vaginalis* in a logarithmic scale. RA, rheumatoid arthritis.

and RA-associated *Prevotella* had a symbiotic relationship in gut microbiome. Further studies are required to reveal the role of the *G. vaginalis* in the gut microbiome of patients with RA.

Liu *et al* also mentioned that female patients with RA confer more severe inflammation profiles (i.e., 28-joint Disease Activity Score (DAS28)) than male patients.[8] Assessing whether *Gardnerella* is responsible for the sexual difference of RA severity, we performed association tests between the relative abundance of *G. vaginalis* and DAS28-CRP, but no significant association was found ($P=0.91$, $r=-0.013$). This result indicates that *Gardnerella* in gut microbiome may not be related to RA severity in female samples.

We hoped that researches focusing on *G. vaginalis* would lead to further clarification of aetiology of RA.

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# Questions on 'Sequencing of the MHC region defines *HLA-DQA*1 as the major genetic risk for seropositive rheumatoid arthritis in Han Chinese population' by Guo *et al*

We read with great interest the paper by Guo *et al*[1] addressing the HLA association with seropositive rheumatoid arthritis (RA) in Han Chinese. The authors reported that aspartic acid at position 160 of HLA-DQα1 (HLA-DQα1:160D) was the major risk factor. It was accompanied by asparagine at position 37 of HLA-DRβ1 (HLA-DRβ1:37N), which was protective. These results were obtained by targeted sequencing in 961 cases and 1812 controls distributed in discovery and validation stages. The underlying assumption is that sequencing had uncovered new susceptibility HLA alleles. Specifically, HLA-DQα1 has not previously been associated with RA, whereas the most associated HLA alleles and amino acids were those included in the shared epitope (SE) of HLA-DRB1.[2–4] SE alleles that have been associated with increased RA risk in all the ethnic groups analysed including the Han Chinese and other Asian ethnicities.[2–5] The new results are, therefore, of an extraordinary novelty and need to be considered with attention.

A careful analysis shows reasons for concern due to internal inconsistencies in the Guo *et al* study.[1] These inconsistencies include amino acids at DQα1:160 that do not sum up: the frequencies of the three amino acids (D, A and S) were 0.20, 0.22 and ≈0.02 in controls and 0.36, 0.37 and ≈0.01 in patients with RA, respectively (table 1). The three amino acids did not add up to 1.0 as required given that they are the only amino acids at this position. Also, the OR in cases/controls of the DQα1:160 amino acids was inconsistently described: two of the amino acids were described as increased in patients with RA, DQα1:160D with OR=2.36 and DQα1:160A with OR=2.27 (table 1). These ORs are impossible considering the low frequency of the third (DQα1:160S) amino acid. None of these inconsistencies can be attributed to a typographical error because they appear in multiple places. In addition, the DRβ1:37N amino acid was reported as associated with protection from RA with OR=0.49 and $p=5.81\times10^{-16}$, but its frequency was identical in patients with RA and controls (table 1). This puzzling result is unlikely to be a typographical error because the equality between patients and controls is reported in three supplementary tables and because the omnibus test performed by Guo *et al* did not find DRβ1:37N among the DRB1 amino acids associated with RA (supplementary table 10 in Guo *et al*). On the contrary, the most associated DRB1 amino acids (page 776 and supplementary table 10 in Guo *et al*) were the same reported in other studies that correspond to the SE, which are the 11 and 13 amino-acid positions and the DRB1*04:05 allele.[2–5] Besides these internal inconsistencies, the frequency of the DQA1 alleles containing the DQα:160A amino acid was much lower in Guo *et al* than in other studies including those done in Asians (table 1).[6] These inconsistencies are worrisome and ask for clarification.

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**Table 1** Inconsistencies in the frequencies of the top associated amino acids from Guo *et al*[1]

| | Guo *et al* | | | Other studies* | | |
|---|---|---|---|---|---|---|
| | Frequency† | | | Hong Kong Chinese | Koreans§ | Japanese |
| Amino acids | Controls | Patients with RA | OR‡ | n=1064 | n=1209 | n=3078 |
| DQα1:160D | 0.20 | 0.36 | 2.36 | 0.21 | 0.22 | 0.31 |
| DQα1:160A | 0.22 | 0.37 | 2.27 | 0.78 | 0.76 | 0.66 |
| DQα1:160S | 0.02 | 0.01 | | 0.01 | 0.02 | 0.03 |
| DRβ1:37N | 0.23 | 0.23 | 0.49 | | | |

*Taken from the Allele Frequencies Net Database (http://allelefrequencies.net).
†Frequencies taken from Guo *et al.* Figure 3 and supplementary tables 3, 5 and 8 for DQα1 amino acids and from supplementary tables 4, 6 and 8 for DRβ1:37N.
‡OR reported in pages 775 and 776 of Guo *et al* for DQα1 and DRβ1:37N, respectively.
§Results for Koreans were combined from four studies.
RA, rheumatoid arthritis.

# Response to: 'Questions on 'Sequencing of the MHC region defines *HLA-DQA*1 as the major genetic risk for seropositive rheumatoid arthritis in Han Chinese population' by Guo *et al*' by Regueiro and Gonzalez

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We appreciate Dr Gonzalez's interest and comments on our recent publication 'Sequencing of the major histocompatibility complex (MHC) region defines human leukocyte antigen (HLA)-DQA1 as the major genetic risk for seropositive rheumatoid arthritis in Han Chinese population'.[1 2] Dr Gonzalez's comments provide us with an opportunity to clarify and discuss the frequencies of amino-acids at position DQα1:160 and the protective association of DRβ1:37N in rheumatoid arthritis (RA), and to improve our study.

One of the concerns Dr Gonzalez expressed is the frequencies of three amino-acids (Asp (D), Ala (A) and Ser (S)) at DQα1:160 do not sum up to 1.0, that is, 0.20, 0.22 and ≈0.02 in controls; 0.36, 0.37 and ≈0.01 in RA patients. The explanation is that the 'minor frequencies' was set as default for all variants in PLINK. The original frequencies of three amino-acids (D, A and S) were 0.20, *0.78* (1–0.22) and ≈0.02 (the sum is 1.00) in controls and 0.36, *0.63* (1–0.37) and ≈0.01 (the sum is 1.00) in cases, respectively. In our original paper the frequencies of DQα1:160A inhealthy controls were similar to those reported in other Asian studies.[3] These results do not affect the calculation of p value, but do affect the odd ratio (OR) calculation. Indeed, by the omnibus test DQα1:160A showed a protective effect (OR=0.46, $p=2.72 \times 10^{-35}$, online supplementary table 10 in Guo *et al*).[2] We appreciate Dr Gonzalez *et al* for this important point and have made a correction for our publication, in which all variants have been presented according to original frequencies instead of minor frequencies.[4]

Regarding the protective effect of DRβ1:37N, although the identified amino-acid DRβ1:37N did not show any significant association in univariate regression analysis, it reached second strong statistical significance after conditioning on DQα1:160D in both discovery and validation stages, indicating an independent association. This phenomenon could be potentially explained by the Simpson's paradox, a striking observation that an association between two variables at the population-level might increase or decrease in quantity, or even change direction within the subgroups, depending on the set of variables being controlled,[5 6] and has been reported in several genetic association studies.[7 8] Notably, the DRβ1:11D also showed an independent protective effect and was in high linkage disequilibrium (LD) with DRβ1:37N (r2=0.62; online supplementary tables 8 and 9 in Guo *et al*).

Regarding other DRB1 variants, as the author indicated, by omnibus test we replicated the findings reported in previous studies,[9–12] including the position 11 and 13 at DRβ1, and the allele *DRB1*04:05*. However, our study focused on single nucleotide polymorphisms (SNPs), classical HLA alleles and the individual amino-acid variants rather than amino-acid positions, because a particular amino-acid(s) may have potential biological function(s). Furthermore, different amino-acids at same position may insert different functions.[13] Taking this into consideration, DQα1:160D remained the top association in omnibus test ($OR=2.30$, $p=1.82 \times 10^{-38}$) (online supplementary table 10 in Guo *et al*). Furthermore, consistent with our findings, Hirata *et al*[14] have also reported that one of DQα1:160D encoding allele *DQA1*0303* was a strong risk for susceptibility to RA in Japanese population ($OR=2.65$, $p=2.0 \times 10^{-173}$, shown in table 1 in Hirata *et al*).

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# Reactive arthritis, a missing link: comment on the recent article from Sepriano *et al*

The recent paper from Sepriano *et al* provides an extremely important new insight on the concept of axial spondyloarthritis (axSpA).[1] Clearly, the *Gestalt* of axSpA is heterogeneous, with three recognisable clinical entities labelled as: 'pure axial SpA', 'axial SpA with peripheral signs' and 'axial SpA at risk'. The finding given in the paper suggests a larger overlap between axSpA and pSpA than anticipated at the time when the Assessment of SpondyloArthritis international Society (ASAS) criteria were developed. Thus, the question arises as to how accurately the three recognisable clinical entities of the ASAS classification criteria represent the diseases entities originally lumped together in the historical concept of SpA.

