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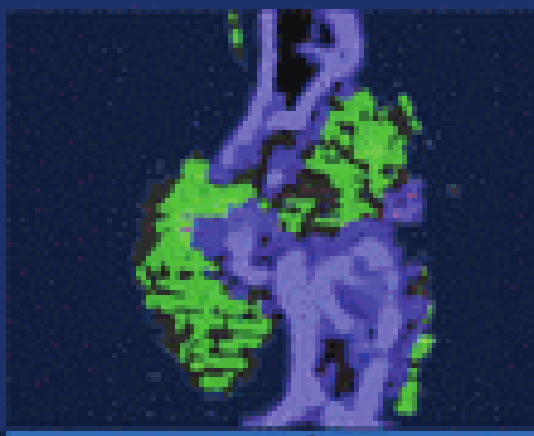

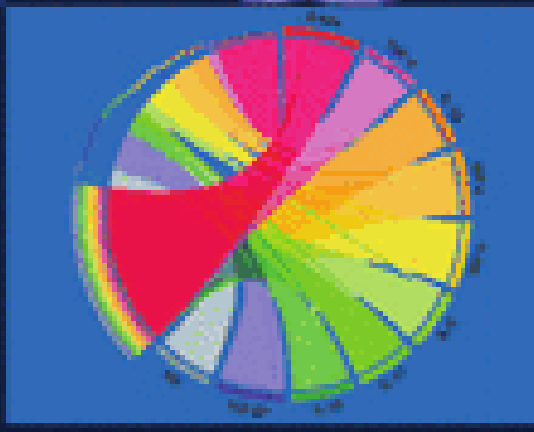

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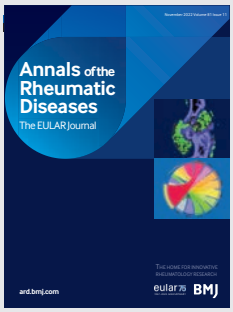

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Defining the targets in SLE management: insights and unmet gaps

Margherita Zen, Mariele Gatto, Andrea Doria

Over the last decade, the application of new therapeutic strategies and the availability of newer molecules have 'raised the bar' regarding goals and expectations for systemic lupus erythematosus (SLE) management, and there is now consensus that disease remission should be the ultimate goal when treating patients with SLE, while low disease activity (LDA) could be considered a suitable alternative outcome only when remission cannot be achieved,[1] typically in patients with refractory disease.

It has to be pointed out that experts consider very important to have a unified definition of remission and LDA in order to be able to compare the results of different studies. This is the reason why a considerable effort in establishing an agreed-upon definition of remission and LDA has been shared among researchers in the last few years.[2–4] In 2015, the Definitions Of Remission In SLE (DORIS) Task Force has been set up, and in 2021, the DORIS definition of remission has been published.[5] Clinical remission in SLE can be conceptualised as the absence of clinical manifestations or urinary or haematological abnormalities due to active immune pathways. The concept underlying such definition is that patients in a sustained state of remission would not experience pathological consequences of the disease over time. In the same years, the Asia Pacific Collaboration group proposed and validated the lupus low disease activity state (LLDAS) definition.[6] Since then, a huge amount of data regarding prevalence, durability, and protective effect of remission and LLDAS on disease outcomes such as damage, quality of life and flare rate has been accrued.[7–13] These studies validated the definitions of DORIS remission and LLDAS worldwide.

Nevertheless, there are setting in which the application of these definitions is not possible. This is the case of the study by Ugarte-Gil *et al*,[14] carried out in the SLICC cohort, where data on physician global assessment (PGA) were lacking. What should researchers have done in this case? Give up and not evaluate this topic in their cohort? The authors decided to use an alternative definition of remission, namely the one proposed by Zen *et al* (Padua definition), which was already validated in different settings,[11 12 15] and to modify the LLDAS definition by excluding PGA. Does this choice limit the relevance of their results? The paper by Ugarte-Gil *et al* suggests that, despite the great effort made by the DORIS Task Force and the good performance of the LLDAS, it is unlikely that a single definition of remission (the DORIS) and LDA (the LLDAS) will be used all over in the next years. Although debatable, the blossoming of a number of definitions of remission and LDA for SLE is not an unusual finding, resembling other diseases, including rheumatoid arthritis (RA), where a number of definitions of remission and LDA exist and are currently used.[16]

The results presented by Ugarte-Gil *et al*[14] also enforce the long and controversial debate regarding the importance of including PGA in the definition of remission and LDA. PGA is generally regarded as the gold standard for evaluating disease activity in SLE. However, PGA has a substantial inter-rater variability partly related to variations among rheumatologists in weighing different organ manifestations and serological activity.[17] In addition, the PGA International Standardisation Consensus in SLE study has recently demonstrated a major need for both standardisation and training in the scoring of PGA, suggesting that only expert physicians can efficiently rate the PGA, and it should be preferably scored by the same rater at each visit, making its use particularly challenging in multicentre and longitudinal studies.[18] It has also to be pointed out that in a multicentre study evaluating different definitions of remission, the addition of PGA to clinical SLE Disease Activity Index 2000 (SLEDAI-2K) did not increase the performance of SLEDAI-2K in predicting damage accrual,[19] and no studies ever evaluated the correlation between PGA and damage accrual, according to a recent review.[17] Thus, since the choice of a target for treatment should be based on the interpretation of measures that are potential predictors of a bad outcome, PGA seems not essential in defining remission and LDA in SLE.