The unifying historical concept of SpA lumps together an interrelated yet heterogeneous group of disorders which includes ankylosing spondylitis (AS), psoriatic arthritis, arthropathy of inflammatory bowel diseases (ulcerative colitis and Crohn's disease), reactive arthritis (ReA), undifferentiated SpA and juvenile SpA.[2] ReA is characterised by preceding infections of the urogenital, gastrointestinal and respiratory tract, and these are best explored for *Chlamydia trachomatis* and *Chlamydia pneumoniae* infections for the joint and spine manifestations.[3] Preceding infection of urethritis/cervicitis or diarrhoea within 1 month prior to the onset of arthritis/enthesitis/dactylitis is included in the ASAS criteria for peripheral SpA but not in those for axial SpA. Baseline patient characteristics and the final latent class analysis models in the SPondyloArthritis Caught Early (SPACE) and DEvenir des Spondylarthopathies Indifférenciées Récentes (DESIR) cohorts do not mention preceding infections.[1] Thus, the latent class and transition analyses neglect infections, although ReA typically manifests with peripheral arthritis as well as enthesitis, tendinitis, bursitis and inflammatory low back pain.[4] Moreover, remitting and chronic ReA may evolve into sacroiliitis in 14%–49%, and into AS in 12%–26% of patients, depending on the triggering infection; a minority of patients even manifest radiological sacroiliitis during the first known attack or arthritis (compare ref 5). Importantly, the causative infections are often asymptomatic or mild, or they may precede the arthritis by several years. Therefore, these silent infections may not appear in medical history and are only discovered by targeted investigation, such as has been demonstrated, for example, for *C. trachomatis* and *C. pneumoniae* (compare ref 6).

Diseases are defined and categorised in a variety of ways: by the symptoms with which they present (syndromic), their underlying causes (aetiological), the biological mechanisms involved (pathogenic), available treatments, historical precedent and through diagnostic exclusion.[7] Understanding gut microbiota–host genetic relationships may contribute to clarification of the pathogenesis of postinfectious SpA and pave the way from symptomatic to aetiological classification.[8] Of note, in the study from a geographic region with a high prevalence of ReA (Guatemala), prospectively included adult subjects with preceding infections developing arthritis classified as pSpA, and control subjects not developing arthritis, both had radiographic sacroiliitis in 56% and 50% of individuals, respectively; thus the postinfectious pSpA would presumably meet the *Gestalt* of 'axial SpA with peripheral signs'.[8]

In conclusion, in recent years the ASAS classification criteria for axial SpA have provided an important contribution to education, research and clinical trials addressing earlier diagnosis, outcome measurements and new treatments for axial SpA. Nonetheless, future classification sets which specify relevant infectious triggers should be useful in advancing classification and related treatment studies, thus giving increased validity also for geographic regions outside Europe which display a higher prevalence of ReA.

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# Response to: 'Reactive arthritis, a missing link: comment on the recent article from Sepriano *et al*' by Zeidler and Hudson

Zeidler and Hudson[1] break a lance on the entity of reactive arthritis when dealing with classification criteria for spondyloarthritis (SpA), axial or peripheral. We would like to thank our colleagues for their insightful comments and reassure them that we do not forget reactive arthritis as an entity or put it aside.

It is important to make clear that Zeidler and Hudson argue about a causal pathophysiological relationship: an infection that causes a disease (arthritis) with features resembling the phenotype (*Gestalt*) of SpA. However, we did not talk about underlying pathophysiology but rather about clustering patients on the basis of communal features.[2] Our starting point was young patients presenting with chronic back pain. We left the pathophysiology of SpA undiscussed.

When fitting reactive arthritis into this concept, it would probably start with those 10% of patients who do not recover spontaneously (or after symptomatic treatment) from reactive arthritis, but will develop persistent disease. These patients will likely be captured by the criteria for peripheral (arthritis, enthesitis, dactylitis) or axial SpA because they will have a phenotype resembling SpA. As such, there is nothing new under the sun.

Admittedly, if Zeidler and Hudson's plea pertains to an overarching umbrella concept that includes both semiacute (self-limiting reactive arthritis) as well as chronic SpA, our concept will probably not fit. It is, however, questionable whether such an 'umbrella-concept' would truly help in understanding the already heterogeneous presentation of 'chronic SpA'.

Still, we agree that infections deserve persistent attention as potential causes of SpA.

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# Secukinumab efficacy in patients with PsA is not dependent on patients' body mass index

We read with interest the recently published paper from McGonagle *et al*[1] analysing the role of interleukin (IL)-17A in axial spondyloarthritis and psoriatic arthritis (PsA). The meta-analysis and functional study provided by the authors highlighted the efficacy of IL-17A block by secukinumab in the treatment of PsA. However, there is no mention of the role of body mass index (BMI), if any, in influencing the clinical response to secukinumab, given the lack of published data. PsA is a chronic inflammatory arthritis burdened by a series of metabolic comorbidities. Among them, obesity is very common in PsA, with a prevalence of 27%, as confirmed by a recent Spanish work.[2] Obesity in PsA has been associated with higher disease activity and a worse effectiveness of biologic treatment in PsA. This has been certainly proven for anti-tumour necrosis factor (TNF)-α as demonstrated by different studies reporting, in obese patients, a reduced treatment response and adherence. In particular, results coming from DAN-BIO and ICE-BIO registries[3] point out that obesity is a risk factor for anti-TNF withdrawal due to poor response. Although a recent multicentric, retrospective study in Spain has shown that obese subjects with psoriasis have a poor therapeutic response to secukinumab,[4] no data are currently available for secukinumab in obese patients with PsA.

Our studies focused on the relationship between BMI and clinical response to secukinumab in PsA. We prospectively analysed 100 patients with PsA (57% female, median age 53 (49.2–55.0 years) satisfying Classification Criteria for Psoriatic Arthritis (CASPAR) criteria[5] for PsA, afferent to our clinics, who were treated with secukinumab. Patients were divided into two groups based on BMI (BMI<25 normal weight and BMI≥25 overweight/obese). In the normal-weight group, 75% were female; the median age was 50.5 (41.0–54.6); the median BMI was 22 (20.2–23.3); and the median Disease Activity in PSoriatic Arthritis (DAPSA) was 19.19 (15.6–24.2). The features of the overweight/obese patients were similar to those of the normal-weight group (48% were female, median age 54 (50–59), median BMI 29 (27.4–30.1) and median DAPSA 21.2 (19.0–24.4)). Clinical response to therapy, evaluated as the achievement of low disease activity or remission according to DAPSA, was recorded 6 months after starting treatment. After 6 months of treatment, the variation of the DAPSA was inversely related to BMI: overweight/obese patients had in fact a better response to secukinumab compared with normal-weight patients (figure 1A,B). By using a correlation coefficient (Statistical Package for Social Science (SPSS)) to analyse the degree of association between BMI and DAPSA, we confirmed that BMI and DAPSA were inversely related in patients with PsA (p=0.05) in our study.

Interestingly, analysis of serum levels of IL-17 in 20 obese patients compared with 20 non-obese patients showed significantly higher serum levels of IL-17 in the former (figure 1C), indicating IL-17 as a key cytokine driving inflammation in obese patients with PsA. As far as we are concerned, these are the first data about clinical response to secukinumab in obese patients with PsA. Obesity has been shown to promote the expansion of IL-17-producing T cells in adipose and peripheral tissues.[6] In addition, in patients affected by metabolic syndrome, the levels of IL-17R expression in the liver or muscles are correlated with insulin resistance.[6] Our results support the relevance of IL-17 in driving systemic inflammation in obese patients with PsA, also providing evidence that obese patients may have a better response to secukinumab compared with non-obese patients. Interestingly, this effect was not influenced by the secukinumab dosage. In conclusion, although further studies are required to confirm our data, these findings indicate a close relationship between IL-17, obesity and PsA, possibly supporting the idea that obesity might be one relevant clinical factor driving the choice of secukinumab in overweight/obese patients.

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A

| | | | continuous BMI | DAPSA T6 |
|---|---|---|---|---|
| Kendall's tau coefficient | Continuous BMI | correlation coefficient | 1,000 | 0.135* |
| | | 1-TAIL SIGNIFICANCE | - | 0.028 |
| | | N | 100 | 93 |
| | DAPSA T6 | correlation coefficient | 0.135* | 1,000 |
| | | 1-TAIL SIGNIFICANCE | 0.028 | - |
| | | N | 93 | 93 |
| Spearman's rho | Continuous BMI | correlation coefficient | 1,000 | 0.192* |
| | | 1-TAIL SIGNIFICANCE | - | 0.032 |
| | | N | 100 | 93 |
| | DAPSA T6 | correlation coefficient | 0.192* | 1,000 |
| | | 1-TAIL SIGNIFICANCE | 0.032 | - |
| | | N | 93 | 93 |

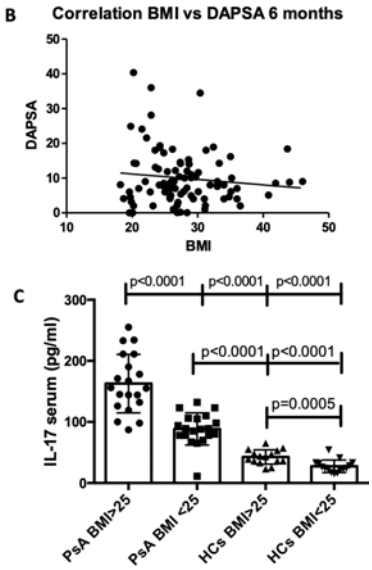


**Figure 1** Correlation between BMI and DAPSA and serum IL-17 levels in PsA obese and non-obese patients. (A) Correlation between BMI and DAPSA by Kendall's tau coefficient and Spearman's rho tests (*statistical significance is for values of 0.05, one tail). (B) Graphical representation of correlation between BMI and DAPSA by Spearman test. (C) Analysis of serum levels of IL-17 in 20 obese and 20 obese and 20 non-obese patients compared to 30 healthy controls. BMI, body mass index; HC, health control; IL, interleukin; PsA, psoriatic arthritis.

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# Prevalence of comorbidities and risk factors in spondyloarthritis: results of a cross-sectional study

Spondyloarthritis (SpA) includes axial and peripheral SpA, according to the Assessment of SpondyloArthritis international Society classification criteria.[1 2] An increasing number of research has focused on the comorbidities and risk factors in SpA. The first and latest international cross-sectional ASAS-COMOSPA study, published in the *Annals of the Rheumatic Diseases*, has proposed some comorbidities such as cardiovascular diseases, osteoporosis, cancers, infections and gastrointestinal diseases. This research has also proposed the risk factors for cardiovascular diseases, cancers and osteoporosis.[3] Other studies have also focused on the cardiovascular diseases among patients with SpA.[4–6] However, as for heart comorbidities, valvular heart disease was not mentioned in the ASAS-COMOSPA study, while it has been reported to be associated with SpA in a number of recent studies, based both on epidemiology and pathology.[7–9] As for risk factors for cardiovascular diseases, hyperuricaemia was not included as well, while uric acid has also been proven to be related to cardiovascular disease in the recent years.[10–13] Since there are still some comorbidities and risk factors to be evaluated, we proposed a research to include valvular heart disease as a comorbidity of SpA and hyperuricaemia as a risk factor of cardiovascular disease among patients with SpA.

From 2016 to 2018, we conducted a cross-sectional study of 202 SpA diagnosed by rheumatologists in the Chinese Shenzhen Second People's Hospital. We extracted data from the hospital's information systems by searching medical files at the Department of Rheumatology and Immunology, the Department of Ophthalmology, and the Department of Gastroenterology. All the medical files were examined to see if the diagnosis of axial or peripheral SpA could be confirmed.

Once the diagnosis of axial and peripheral SpA was confirmed, the following categories of data were collected: demographics and disease characteristics, extra-articular manifestations, comorbidities, risk factors for comorbidities, and sacroiliac joint image. The Bath Ankylosing Spondylitis Disease Activity Index,[14] the Ankylosing Spondylitis Disease Activity Score calculated with CRP,[15 16] and the Bath Ankylosing Spondylitis Functional Index were collected.[17] Medications including non-steroidal anti-inflammatory drug (NSAID), corticosteroids, conventional synthetic disease-modifying antirheumatic drugs (DMARDs) and tumour necrosis factor inhibitor (TNFi) were also collected. Extra-articular manifestations included uveitis, psoriasis and inflammatory bowel disease (IBD) (Crohn's/ulcerative colitis). Comorbidities included cardiovascular disease, diabetes, valvular heart disease and gout. Risk factors for comorbidities of cardiovascular disease included smoking, hypertension, hyperuricaemia and hyperlipidaemia. Hypertension was defined as a history of hypertension or antihypertensive therapy or blood pressure (BP) >140/90 mm Hg, or BP >130/80 mm Hg in the case of history of diabetes or renal insufficiency. Hyperuricaemia was defined as uric acid >416 µmol/L in men and >357 µmol/L in women.[18–20] Hyperlipidaemia included factors of triglycerides, low-density lipoprotein (LDL) cholesterol and total cholesterol.[3 12] Sacroiliac joint image was evaluated as radiographic SpA or non-radiographic SpA. Radiographic SpA was defined as grade II bilaterally or grade III–IV unilaterally, according to the Modification of the New York Criteria for radiography.[21] It can also be defined as an erosion score and/or joint space score of 2 or higher in any of the 24 regions of both joints for CT.[22] Non-radiographic SpA was defined as sacroiliac joint image without structural sacroiliitis described above.[23] Data were analysed with the use of the statistical packages R

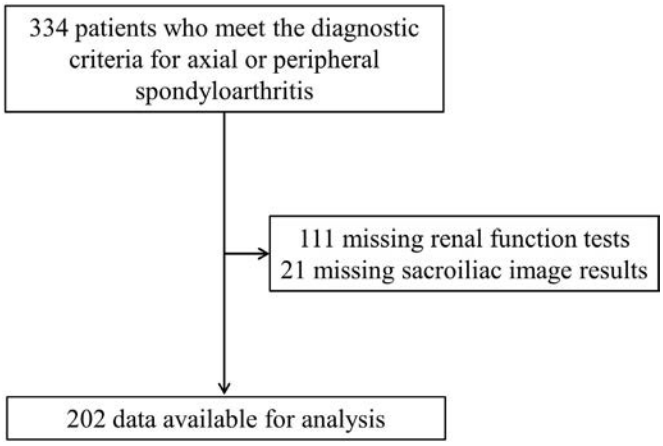


**Figure 1** Study profile.

**Table 1** Demographics and disease characteristics of the 202 patients of this study

| Number of patients | 202 |
|---|---|
| Age (years) | 38.2±12.6 |
| Gender (male) | 147 (72.8%) |
| HLA-B27 | 177 (88.9%) |
| Smoking status (current) | 35 (17.3%) |
| Smoking status (ever) | 39 (19.3%) |
| Alcohol | 27 (13.4%) |
| Disease duration (months) | 87.5±86.6 |
| Uric acid (umol/L) | 380.2±107.7 |
| BASDAI | 3.3±1.9 |
| ASDAS-CRP | 2.6±1.1 |
| BASFI (0–10) | 1.8±2.2 |
| Axial involvement (any) | 181 (89.6%) |
| Peripheral involvement (any) | 104 (51.5%) |
| Axial involvement (only) | 103 (51.0%) |
| Peripheral involvement (only) | 25 (12.4%) |
| Mixed (axial and peripheral involvement) | 84 (41.6%) |
| Enthesitis involvement | 25 (12.4%) |
| Dactylitis | 9 (4.5%) |
| Uveitis | 35 (17.3%) |
| Psoriasis | 10 (5.0%) |
| IBD | 4 (2.0%) |
| Diarrhoea | 21 (10.4%) |
| NSAID (ever) | 107 (53.0%) |
| Corticosteroids (past) | 27 (13.4%) |
| Conventional synthetic DMARDs (past) | 49 (24.3%) |
| TNFi (past) | 21 (10.4%) |
| Conventional synthetic DMARDs (onset) | 53 (26.2%) |
| TNFi (onset) | 63 (31.2%) |

All results are presented as mean±SD for continuous variables and percentages for categorical variables.

ASDAS, Ankylosing Spondylitis Disease Activity Score-CRP; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; CRP, C reactive protein; DMARDs, disease-modifying antirheumatic drugs; HLA-B27, human leucocyte antigen B27; IBD, inflammatory bowel disease; NSAID, non-steroidal anti-inflammatory drug; TNFi, tumour necrosis factor inhibitor.