One strength of the DORIS remission and the LLDAS is the inclusion of a therapeutic item in their definition, unlike what was done in RA. In particular, the inclusion of a cut-off of glucocorticoid (GC) dose is very reasonable, as higher doses of GCs can disguise an active disease.

However, the treat-to-target (T2T) approach in SLE has to be considered a stepwise process where the first step should be the achievement of clinical remission, or clinical LDA, both meant as control of disease activity irrespective of the treatment (GCs, immunosuppressants, biologics) at least in the short term,[20] since the first driver of damage and poor outcome still remains the disease activity itself. As a second step, the decrease in GC dosage and even their withdrawal should be always attempted. In this regard, it is worthy to note that a significant heterogeneity in GC administration across different cohorts has been observed,[21] also in the management of patients in clinical remission or LDA.

Notably, the study by Ugarte-Gil *et al*[14] highlights that the feasibility of achieving and maintaining the targets—remission or LLDAS—not only depends on the definition used, but also on genetic, geographical and socioeconomic factors of the population(s) studied (figure 1). Ethnicity influences SLE outcomes, having Caucasians a better prognosis compared with Afro-Caribbeans and Asians; a low socioeconomic status is associated with worse SLE damage, greater mortality and poorer quality of life.[22] In addition, the type of healthcare system and its financial resources might highly affect the availability of therapeutic options, in particular more expensive drugs, and the access to care, thus influencing the probability of achieving the targets. In RA, it was shown that the macroeconomic environment is an important determinant of the access to biological disease-modifying antirheumatic drugs. The limited use of biologics was attributed to a low/difficult access to specialists and the cost of biologics, which contributes to restrictive guidelines for their use and administrative constraints, most likely related to the gross domestic product in each country,[23] but also to the type of healthcare regimen.[24] A review on the healthcare quality in SLE carried out in USA showed that access to care, health insurance, and financial and organisational arrangements of the health

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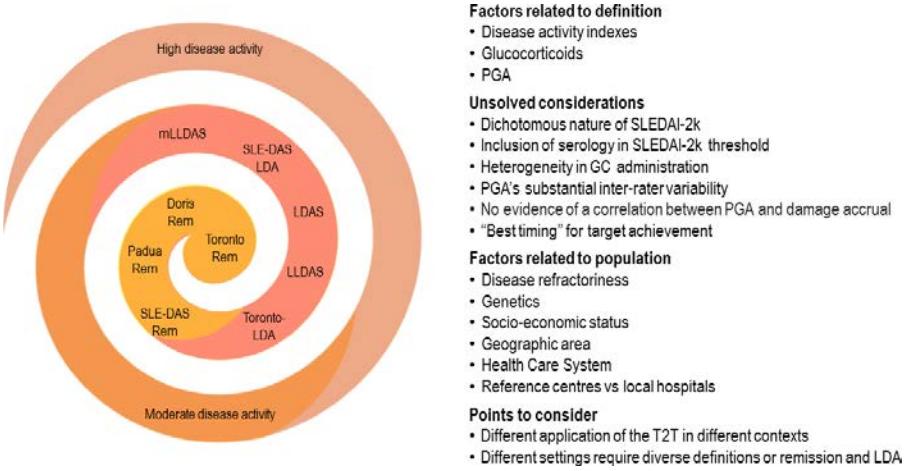

Figure 1 Factors influencing the attainment of the T2T goals remission and LDA. Toronto rem, Toronto lupus Cohort definition of remission, Polachek *et al*.[3]; DORIS rem, DORIS definition of remission, van Vollenhoven *et al*.[5]; Padua rem, Padua lupus Cohort definition of remission, Zen *et al*.[2 12]; SLE-DAS rem, SLE-DAS definition of remission, Jesus *et al*.[33]; Toronto-LDA, Toronto lupus Cohort definition of LDA, Polachek *et al*.[3]; LLDAS, lupus low disease activity state, Franklyn *et al*.[6]; LDAS, low disease activity status, Ugarte-Gil *et al*[4]; SLE-DAS LDA, SLE-DAS definition of low disease activity, Assunção *et al*.[34]; mLLDAS, modified LLDAS, Ugarte-Gil *et al*.[14] GC, glucocorticoid; LDA, low disease activity; PGA, physician global assessment; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; T2T, treat-to-target.

system represent relevant determinants of different disease outcomes in SLE, including disease activity and damage.[25] The fact that the Italian National Health Service provides free and equal access for all citizens to all healthcare services, including primary, hospital and emergency care, as well as visits, diagnostic procedures and therapies, including biologics and other innovative drugs, might have contributed to the high attainment of remission observed in Padua Lupus cohort[2 7 12] and in other Italian studies.[15 19 26] Thus, these observations suggest that not only disease phenotypes but also geographic and socioeconomic factors might drive the choice of the most reasonable and appropriate target in different populations. Accordingly, different settings could require diverse definitions of remission and LDA.