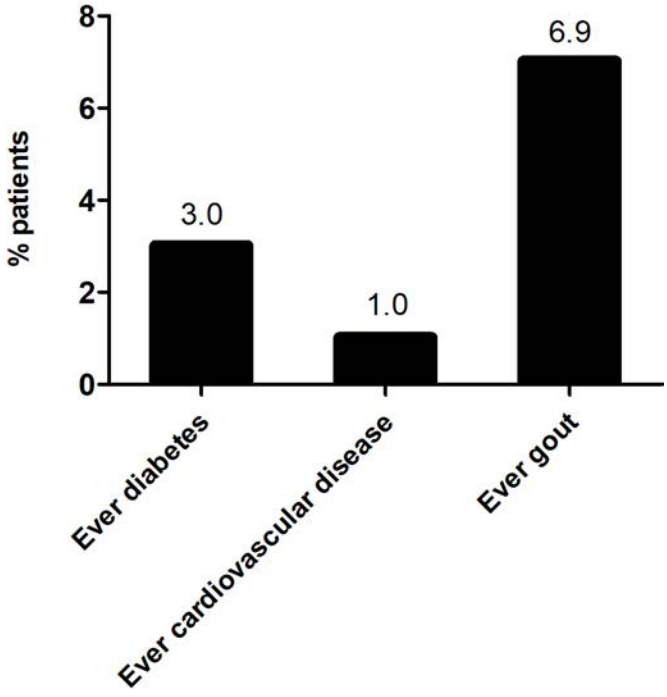


Figure 2 Prevalence of comorbidities.

(The R Foundation; http://www.r-project.org; version 3.4.3) and EmpowerStats (www.empowerstats.com; X&Y Solutions Inc).

Between 1 January 2016 and 31 December 2018, 202 individuals were included in this study. Individuals who were not examined for renal function (n=111) and those who were not examined for sacroiliac joint imaging (n=21) were not included. The study profile of screening patients for analysis is presented in figure 1.

The demographics and disease characteristics are listed in table 1. More patients presented axial involvement (89.6%), and fewer patients presented only peripheral involvement (12.4%). More patients ever accepted NSAIDs (53.0%), and fewer accepted TNFi (10.4%) compared with conventional synthetic DMARDs (24.3%). However, more and more patients began to accept TNFi (31.2%) as an optimal therapy, instead of conventional synthetic DMARDs (26.2%).

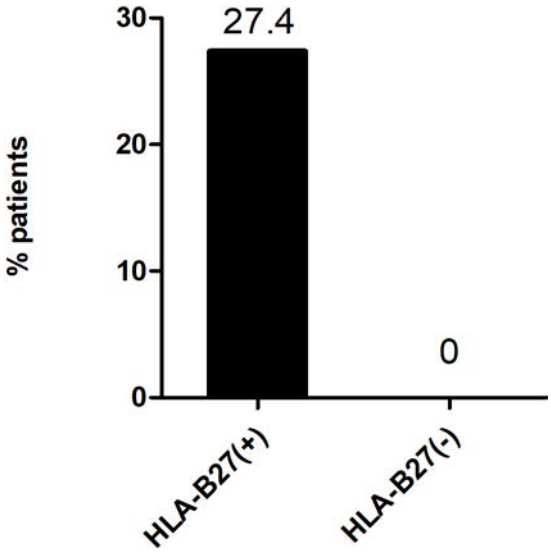


Figure 3 Valvular heart disease in HLA-B27-positive and HLA-B27-negative subgroups. HLA-B27, human leucocyte antigen B27.

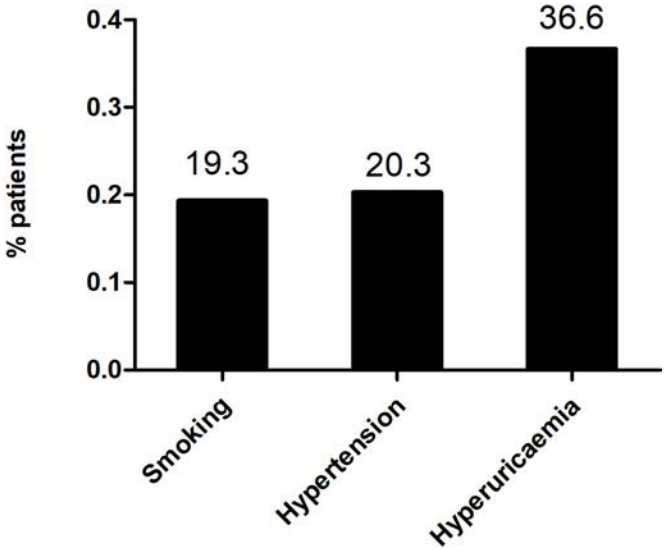


Figure 4 Prevalence of detected risk factors for comorbidities.

The most common extra-articular manifestation was uveitis (17.3%), while the least common was IBD (2.0%). Diarrhoea was relatively frequent, which was 10.4% of the study population.

The prevalence of comorbidities is presented in figure 2. The prevalence of cardiovascular disease in the study population was 1.0%, while that of diabetes was 3.0%. Of the patients 6.9% ever suffered gout. Cardiac ultrasonography evaluation was performed on 124 of the 202 patients, and the prevalence of valvular heart disease in this population was 24.2%. When evaluated by human leucocyte antigen B27 (HLA-B27)-positive and HLA-B27-negative subgroups, respectively, HLA-B27-positive subgroup presented more patients suffering from valvular heart disease (27.4%), which had statistical significance (p=0.014). This is presented in figure 3.

The prevalence of detected risk factors for comorbidities is presented in figure 4. Conventional risk factors for cardiovascular disease (ie, BP, LDL cholesterol) were detected in this study. Of the patients in this study, 19.3% ever smoked, 20.3% reported to have ever suffered from hypertension, 36.6% had hyperuricaemia, and 30.8% had hyperlipidaemia (hyperlipidaemia detection was conducted in a scope of 133 patients).

Uric acid, a specific risk factor, was evaluated in this study with a mean±SD of 380.2±107.7 umol/L. Uric acid was evaluated by radiographic SpA and non-radiographic SpA, with the radiographic subgroup (n=141, 69.8%) presenting a mean±SD of 397.7±106.6 umol/L and the non-radiographic subgroup (n=61, 30.2%) presenting a mean±SD of 339.8±99.8 umol/L, which had statistical significance (p<0.001). The prevalence of hyperuricaemia in the radiographic SpA subgroup was 40.4%, while in the non-radiographic SpA subgroup was 27.9%; these are all presented in figure 5.

Our study represents the characteristics of Chinese SpA population and reports some unnoticed and specific aspects, as a supplement to ASAS-COMOSPA study.

Although there were a lot of common demographics and disease characteristics of SpA presenting in this study as the ASAS-COMOSPA study, there were noteworthy aspects in extra-articular manifestation, comorbidities, risk factors for comorbidities and sacroiliac joint image.

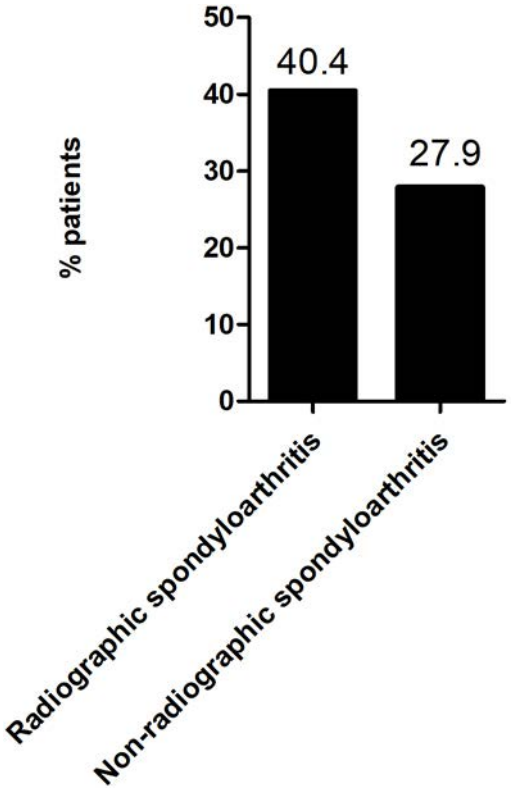


**Figure 5** Prevalence of hyperuricaemia in radiographic spondyloarthritis and non-radiographic spondyloarthritis subgroups.

IBD was less common in Chinese population compared with the worldwide population shown in the ASAS-COMOSPA study. Diarrhoea was a frequent symptom of IBD. In this study of Chinese population 10.4% had diarrhoea, while diarrhoea was not mentioned in the ASAS-COMOSPA study. IBD was diagnosed using endoscopic and histological measures. As for reports about IBD, endoscopic manifestations were not consistent with symptoms such as diarrhoea and stool frequency. Endoscopically inactive disease was not associated with complete normalisation of diarrhoea.[24] Diarrhoea, reflecting gut inflammation,[25] was a noteworthy symptom of IBD among patients with SpA.

Valvular heart disease, which is also a common heart comorbidity of SpA, was relatively higher (24.2%) in this study, compared with what has been reported in the heart failure population,[26] which was also neglected by the ASAS-COMOSPA study. The prevalence of valvular heart disease in HLA-B27-positive subgroup is different from that in the HLA-B27-negative subgroup. We are going to discuss the association between valvular heart disease and HLA-B27 in a subsequent article. The prevalence of gout was higher in our study than the general population of most countries in the world.[27]

Uric acid, which had been proven to be a new cardiovascular risk factor,[28 29] turned out to be relatively higher than the general population,[30] and was different between the radiographic and non-radiographic subgroups. We will later discuss the association between uric acid and sacroiliac radiographic image in another article.

Further studies confirming the impact of comorbidities and risk factors in patients with SpA are required. If confirmed, these studies would lead to standardised assessments of patients with SpA.

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# Glucocorticoid withdrawal in lupus – to do or not to do?

I read with great interest, the recently published article in your journal titled "Withdrawal of low-dose prednisone in SLE patients with a clinically quiescent disease for more than 1 year: a randomised clinical trial" by Mathian *et al*.[1] The discussion on the feasibility of completely stopping glucocorticoids in systemic lupus erythematosus (SLE) has been ongoing and it poses a practical challenge to every physician treating SLE. Besides the adverse effects, studies also suggest that long term glucocorticoid therapy for remission maintenance in SLE leads to increased accrual of organ damage.[2] The effect of withdrawal of glucocorticoids on SLE remission has been studied in a number of observational studies. Data from a randomised trial was lacking except for a small pilot study (SIMPL) conducted by Galbraith *et al* where the effect of glucocorticoid withdrawal on lupus nephritis remission maintenance was studied in 15 patients.[3]

The above-mentioned study by Mathian *et al* (CORTICOLUP study) provides scientific evidence addressing this issue. The study has successfully shown the superiority of glucocorticoid maintenance therapy over its withdrawal both in terms of all flares and severe flares.[1] The follow-up period was only 52 weeks, yet the number of flares in the withdrawal group were quite high (27% vs 7%) with a HR of 0.2. The results of the CORTICOLUP study are in contrast to the results of previous studies.[3 4]

Majority of the patients in the CORTICOLUP study in either groups (28 and 25 in maintenance and withdrawal groups, respectively) were not on any immunosuppressive therapy besides hydroxychloroquine, and 11 patients were not on hydroxychloroquine.[1] It would be interesting to know if there was any difference in the flare rates among those who were on immunosuppression compared with others. Another avenue to explore is the time of steroid withdrawal. A retrospective study by Tani *et al* suggests that the time interval from the last flare to the steroid withdrawal may also play a role in future flares.[5] Interestingly, the quiescent time was more in the withdrawal group in CORTICOLUP study, although not significant.[1]

Research is ongoing to study the steroid sparing effects of biologics in remission induction. Belimumab, epratuzumab, and tabalumab have been successfully shown to reduce the steroid doses by 25% or more in remission induction in a metanalysis by Oon *et al*.[6] The use of biologics and other immunosuppressants as steroid sparing therapy for remission maintenance in SLE is yet to be explored. In the absence of randomised trials favouring steroid withdrawal, the question remains – to withdraw or not to withdraw?

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# Response to: 'Glucocorticoid withdrawal in lupus: to do or not to do?' by Acharya

We thank Acharya for her interest in our study showing that in patients with systemic lupus erythematosus (SLE) with a quiescent disease and a stable treatment regimen, for at least 1 year, withdrawal of 5 mg of prednisone was associated with a four-fold increase (ie, 27%), in the risk of flare onset, as defined by the SELENA-SLEDAI flare index and the British Isles Lupus Assessment Group index during a 1-year follow-up.[1 2] Acharya states that these findings contrast with those from two previous published studies on the same subject.[3 4] The latter studies are, however, not comparable to ours. As pointed out by Acharya, the Steroids In the Maintenance of remission of Proliferative Lupus nephritis (SIMPL) trial was a small pilot study, including only 15 patients, that was however not designed to assess the efficacy or safety of maintaining low-dose prednisone administration.[3] With respect to the report of Moroni *et al*, in their study treatment withdrawal in patients with SLE with nephritis included not only glucocorticoids but also immunosuppressants.[4] We would like to argue that the results of the CORTICOLUP trial are consistent with those of recently published observational studies, indicating that treatment with low-dose glucocorticoids prevents relapse in about one-fifth to one-third of patients with SLE with no or very low disease activity.[5 6]

As suggested by Acharya, the results might have been different had the majority of patients been on immunosuppressant therapy. We, of course, do acknowledge that the results of the CORTICOLUP study cannot be extrapolated to all patients in remission. The percentage of patients treated with an immunosuppressant in the CORTICOLUP study, amounting to 27%, reflects the clinical practice of our team. Moreover, our practice is comparable to that of other teams.[5 7] Yet, the indication of an immunosuppressive treatment is not evidence based, especially in patients in remission, and depends to date on the decision of the physician and therefore varies according to his/her convictions. Finally, in the interaction analysis shown in figure 3 in our study,[2] there was no significant interaction between the effect of prednisone maintenance and immunosuppressants or hydroxychloroquine.

To conclude, like Acharya, we believe that prescribing an immunosuppressant or a biologic might reduce the use of prolonged glucocorticoid therapy to prevent relapse of the disease. However, this belief has to be proved and balanced with the infectious and oncological risk possibly brought about by long-term exposure to this type of medication.

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# Comments on the article: "Withdrawal of low-dose prednisone in SLE patients with a clinically quiescent disease for more than 1 year: a randomised clinical trial"

We recently have read with great interest the article written by Mathian *et al* entitled "Withdrawal of low-dose prednisone in SLE patients with a clinically quiescent disease for more than 1 year: a randomised clinical trial" published in 19 December 2019.[1] The study assessed development of flares in patients with systemic lupus erythematosus (SLE) with clinically inactive disease course on maintenance versus withdrawal of 5 mg/day prednisone over a 12-month period. This study showed that withdrawal of this low-dose glucocorticoid was associated with a fourfold increase in the risk of flares. Although this study is impressive and provides the strongest evidence regarding the efficacy of low-dose prednisone in the prevention of the disease flares, there are some concerns that may endanger the validity of the study.

First, as this study aimed at comparison of risk of flares patients with SLE experience in each of the two groups, it was expected that all factors determined so far in the literature, to be associated with increased risk of the disease flares, be considered and compared between the groups, if it is possible. African-American race (OR of 1.8 compared with Caucasian ethnicity), disease onset ≤25 years (HR of 2.14) and serum BLyS levels ≥2 ng/mL (HR of 1.5–1.9 to experience severe flares in the following 12 months) are risk factors not considered in the current study.[2–4] As confounding potential of these factors cannot be ruled out in this study, the causative relationship between withdrawal of prednisone and higher flare risk would be in question. Although the serum BLyS levels of the study participants at the baseline could not be evaluated anymore, the first two mentioned factors could simply be measured and added to the study. Adjustment of these potential confounding factors remarkably increases the credibility of the results in this study.

Second, masking the nature of the treatment course known as blinding is a critical methodological feature of randomised clinical trials (RCTs). A pilot study with a close design with similar purpose conducted by Galbraith *et al* showed that blinding is totally applicable in this setting by over-encapsulation of both prednisone and placebo tablets.[5] Since there was no placebo group, the findings of this open-label trial could have been influenced by two problems. First, some of the patients in the withdrawal group might have failed their adherence to immunosuppressive drugs following discontinuation of prednisone, as they might have thought that their disease status is better than the other group and it is not necessary to strictly follow the medications. The second problem is that some other patients might develop emotional stress after discontinuation of prednisone rooted from this fact that they are not receiving maintenance treatment anymore while a majority of other patients undergo long-term low-dose glucocorticoid treatment. As both these events, poor compliance to treatment and emotional stress are considered to be associated with an increased risk of flares, blinding should had been performed.[6]

Notwithstanding the foregoing, this study has provided a fascinating evidence and it is the sole RCT study conducted with appropriate population size concerning long-term use of maintenance glucocorticoid in patients with SLE. However, whether to administer or discontinue this low-dose glucocorticoid requires further studies to validate the results of the current study. Also, there is a need for studies that assess other dimensions of withdrawal of glucocorticoids in patients with SLE, as the goal of low-dose corticosteroid maintenance is not just to prevent the disease flares.