Another aspect that emerges as a possible driver of unbalanced outcomes in multicentre studies is the different application of the T2T in different contexts. In reference rheumatology centres, the strategy of treating to the target—remission whenever possible, LDA in refractory patients—with treatment adjustments until the target is reached and maintained, using antimalarials in all patients who tolerate them, immunosuppressants if needed, with the possibility of an early use of biologics, aiming at the lowest doses of GCs, is already a reality. Thus, it is hard to set up a clinical trial investigating T2T as opposed to a standard approach only aimed at symptomatic control, which would lead to an ethically unacceptable undertreatment. Where the T2T strategy still needs to be implemented is in local hospitals, which are often those with economic restrictions and/or without lupus clinics. In addition, we are looking forward to seeing the results of the randomised 'LUPUS-BEST' trial for the application of the tight control T2T approach, with a glimpse on overtreatment-related drawbacks.[27] An important issue would also be to understand whether there is a 'best timing' for the achievement of the target in different SLE manifestations, similarly to what has already been demonstrated in lupus nephritis,[28] which could guide the intensity, escalation, and de-escalation of treatment, and how to better define 'refractory' SLE.

Besides these considerations, the heterogeneity among disease manifestations makes the definition of LDA challenging, and we wonder whether the LLDAS or the LDA-Toronto Clinic definition[3] really capture all patients in LDA. In clinical practice, we consider our patients in LDA when they have mild clinical manifestations or mild urinary or haematological abnormalities. The entity of these manifestations should be as 'mild' as they are acceptable in terms of residual damage progression they can be responsible for. Indeed, the choice to settle for LDA is based on the balance between the residual disease activity and the increase in therapy, which would be required to completely abolish disease activity in that particular patient. Serology has relatively little value in the definition of disease activity and remission, as well as in driving therapy; thus, to include serology in the definition of LDA is questionable.

Defined as such, clinical LDA is not frequently observed in patients with SLE. LDA can be transiently present in the relapsing-remitting pattern of disease activity, in the phases of transition from remission to disease activity and vice versa, or in cases of chronic active disease with mild manifestations, the less frequent pattern of SLE.[29 30] Thus, the high prevalence of LDA reported in different cohorts using the LLDAS definition is related to the inclusion of patients in remission within the LDA group. The overlap between LLDAS and remission was reported in several studies, and was confirmed by the study of Ugarte-Gil *et al*.[14] Recently, in a prospective cohort study carried out in 1707 patients from 13 international centres, LLDAS overlapped with the 2021 DORIS definition of remission in 75% of total visits, meaning that only 25% of visits represented patients in LLDAS but not in remission.[31]

Another issue is that not all patients in LDA can be captured by LLDAS. In fact, the definition of LLDAS excludes patients with major organ system involvement, including renal, cardiopulmonary, central nervous system, vasculitis, fever, haemolytic anaemia and gastrointestinal activity. Thus, through the LLDAS, one should exclude the possibility of LDA in renal, serosal and vasculitic domains. Moreover, since the threshold of the SLEDAI-2k in LLDAS has been set at four points including serology, patients with mild arthritis (four points in the SLEDAI-2k) or skin manifestations (two points) but with positive serology are precluded from being classified as in LDA by the LLDAS. This means that LLDAS can also miss patients with mild skin and joint disease.

As LDA suffers from a lack of defined borders discriminating it from remission, some effort is still needed to clearly depict what LDA is in lupus, including its definition and prevalence. A new measure of disease activity, the SLE disease activity score, has been proposed to overcome these limitations, related to the dichotomous nature of the SLEDAI-2k,[32] with cut-offs for remission[33] and LDA also derived and validated.[34] It would be interesting to see its performance in capturing patients with clinical LDA in different cohorts.

In conclusion, the identification of beneficial targets, such as remission and LDA, represents an important step in

the application of the T2T strategy in SLE, with the ultimate goal of decreasing damage progression and improving quality of life.[35] The application of a unified definition of remission and LDA would be ideal to directly compare results from different cohorts as well as from randomised control trials for new drugs in the research setting; however, in clinical practice, different definitions could suit better diverse patient cohorts, taking into account variation in ethnicity, demographics and access to care, which might impact on T2T applicability.

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Diagnostic evaluation of the sacroiliac joints for axial spondyloarthritis: should MRI replace radiography?