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# Response to: 'Comments on the article: "Withdrawal of low-dose prednisone in SLE patients with a clinically quiescent disease for more than 1 year: a randomised clinical trial"' by Mousavi and Taherifard

We thank Mousavi *et al* for their interest in our study.[1 2] Mousavi *et al* regret that we did not take into account different factors determined in the literature to be associated with increased risk of flare of the disease, such as 'African American ethnicity', disease onset ≤25 years and B Lymphocyte Stimulator (BLyS) serum levels ≥2 ng/mL.[3] However, the studies cited by Mousavi *et al* included active patients with systemic lupus erythematosus (SLE) (eg, with a Safety of Estrogens in Lupus Erythematosus National Assessment–Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) score ≥6 for the Study of Belimumab in Subjects With Systemic Lupus Erythematosus (BLISS)-52 and BLISS-76 trials[4]), while in the CORTICOLUP study only clinically quiescent patients were included. The predictors of relapse are certainly not the same as for active or clinically quiescent patients. Predictors of flares in clinically quiescent patients are poorly known. They include age, disease duration, remission duration, high anti-dsDNA and hypocomplementaemia.[5–7] These factors were assessed in the interaction analysis of the CORTICOLUP study. To answer more specifically to the comment of Mousavi *et al*, demographic classifications such as 'African-American, Caucasians or Hispanics', although requested by some health authorities, are non-relevant in France and other parts of the word. The French population of patients with SLE is widely multiethnic and includes a significant proportion of patients from African and Asian origin who were all represented in the CORTICOLUP. Morever, we think that these considerations do not affect the conclusions of the study because, as a result of the randomisation procedure, the frequencies of the different risk factors of flare mentioned by Mousavi *et al* should not differ between both groups. In this regard, the rate of patients with a disease onset ≤25 years was comparable in the group of patients who were withdrawn (38%) to those who were not (36%, p=0.9 using the $\chi^2$ test).

We, of course, do acknowledge that the results of the CORTICOLUP should be interpreted in light of its open-label design without a placebo group. However, we are not aware of studies showing that emotional stress due to treatment discontinuation is a recognised factor associated with an increased risk of lupus flare. We agree with Mousavi *et al* that we cannot be sure that all patients were actually taking their treatment regimen during the study. However, this limitation is frequent in clinical trials because, apart from a blood assessment of the drugs, it is difficult to be certain of the patients' adherence to treatment.

Yet, the measurement of hydroxychloroquine (HCQ) blood levels is encouraging in this respect. We have reported that very low blood HCQ concentrations can serve as an objective marker for poor adherence to treatment for SLE.[8] Using this assay, and as part of the overall assessment of the CORTICOLUP study, we have performed a post-hoc analysis, whose results do address the concern of Mousavi *et al*. For the patients treated with HCQ, only one patient in the group who were withdrawn from prednisone, and none of the patients in the group who continued prednisone intake, had HCQ blood levels<100 ng/mL at 3, 6, 9 and 12 months of the study. This result confirms the adherence of the patients in the CORTICOLUP study to the treatment.

To conclude, we agree with Mousavi *et al* that the goal of treatment maintenance is not just to prevent the disease flares. However, the reduction in the number of flares is certainly important because a consistent link between the number of flares and organ damage accrual, as well as the quality of life, healthcare cost and work productivity has previously been reported in the literature.[3]

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# Immune-mediated necrotizing myopathies and interstitial lung disease are predominant characteristics in anti-Ku positive patients with idiopathic inflammatory myopathies

We read an interesting study by Spielmann *et al* conducted on a single-centre large French cohort, which identified that anti-Ku autoantibodies were effective biomarkers for two distinct connective tissue diseases (CTDs): anti-Ku-positive patients with elevated serum creatine kinase (CK) levels had a high risk for developing interstitial lung diseases (ILD), while anti-Ku-positive patients with anti-double-strand DNA were at high risk for developing glomerulonephritis.[1] Anti-Ku autoantibodies are associated with various CTDs, such as systemic lupus erythematosus, systemic sclerosis, idiopathic inflammatory myopathies (IIM), mixed CTDs, Sjögren's syndrome, and rheumatoid arthritis. However, few studies have focused on the distinguishing features, especially the pathological features of IIM patients with isolated anti-Ku and anti-Ku coexistence with myositis-specific autoantibodies (MSA).

Here, we retrospectively investigated the characteristics of 1214 IIM patients with anti-Ku autoantibodies, all fulfilling the Bohan & Peter criteria for IIM and admitted to the Department of Rheumatology at China-Japan Friendship Hospital from January 2008 to July 2019. Anti-Ku autoantibodies were detected by line immunoassay (EUROLINE, Germany) and ELISA assay (Enzyme-linked Biotechnology, China) in the sera of 156 patients with anti-nuclear antibodies of titres≥1/160 showing fine speckled patterns on immunofluorescence assay of HEp-2 cells. Finally, 21 patients were confirmed to be anti-Ku-positive by line immunoassay and ELISA assay. Meanwhile, MSA and anti-3-hydroxy-3-methylglutaryl-CoA reductase autoantibody levels in the sera of anti-Ku-positive patients were measured using line immunoassay (EUROLINE, Germany) and ELISA assay (Raybiotech, China). Muscle biopsy was performed in 13 of 21 anti-Ku-positive patients.

The incidence of anti-Ku autoantibodies was 1.73% in our IIM cohort. Twenty out of 21 patients were women. The average age of onset was 42.60±14.35 years. Eight patients were diagnosed with dermatomyositis (DM), 11 with polymyositis (PM), and two overlapping with SSc. Eleven patients (52.4%) showed isolated anti-Ku antibodies, the others (47.6%) coexistence of anti-Ku with MSA. Skin involvement was less common among patients with isolated anti-Ku than that among patients showing coexistence of anti-Ku and MSA (18.2% vs 70%, p=0.03). ILD presented in 76.2% of anti-Ku-positive IIM patients, consistent with the high frequency of ILD reported in previous studies.[1 2] Although there was no significant difference in the incidence of ILD between patients with isolated anti-Ku and anti-Ku coexistence with MSA, patients with isolated anti-Ku had a lower mean percentage of predicted value for FVC and DLco than those with coexistence of anti-Ku and MSA(74.05%±12.84% vs 93.21±18.54%and 59.61±15.41% vs 76.03±14.15%, p=0.035and 0.049, respectively). Increased CK level was observed in 90.9% (10/11) of patients with isolated anti-Ku and 50% (5/10) of those with coexistence of anti-Ku and MSA (table 1).

In the previous studies on French and Japanese cohorts, the musculoskeletal histopathological performance of only 22 IIM patients with anti-Ku-positive was described.[3–5] The main pathological features were muscle fibre necrosis (18/22, 81.8%) and major histocompatibility complex (MHC) class I expression (16/19, 84.2%). In our cohort, 6 of 7 patients with isolated anti-Ku presented typical immune-mediated necrotizing myopathy (IMNM)-like pathological features with predominantly necrotic muscle fibre and CD68$^+$ macrophage endomysial infiltration in accordance with the 2017 European Neuromuscular Centre (ENMC) criteria for IMNM.[6] However, 1 out of 6 patients with coexistence of anti-Ku and MSA presented with typical pathological features of IMNM. Classical pathologic DM such as perifascicular atrophy and normal pathologic performance were observed in anti-TIF1γ- and anti-MDA5-positive

**Table 1** Characteristics of anti-Ku-positive patients with IIM

| Features | Isolated anti-Ku (n=11) | Coexistence of anti-Ku and MSA (n=10) |
|---|---|---|
| Female | 10 (90.9%) | 10 (100%) |
| Age of onset | 45.55±16.45 | 39.00±11.47 |
| Duration(months) | 12(3,72) | 29(4,60) |
| Diagnosis | | |
| DM | 2 (18.2%) | 6 (60%) |
| PM | 8 (72.7%) | 3 (30%) |
| PM+SSc | 1 (9.1%) | 0 |
| DM+SSc | 0 | 1 (10%) |
| MSA | | |
| MDA5 | – | 3 (14.8%) |
| NXP2 | – | 1 (4.8%) |
| TIF1γ | – | 2 (9.5%) |
| Jo-1 | – | 1 (4.8%) |
| PL-12 | – | 1 (4.8%) |
| PL-7 | – | 1 (4.8%) |
| SRP | – | 1 (4.8%) |
| MAA | | |
| Ro-52 | 1 (9.1%) | 5 (50%) |
| PM-Scl 75/100 | 0 | 2 (20%) |
| Muscle Weakness | 8 (72.7%) | 8 (80%) |
| Dysphagia | 2 (18.2%) | 5 (50%) |
| Neck weakness | 1 (8.3%) | 1 (10%) |
| Myalgia | 6 (54.5%) | 4 (40%) |
| Skin involvement* | 2 (18.2%) | 7 (70%) |
| Raynaud's phenomena | 3 (27.3%) | 2 (20%) |
| ILD | 8 (72.7%) | 8 (80%) |
| FVC% of predicted value† | 74.05±12.84 | 93.21±18.54 |
| DLco% of predicted value‡ | 59.61±15.41 | 76.03±14.15 |
| Arthritis | 3 (25%) | 2 (20%) |
| Cancer | 0 | 0 |
| Increased CK | 10 (90.9%) | 5 (50%) |
| Pathological pattern | n=7 | n=6 |
| IMNM | 6 | 1(anti-PL-7 positive) |
| pDM | 0 | 2(anti-MDA5 and -TIF1γ positive) |
| NSM | 1 | 1(anti-Jo-1 positive) |
| Normal | 0 | 2(anti-MDA5 and -TIF1γ positive) |

FVC and DLco value were available for 8 patients with isolated anti-Ku and 7 patients with anti-Ku coexistence of MSA.
*P=0.03.
†P=0.035.
‡P=0.049.
CK, creatine kinase; DLco, carbon monoxide diffusion capacity; DM, dermatomyositis; FVC, forced vital capacity; ILD, interstitial lung disease; IMNM, immune-mediated necrotizing myopathie; MAA, myositis-associated autoantibodies; MSA, myositis-specific autoantibodies; NSM, non-specific myositis; pDM, pathologic DM; PM, polymyositis; SSc, systemic sclerosis.