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ABSTRACT

The possibility of detection of structural damage on magnetic resonance imaging (MRI) of sacroiliac joints raises the question of whether MRI can substitute radiographs for diagnostic evaluation and to a further extent for classification of axial spondyloarthritis (axSpA). In this viewpoint, we will argue that it is time to replace conventional radiographs with MRI for the assessment of structural changes in sacroiliac joints. This message is based on current data on the following questions: (1) How reliable are conventional radiographs in the diagnosis of axSpA overall and radiographic axSpA in particular? (2) How does T1-weighted MRI compare to radiographs in the detection of sacroiliitis? (3) Are there now other (better) MRI sequences than T1-weighted, which might be more suitable for the detection of structural lesions? (4) Which MRI sequences should be performed for the diagnostic evaluation of the sacroiliac joints? (5) Do we have data to define sacroiliitis based on structural changes detected by MRI?

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease predominantly of the axial skeleton, but peripheral joints and entheses can also be affected. The condition usually starts in the sacroiliac (SI) joint, and isolated spinal involvement without affection of the SI joint is rare.[1] In a study of patients with chronic low back pain and maximal symptom duration of 3 years, only 1%–2% of patients with axSpA had spondyloarthritis (SpA)-typical lesions in the spine without affection of the SI joints.[2] This is the reason why in the Assessment of SpondyloArthritis International Society (ASAS) classification criteria of axSpA[3] the presence of sacroiliitis on imaging took one of the central roles.

Like in any chronic inflammatory disease, axSpA has an early phase with bony inflammation and a later stage dominated by structural damage of the bone.[1] Since the publication of the modified New York criteria for classifying ankylosing spondylitis (AS) in 1984,[4] the detection of radiographic sacroiliitis became the cornerstone of the AS diagnosis. In the 1990s of the last century, it became evident that MRI is able to detect active inflammation early in the course of axSpA. Thus, in addition to radiographic sacroiliitis, active inflammation seen on MRI in fluid-sensitive, fat-suppressed pulse sequences such as short-tau inversion recovery (STIR) or T2 with fat saturation was added in the ASAS classification criteria for SpA in 2009.[3] Also, the ASAS–European Alliance of Associations for Rheumatology (EULAR)[5] and the American College of Rheumatology (ACR)–Spondyloarthritis Research and Therapy Network (SPARTAN)–Spondylitis Association of America (SAA)[6] management recommendations for axSpA implemented the presence of sacroiliitis on MRI (as an alternative to radiographic sacroiliitis) as one of the starting points in the treatment algorithms.

The detection of active inflammation in the SI joints, that is, subchondral bone marrow oedema, is the major advantage of MRI. Especially in the early phase of the disease, it is of high diagnostic relevance and an important parameter of disease activity. MRI is able, however, to depict not only active inflammation but also structural lesions such as fat lesions of bone marrow, erosion, fat metaplasia in an erosion cavity or backfill, sclerosis and ankylosis. Both active and structural MRI lesions typical of axSpA seem to be relevant for the diagnosis, and active lesions should be interpreted in the context of structural damage.[7 8] Indeed, subchondral bone marrow oedema without structural changes as detected by MRI seems to be less frequent in axSpA than previously thought.[8–11]

The possibility of detection of structural damage on MRI raises, therefore, the question of whether MRI can substitute radiographs in the diagnosis of axSpA and to a further extent in classification. In many countries and in many patients with possible axSpA, MRI is performed anyway as a part of a routine diagnostic approach that makes this question increasingly clinically relevant. At the same time, discussion of this question would not exclude radiography in a diagnostic process if MRI were unavailable, unfeasible or its application limited by its costs.

Here we will argue that it is time to replace conventional radiographs with MRI for the assessment of structural SI joint lesions when available. Our message is based on current data on the following questions: (1) how reliable are conventional radiographs in the diagnosis of axSpA overall and radiographic axSpA in particular? (2) how does T1-weighted MRI compare to radiographs in the detection of sacroiliitis? (3) are there now other (better) MRI sequences than T1-weighted, which might be more suitable for the detection of structural lesions? (4) which MRI sequences should be performed for the diagnostic evaluation of the SI joints? (5) do we have data to define sacroiliitis based on structural changes detected by MRI?

HOW RELIABLE ARE CONVENTIONAL RADIOGRAPHS IN THE DIAGNOSIS OF (RADIOGRAPHIC) AXSPA?

Radiographic findings of the SI joints are difficult to interpret because the pelvic anatomy is complex,

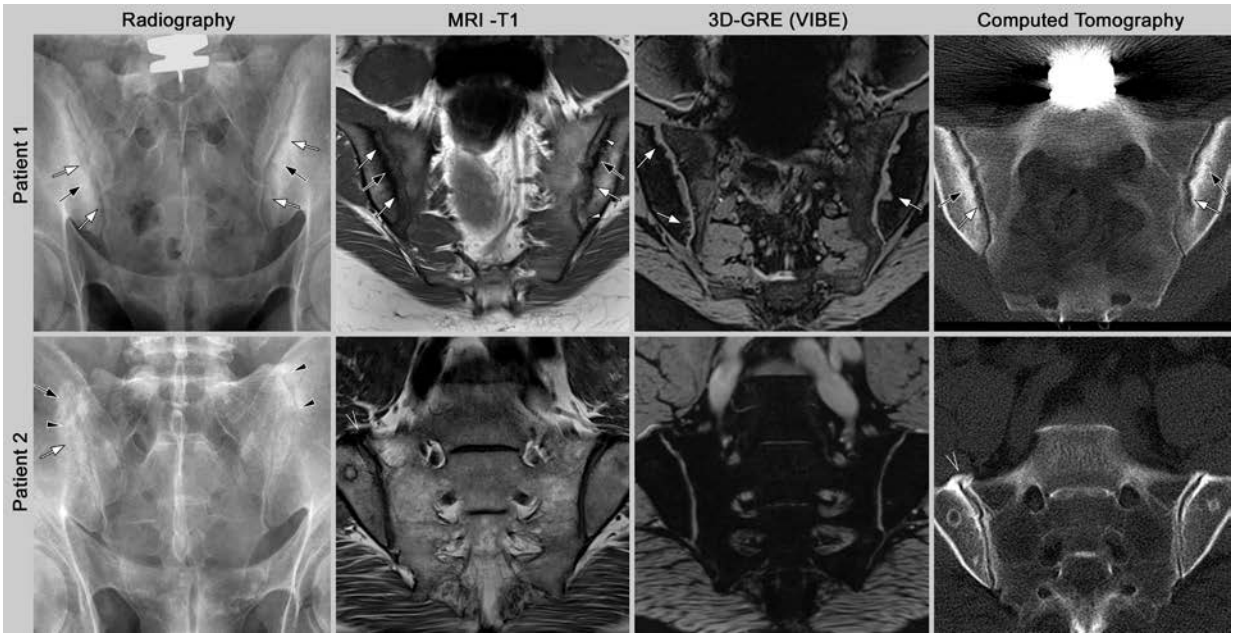

Figure 1 Depiction of structural damage in the SI joints by conventional radiography, MRI and CT. Patient 1: 28-year-old male patient with r-axSpA with bilateral sclerosis (black arrows) and erosion (white arrows) in radiography, MRI and CT. T1-weighted MRI depicts also backfill (arrowheads). 3D-GRE (VIBE) shows erosion in more detail. Patient 2: 46-year-old male patient with sclerosis (black arrows), possible erosion (white arrow) and suspected partial ankylosis (black arrowheads) in radiography. However, MRI and CT do not show structural damage on the SI joint besides osteophyte formation that causes superposition in radiography (open arrowheads). 3D-GRE, three-dimensional gradient echo; r-axSpA, radiographic axial spondyloarthritis; SI, sacroiliac; VIBE, volumetric interpolated breath-hold examination.

the SI joints have an oblique orientation, and superposition of bowel gas can hide or mimic structural bone changes. Therefore, the reliability of positive or negative findings might be better with cross-sectional imaging techniques such as MRI. An earlier study published in 2003 investigated the performance of radiologists (23 participants) and rheumatologists (100 participants) in detecting sacroiliitis[12] in The Netherlands. The whole group performed a training in the interpretation of SI joint radiography and met again after 3 months. Gold standard (radiographic sacroiliitis yes or no) was defined by an expert panel. Sensitivity (84.3%/79.8%) and specificity (70.6%/74.7%) for radiologists and rheumatologists, respectively, were comparably low. These figures were similar at the second meeting after the training.[12]

In another study, there was a considerable difference reported when the results of local readings versus central readings of radiographs of SI joints in patients with axSpA with a disease duration less than 5 years (from the Devenir des Spondylarthropathies Indifférenciées Récentes (DESIR) cohort) were compared: 32 of 109 patients (29.4%) with bilateral obvious sacroiliitis or at least unilateral fusion (by local reading) were rated as negative by central reading and 68 of 579 patients (11.7%) were rated as positive by central reading, although the interpretation of the local readers was negative. Also, the inter-reader agreement even between the central readers was only moderate (kappa=0.54).[13] Thus, applying SI joint radiographs for diagnosing axSpA is rather unreliable.

The problem becomes even larger at the early disease stage or in patients with suspected axSpA[14] that is related to the definition of radiographic sacroiliitis grade 1 (suspicious changes leaving a big room for subjective interpretation) or grade 2 (minimal abnormalities) as compared with sacroiliitis grade 3 (advanced changes with joint space alteration) or 4 (complete ankylosis of the joint).

HOW DOES T1-WEIGHTED MRI COMPARE TO CONVENTIONAL RADIOGRAPHS IN THE DETECTION OF SACROILIITIS?