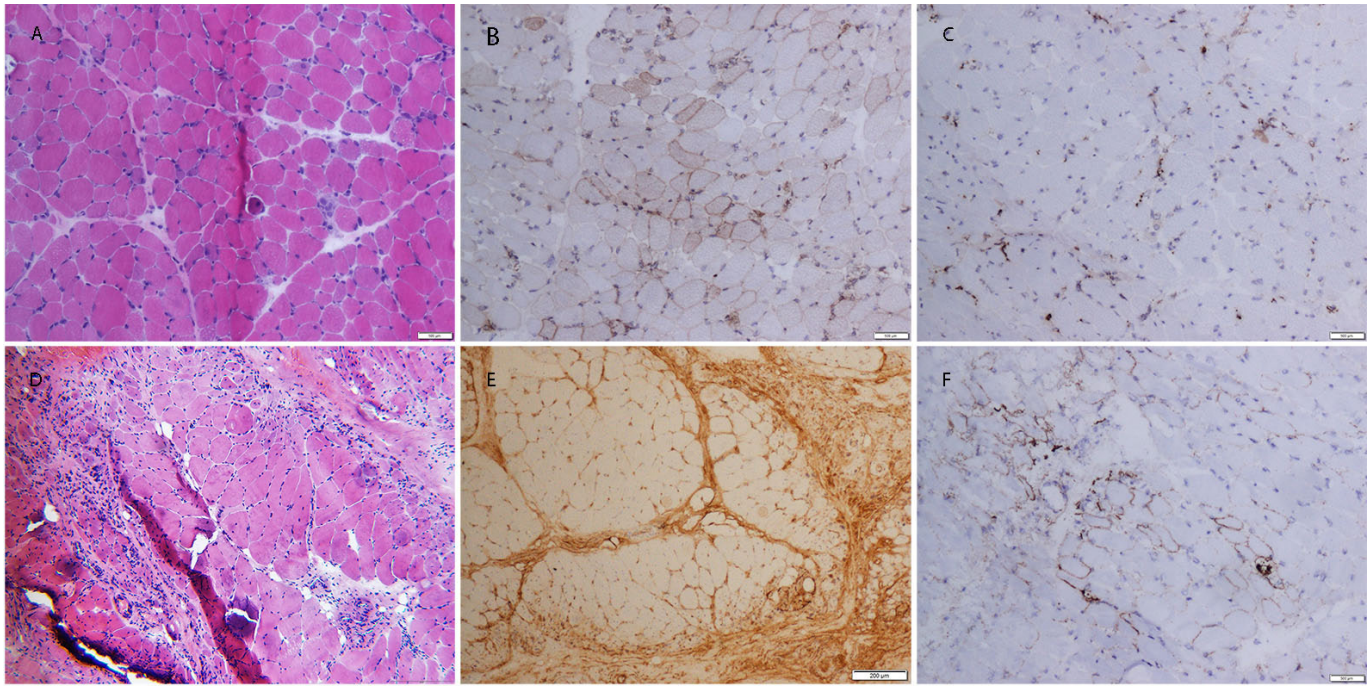


**Figure 1** H&E and immunohistochemistry staining of muscle specimens in anti-Ku-positive patients with idiopathic inflammatory myopathies (IIM). A-C, F IIM patient with isolated anti-Ku: Muscle fibre necrosis, myophagocytosis and regeneration(A), sarcolemmal MHC-I expression(B), $CD68^{+}$ cells scattered endomysial infiltration(C), sarcolemmal membrane attack complex(C5b-9) expression(F). D-E, dermatomyositis patient with anti-Ku coexistence of anti-TIF1γ: perifascicular atrophy(D), sarcolemmal MHC-I expression in perifascicular muscle fibre(E).

patients, respectively. In addition, 1 patient with coexistence of anti-Ku and anti-Jo-1 was diagnosed with non-specific myositis according to the 2004 ENMC classification criteria for IIM(table 1, figure 1).[7]

In conclusion, the presence of anti-Ku autoantibodies is rare among IIM patients. Concomitant ILD and elevated CK level are common features of anti-Ku positive patients. However, the clinical and pathological characteristics are distinct in patients with isolated anti-Ku and those with coexistence of anti-Ku and MSA. Skin rash is more common in patients with coexistence of anti-Ku and MSA, while severe ILD and IMNM are common in patients with isolated anti-Ku. Further studies on the characteristics of anti-Ku-positive IIM using larger cohorts are warranted.

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# Response to: 'Immune-mediated necrotizing myopathies and interstitial lung disease are predominant characteristics in anti-Ku positive patients with idiopathic inflammatory myopathies' by Yang *et al*

We would like to thank Yang *et al*[1] for their rewarding comment on our work, in which we report that patients harbouring anti-Ku autoantibodies with elevated serum levels of creatine kinase (elevated CK) are at risk of interstitial lung disease (ILD), whereas anti-Ku patients with anti-dsDNA are frequently affected by systemic lupus erythematosus and are at risk of glomerulonephritis.[2]

Yang *et al* retrospectively investigated 1214 patients with myositis (defined on Bohan and Peter criteria) in a single Chinese centre. Twenty-one patients (1.7%) had anti-Ku antibodies, defined as a fine speckled pattern seen at immunofluorescence, together with positive commercial assay results.

In accordance with our results, Yang *et al* found that ILD was a predominant characteristic of anti-Ku patients with myositis (76.2%).

Interestingly, using commercial assays, Yang *et al* also reported the frequent (48%) coexistence of anti-Ku with myositis-specific or myositis-associated autoantibodies (MSA/MAA). Moreover, as compared with patients with isolated anti-Ku antibodies, a skin rash was more frequent in these patients, as well as better pulmonary functional test results. In our cohort, anti-Ku antibodies were systematically confirmed using an in-house immunodiffusion technique. Apart from anti-Jo1 and anti-U1-RNP, MSA/MAA status was not available in all our anti-Ku patients. However, when searched for (using D-Tek line immunoassay, Mons, Belgium), the result was generally negative and only two anti-Ku patients with elevated CK tested positive for a coexisting MSA/MAA (table 1). None of them had a dermatomyositis rash. False positivity for MSA/MAA has recently been shown to be common when using commercial assays (14%), anti-Ku being the most frequent false positive specificity of the EuroImmun line immunoassay (3%).[3] Thus, the important report by Yang *et al* highlights the diagnostic challenge posed by the 'anti-Ku syndrome' in view of the limitations of currently available routine tests.

**Table 1** Myositis-specific and associated autoantibodies in our 15 patients with anti-Ku autoantibodies and elevated CK

| MSA and MAA | Anti-Ku patients with elevated CK n=15 |
|---|---|
| Anti-Jo1 | 0/15 |
| Anti-PL7 | 0/13 |
| Anti-PL12 | 0/13 |
| Anti-OJ | 0/9 |
| Anti-EJ | 0/9 |
| Anti-Ha | 0/9 |
| Anti-Zo | 0/9 |
| Anti-KS | 0/9 |
| Anti-U1-RNP | 0/15 |
| Anti-PM/Scl | 1/14 |
| Anti-Mi2 | 0/12 |
| Anti-MDA5 | 1/9 |
| Anti-TIF1γ | 2/9 |
| Anti-NXP2 | 0/9 |
| Anti-SAE | 0/9 |
| Anti-SRP | 0/11 |
| Anti-HMGCR | 0/9 |
| **Total** | **2*** |

*One patient was positive for both anti-MDA5 and anti-TIF1γ; another had anti-PM/scl and anti-TIF1γ.
CK, creatine kinase; MAA, myositis-associated autoantibodies; MSA, myositis-specific autoantibodies.

In this regard, Yang *et al* additionally described the muscle biopsy findings available in 13 of their 21 anti-Ku patients. Noteworthily, the immune-mediated necrotising myopathy (IMNM) pattern, as defined by the 2017 ENMC criteria, was found in 6/7 (86%) of their patients with isolated anti-Ku versus 1/6 (17%) of their counterparts. Similarly, in our cohort, an IMNM pathological pattern was found in 7 of 8 anti-Ku patients who underwent a muscle biopsy (88%) . The sole muscle lesion found in our remaining patient was patchy (not perifascicular) sarcolemmal major histocompatibility complex class I expression.