Two studies compared radiographs with T1-weighted MRI for the detection of structural lesion in the SI joints. The first one took the radiographs as the reference method and reported a sensitivity of 84% and a specificity of 64% for fulfilling the modified New York criteria, with similar figures for the detection of 'chronic sacroiliitis' (=overall structural damage).[15] This study has also demonstrated that the reliability (inter-reader variability) of the detection of structural lesions (erosions, sclerosis and joint space alteration) was better for MRI than for the radiography.[15] In an analysis of the DESIR study, radiographs and MRI T1-weighted images of the SI joints were analysed and compared by two trained readers. Twelve (reader 1) or 10 (reader 2) patients would have been classified as axSpA by radiographs but not by MRI, and 3 (reader 1) and 6 (reader 2) patients were negative in radiography but MRI positive.[16] Thus, taken together in these two studies, there was a moderate to good agreement between radiographs and MRI for scoring structural lesions at the SI joints.

However, no CT as a gold standard for the structural damage detection was available in these studies. Therefore, based on these study data, the question on whether any of the two imaging methods for the detection of structural lesions in the SI joints would be superior to the other could not be answered. This question was addressed in the German SacroIliac Magnetic Resonance Computed Tomography (SIMACT) study.[17] In this prospective study, 110 patients referred to the rheumatologist with chronic low back pain and possible axSpA were included. All patients underwent radiography, low-dose CT and MRI of the SI joints (a T1-weighted sequence was used for this analysis). All images were scored by three readers (radiologists),

with the low-dose CT as standard of reference for the detection of structural SI joint lesions: erosions, joint space changes (including ankylosis) and sclerosis. MRI showed a better absolute agreements with CT compared with radiography for erosion (88.2% vs 70.9%), joint space changes (92.7% vs 80.9%) and overall positivity for the presence of structural damage (89.1% vs 70.0%), but not for sclerosis (83.6% vs 86.4%, respectively). Furthermore, the reliability (inter-reader variability) of structural damage detection was better on MRI for erosions and joint space alteration, but not for sclerosis.[17] It should be noted that the study was performed in a specialised SpA centre with experienced readers; therefore, the performance of the discussed imaging methods might be different in non-specialised centres.

In another study from China, it was shown again that CT has the best sensitivity and specificity for the detection of structural lesions in the SI joints, but closely followed by MRI in patients with non-radiographic axSpA who were, by definition, negative on conventional radiographs.[18]

In a most recent study, the German group compared the value of different imaging approaches of the SI joints for the diagnosis of axSpA, using the clinical diagnosis by experts as reference standard: conventional radiographs, CT, MRI (both STIR and T1), conventional radiographs+MRI and CT+MRI.[10] The obtained results confirmed radiography to be inferior to MRI: radiographs showed with 66.3% the lowest sensitivity compared with MRI (82%) and CT (76.4%) and also a lower specificity of 67.6% vs MRI (86.5%) and CT (97.3%). Also, the inter-rater reliability was lowest for radiographs (kappa=0.517), followed by MRI (kappa=0.665) and CT (kappa=0.875). In daily clinical practice, MRI is often ordered after radiography in case of negative or equivocal results. Consequently, both imaging modalities are assessed simultaneously. In the referenced study, the combination of radiography with MRI could not outperform MRI alone. An analysis of different scenarios showed that the current clinical standard with MRI done only in patients with no definite radiographic sacroiliitis according to the modified New York criteria for AS had the highest sensitivity (86.5%) but poor specificity (66.2%), indicating a substantial risk of overdiagnosis. Raising the positivity threshold of radiographs to at least grade 3 unilaterally increased the specificity of the approach to 81.1% but decreased the sensitivity to 79.8%. A strategy with MRI as the only imaging method had a sensitivity of 82% and a specificity of 86.5%.[10]

However, these studies also demonstrated that MRI (with T1-weighted and STIR sequences only) is not as good as CT, especially for the differentiation between sclerosis and erosions, raising the question whether the MRI technique can be improved for this purpose.

ARE THERE NOW OTHER (BETTER) MRI SEQUENCES THAN T1-WEIGHTED, WHICH MIGHT BE MORE SUITABLE FOR THE DETECTION OF STRUCTURAL LESIONS?

Technical progress is moving fast, and it can be expected that new interesting imaging methods will emerge over the coming years. However, in the context of this discussion, erosion-sensitive three-dimensional gradient echo (3D-GRE) MRI sequences (such as volumetric interpolated breath-hold examination (VIBE)) are of special interest that are available on nearly all currently installed MR machines. Figure 1 presents examples of 3D-GRE images in comparison to T1-weighted images, CT and conventional radiography.

Two studies have been published comparing 3D-GRE and the T1-weighted MRI sequences, focusing on the scoring of erosions and using again low-dose CT as a gold standard. In the first study, patients from the SIMACT trial (110 subjects) were analysed by adding the 3D-GRE images.[19] Reading of the images was done by two experienced radiologists. The 3D-GRE sequence had better sensitivity than the T1-weighted one (95% vs 79%, respectively) with a similar specificity (93% each). 3D-GRE identified approximately 20% more patients with erosion than the T1, 36 vs 30 of 38 patients with positive low-dose CT findings.

In the second study with a comparable study design, the sensitivity of 3D-GRE was again higher than that for T1-weighted MRI (71.2% vs 63.4%, respectively) with a similar specificity of 87.3% vs 88%, respectively.[20] Thus, based on these data, the 3D-GRE sequence comes close to CT in the detection of erosions in the SI joints and is superior to the T1-weighted sequence. However, for the detection of fat lesions and fat metaplasia in an erosion cavity representing repair fibrous tissue (also called backfill), the T1-weighted sequence is still indispensable.

While the strengths of 3D-GRE lie in a short acquisition time, thin slices and good contrast of erosion and bone, it still cannot depict the cortical bony surface directly, in contrast to CT. Furthermore, clear-cut definitions for erosions and their positivity in the clinical context are warranted to avoid overcalling of minor physiological or degenerative irregularities of the cortical bone and their misinterpretation as erosions related to axSpA.

WHICH MRI SEQUENCES SHOULD BE PERFORMED FOR THE DIAGNOSTIC EVALUATION OF THE SI JOINTS?

Four sequences for MRI of the SI joints have been recommended by the radiologists of the European Society of Skeletal Radiology Arthritis Subcommittee in 2015[21]: (1) semicoronal oblique T1-weighted (for fat lesions, erosions and ankylosis); (2) semicoronal STIR (or another T2-weighted sequence with suppressed fat signal, for bone marrow oedema); (3) semicoronal cartilage (erosion sensitive) sequence (such as 3D-GRE/VIBE); and (4) a second T2-weighted semiaxial sequence with suppressed fat signal, also for bone marrow oedema. These sequences are also included in the international consensus developed by ASAS and SPARTAN that has been recently presented at the Annual European Congress of Rheumatology–EULAR 2022 (POS0989). Based on the evidence presented earlier, this would currently be the mandatory MRI sequence protocol for the detection of bone marrow oedema and structural lesions of the SI joints for the diagnosis of axSpA. Interdisciplinary work involving both radiologists and rheumatologists would be necessary to overcome barriers hampering the implementation of these recommendation in daily clinical practice.

DO WE HAVE ENOUGH DATA TO DEFINE SACROILIITIS BASED ON STRUCTURAL CHANGES DETECTED BY MRI?

The current ASAS definition of a ‘positive MRI of SI joints’ was intended for use as part of the ASAS classification criteria only, thus, not for diagnosis. Therefore, even the latest definition update relies on the presence of bone marrow oedema that should be highly suggestive of axSpA.[7] However, structural damage is considered important contextual information for the interpretation of whether bone marrow oedema is suggestive of axSpA or not. In the clinical setting, when making the diagnosis, structural damage on MRI could be the leading finding resulting in a diagnosis of axSpA even if active inflammation was absent at the time point of the investigation. It is currently under debate if structural damage detected by MRI could substitute structural damage detected by conventional radiographs also in the context of classification criteria.

A systematic literature research on the performance of MRI in the diagnosis of axSpA was published in 2019 but included publications only until March 2017.[22] Many studies have been performed in the last decade and many after this deadline. For example, in the study by Weber *et al* from 2015, erosions in >2 SI-joint quadrant performed best,[23] and in the study by Baraliakos *et al* from 2021 analysing STIR and T1-weighted sequences, the combination of bone marrow oedema and erosions had the highest predictive value for the diagnosis of axSpA.[11]

In the original definition of a positive SI joint MRI by ASAS from 2009[24] and in the updated definition from 2016,[25] structural lesions of the SI joints such as erosion, fat lesion, sclerosis and ankylosis were described, but it was felt by the ASAS group that more data were needed for a definition of a structurally positive or negative MRI. The same was the case for the EULAR recommendation for the use of imaging in the diagnosis and management of SpA in clinical practice.[26] However, the ASAS MRI working group—including most of the researchers in this field, both rheumatologists and radiologists—published then in 2019 the following definitions for MRI SI joints lesions reflecting structural change: erosion is defined as a defect in subchondral bone associated with full-thickness loss of the dark appearance of the subchondral cortex at its expected location, with loss of signal on a T1-weighted non-fat suppressed sequence compared with the normal bright appearance of adjacent bone marrow. Moreover the definition for fat lesion was as follows: bright signal on a T1-weighted non-fat-suppressed sequence that is brighter than normal bone marrow, which meets the following requirement: (1) homogeneously bright, (2) located in a typical anatomical area (subchondral bone) and (3) sharply defined along its non-articular border with normal bone marrow.[7] In a follow-up publication by the working group, structural damage on MRI T1-weighted of SI joints typical of SpA was defined as an ASAS-defined erosion (see aforementioned) in ≥3 SI joint quadrants or at the same location in ≥2 consecutive slices or by an ASAS-defined fat lesion in ≥5 SI joint quadrants or in ≥3 consecutive slices.[8] In the latter study, in contrast to the majority of other publications, the positive predictive validity of the MRI definitions for a diagnosis of axSpA has been analysed after a mean follow-up of 4–5 years that strengthened the main study outcome: the diagnosis of axSpA.[8]