Overall, these data emphasise that interstitial lung disease is a predominant feature in anti-Ku patients with myositis and, importantly, highlight that IMNM might be part of this anti-Ku syndrome.

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# Efficacy of dupilumab reveals therapeutic target for IgG4-related disease: simultaneous control of inflammation and fibrosis

We read with great interest the article from Simpson *et al* on the clinical efficacy of dupilumab in a patient with IgG4-related disease (IgG4-RD).[1] Glucocorticoid is currently the first-line induction therapy for IgG4-RD.[2] Since it generally suppresses acquired immune cells, we could not know the therapeutic targets in IgG4-RD. So far, the efficacy of rituximab, targeting B cells, has been discussed,[3] but we reconfirm that type 2 helper T (Th2) cells can be one of the therapeutic targets in IgG4-RD by this article. Tanaka *et al* previously disclosed that the expression of Th2 cytokine mRNA was elevated in the labial glands from patients with IgG4-RD, compared with the patients with Sjögren's syndrome and healthy controls.[4] We have shown that the levels of serum interleukin (IL)-5 were elevated according to the disease progression,[5] and the ST2$^+$ memory T produced large amount of IL-5.[6] Because ST2 is the receptor of IL-33, which could lead to differentiation from naïve T to Th2, we had considered that IL-5 would be a therapeutic target for IgG4-RD. Based on the hypothesis, we treated with mepolizumab—a humanised anti-IL-5 monoclonal antibody—for several IgG4-related dacryoadenitis and sialadenitis (IgG4-DS) patients with bronchial asthma. They experienced several relapses or presented with difficult for tapering glucocorticoid. As a result, bronchial asthma was improved, and the peripheral counts of eosinophils promptly led to 0 in all patients. However, the enlargement of lacrimal and salivary glands was not changed, and the serum IgG4 concentration also unchanged or slightly decreased. The steroid tapering effect was limited and mepolizumab could not lead to overall control in IgG4-DS.

For this reason, it was presumed that IL-4, among the Th2 cytokines, was more involved in the pathogenesis of IgG4-RD. IL-4 is indispensable cytokine for the formation of germinal centres, and in especially IgG4-RD, it is also important in humoral immune reactions including IgG4 production by the contact between follicular helper T cells and B cells.[7] So, in the long term, we want to focus not only on clinical improvement but also on changes in immunological findings including globulin levels in the case presented by Simpson. In addition, dupilumab can also inhibit IL-13 signal because IL-13 receptor uses IL-4Rα. Clinical efficacy of dupilumab was initially confirmed in atopic dermatitis.[8] In atopic dermatitis, IL-13 leads to self-proliferation of fibroblasts via periostin.[9] Ohta *et al* reported that the production of both IL-13 and periostin was detected in the involved organs of IgG4-RD.[10] It is possible that IL-13 is one of the key player cytokines in the mechanism of the fibrosis in IgG4-RD. For this reason, dupilumab has a potential to suppress the progression of the fibrosis in IgG4-RD. It is necessary to perform large-scale clinical trials for the evidence of long-term efficacy and safety.

In 2016, we reported the IgG4-RD case that abatacept was effective in this journal.[11] The patient has been treated safely with abatacept for more than 5 years without the relapse. Although the number of patients treated with abatacept has increased since then, no patient experienced secondary non-response. If it can be proved the long-term maintenance and safety in dupilumab administration, it is probably regarded as one of the T cell-targeted biologics for IgG4-RD. Abatacept regulates T cells as a whole, and dupilumab suppresses only Th2 cells. We will confirm that the regulation of T cells is important in IgG4-RD. In the future, we expect to compare the efficacy and safety in abatacept and dupilumab for IgG4-RD.

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# MRI and ultrasonography are useful tools for a non-invasive diagnosis of IgG4-related disease

We read with much interest the 2019 classification criteria for IgG4-related disease by the American College of Rheumatology and the European League Against Rheumatism.[1]

This new classification driven by scientific evidence and research provides a substantial amount of new information, which will considerably improve the identification and management of patients with IgG4-related disease.

Despite the fact that radiology is integrated into the diagnostic criteria, only CT and/or positron emission tomography-CT are mentioned. We humbly suggest that the use of ultrasonography and/or MRI is missing. MRI is considered to be a relevant tool for diagnosing IgG4-related disease in the majority of organs included in the entry criteria such as the pancreas, bile ducts, orbits, lacrimal glands, major salivary glands, pachymeninges or thyroid gland.[2 3] Its diagnostic accuracy is superior to that of CT and/or PET-CT for almost all organs, especially when imaging head and neck, orbital or brain IgG4-related disease. For example, detecting pachymeningitis with CT is challenging, whereas MRI is very sensitive.[4] Moreover, MRI has proved to have high specificity to diagnose IgG4-related ophthalmic disease, in front of an enlargement of the infraorbital nerve.[5 6] Advanced MRI techniques such as diffusion-weighted imaging, have excellent accuracy in distinguishing IgG4-ROD from lymphoma.[7] Similarly, ultrasonography has been reported to easily detect changes in major salivary glands affected by IgG4-related diseases, even for inexperienced observers.[8]

One of the major points of the 2019 classification criteria for IgG4-related disease is that a positive diagnostic of IgG4-related disease can be achieved without invasive, tissue-based pathological confirmation. The update implies that non-invasive techniques such as imaging should have the best accuracy possible. CT and PET-CT have excellent sensitivity to detect lesions compatible with IgG4-related disease. However, MRI and ultrasonography have an even higher specificity in most organs. Moreover, MRI and ultrasonography are non-radiating techniques as opposed to CT and PET-CT.

Therefore, we believe that MRI and ultrasonography should be mentioned as first-line radiological examination choices in patients with a suspected diagnosis of IgG4-RD, especially for the head and neck and brain.

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# Features of polymyalgia rheumatica–like syndrome after immune checkpoint inhibitor therapy

We read with great interest the article published by Braaten and colleagues[1] describing inflammatory arthritis (IA) induced by immune checkpoint inhibitors (ICIs) persisting after immunotherapy cessation. With a growing number of patients treated with ICI, more and more immune-related adverse events are described and a classification has been recently proposed for IA under ICI.[2] It questions whether IA under ICI shows distinctive features from well-defined rheumatological conditions. Here, we report a series of 14 patients who developed polymyalgia rheumatica (PMR)–like syndromes under ICI and compared them with a series of 43 patients with classical PMR seen in the same tertiary centre.

We included patients with rhizomelic pain under ICI from our tertiary department of rheumatology (AP-HP, Université Paris-Saclay) and the pharmacovigilance registry of the Gustave Roussy Cancer Institute. The diagnosis of PMR was based on trained clinicians' assessment. Among 14 patients, 11 fulfilled the EULAR/ACR 2012 criteria for PMR. The comparison between PMR-like syndromes and classical PMR showed a difference in sex ratio and a higher frequency of peripheral arthritis in the ICI group (57% vs 28%) (table 1). C reactive protein (CRP) was positive (>5 mg/L) in most cases in both groups: 92.3% and 88.1% in ICI group and classical PMR, respectively. Among the five patients of the ICI group who underwent $^{18}$F-FDG PET/CT imaging before rheumatological treatment, three showed rhizomelic peri-articular $^{18}$F-FDG PET uptakes associated to a volar FDG uptake at the hands.[3] Thirteen patients received glucocorticoids with eight good responders. Among the five other patients, one received methotrexate, three received tocilizumab (one who responded, one who had primary failure and one who had drug-induced hepatitis) and one healed after ICI disruption.

To sum up, the main finding of this study is the higher prevalence of peripheral arthritis in PMR-like syndromes induced by ICI. The frequency of increased CRP was the same. Lastly, the therapeutic strategies remain the same as what is proposed in classical PMR, but further studies are mandatory to define the optimal treatment strategy and notably the room for biologic disease-modifying antirheumatic drugs.

**Table 1** Characteristics of ICI+PMR compared with ICI–PMR

| | ICI+PMR (n=14) | ICI–PMR (n=43) |
|---|---|---|
| Women (%) | 2 (14.3) | 26 (39.5) |
| Patients over 50 years old (%) | 13 (92.9) | 40 (93) |
| Peripheral arthritis (%) | 8 (57.1) | 12 (27.9) |
| CRP >5 mg/L (%) | 12/13 (92.3) | 37/42 (88.1) |
| Median CRP (mg/L) | 69 (<5–150) | 32 (6–170) |
| GCs sensitivity (excluding high-dose steroid–dependent patients) (%) | 8/13 (61.5) | – |

CRP, C reactive protein; GC, glucocorticoids; ICI, immune checkpoint inhibitor; PMR, polymyalgia rheumatica.

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