Another interesting finding of possible relevance in this context was reported recently when investigating which structural SI lesions on CT performed best for the diagnosis of axSpA.[27] For this, the SI joint was segmented in ventral, middle and dorsal parts, and it was found that the presence of erosion and ankylosis differentiated best between axSpA and non-SpA; however, this was the case only for the middle and dorsal parts and not for the ventral regions of the joint that are typically prone to mechanical stress. Similar findings had been reported by the group previously for the location of bone marrow oedema, especially for the differential diagnosis of axSpA with osteitis condensans ilii.[28] Thus, it should be further clarified whether these findings should be included for a positive definition of structural MRI lesions in the SI joint. Sclerosis was frequently present both in patients with axSpA and in patients without SpA in this study and did not differentiate between the two groups. Sclerosis was also not selected as a differentiating feature between axSpA and non-SpA by the ASAS MRI working group.[7 8] In the dorsal part of the SI joint, the ligament insertion might result into a physiological variation of the contour of the cortical bone that should be taken into account when interpreting erosions in this location.

Based on the data presented here, structural SI joint lesions should be evaluated by MRI in T1-weighted and 3D-GRE sequences, while conventional radiographs should only be used if MRI is not available. Conventional radiography is neither specific nor sensitive for the diagnosis of axSpA and has a low rate of agreement among readers. (Low-dose) CT has good sensitivity and specificity for structural lesions; however, the radiation exposure (even if low-dose CT is comparable to that of conventional radiographs) and its lack for depicting bone marrow changes (ie, oedema and fat) does not recommend its use as first-line imaging. ASAS, together with SPARTAN, has embarked on a study for the re-evaluation of the ASAS classification criteria for axSpA: 1000 patients referred to a rheumatologist because of possible axSpA worldwide are being enrolled and undergo a thorough clinical, laboratory and imaging (MRI and radiographs in all patients) investigation. It is likely that recruitment will be finished in 2022. Whether it will be necessary to use these data for some fine-tuning of the recommendations made above or whether the available data we have now are already sufficient (yes, in the opinion of the authors) to redefine the role of radiographs and MRI in the diagnosis (and potentially also classification) of axSpA should be decided soon. There is a major clinical demand for this. The past decade has seen a continuous discussion and training regarding diagnosis and differential diagnosis of bone marrow oedema of the SI joints by STIR MRI. This training should now also include evaluation of structural lesions on MRI.

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CLINICAL SCIENCE

Infections in patients with rheumatoid arthritis receiving tofacitinib versus tumour necrosis factor inhibitors: results from the open-label, randomised controlled ORAL Surveillance trial

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ABSTRACT

Objectives To characterise infections in patients with rheumatoid arthritis (RA) in ORAL Surveillance.

Methods In this open-label, randomised controlled trial, patients with RA aged≥50 years with ≥1 additional cardiovascular risk factor received tofacitinib 5 or 10 mg two times per day or a tumour necrosis factor inhibitor (TNFi). Incidence rates (IRs; patients with first events/100 patient-years) and hazard ratios (HRs) were calculated for infections, overall and by age (50–<65 years; ≥65 years). Probabilities of infections were obtained (Kaplan-Meier estimates). Cox modelling identified infection risk factors.

Results IRs/HRs for all infections, serious infection events (SIEs) and non-serious infections (NSIs) were higher with tofacitinib (10>5 mg two times per day) versus TNFi. For SIEs, HR (95% CI) for tofacitinib 5 and 10 mg two times per day versus TNFi, respectively, were 1.17 (0.92 to 1.50) and 1.48 (1.17 to 1.87). Increased IRs/HRs for all infections and SIEs with tofacitinib 10 mg two times per day versus TNFi were more pronounced in patients aged≥65 vs 50–<65 years. SIE probability increased from month 18 and before month 6 with tofacitinib 5 and 10 mg two times per day versus TNFi, respectively. NSI probability increased before month 6 with both tofacitinib doses versus TNFi. Across treatments, the most predictive risk factors for SIEs were increasing age, baseline opioid use, history of chronic lung disease and time-dependent oral corticosteroid use, and, for NSIs, female sex, history of chronic lung disease/infections, past smoking and time-dependent Disease Activity Score in 28 joints, C-reactive protein.

Conclusions Infections were higher with tofacitinib versus TNFi. Findings may inform future treatment decisions.

Trial registration number NCT02092467.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Patients with rheumatoid arthritis (RA) have an increased susceptibility to infections due to multiple factors, including age, disease activity, comorbidities and RA treatments.

WHAT THIS STUDY ADDS

⇒ In patients with RA aged≥50 years and with ≥1 additional cardiovascular risk factor, dose-dependent increases in the incidence and risk of all infections, serious infection events (SIEs) and non-serious infections (NSIs) were observed with tofacitinib (5 mg two times per day (recommended dosage for RA) and 10 mg two times per day) versus tumour necrosis factor inhibitors (TNFi).

⇒ Across treatment groups, the incidence of all infections and SIEs were increased in patients aged≥65 versus 50–<65 years, with increased risks more pronounced with tofacitinib 10 mg two times per day versus TNFi in older patients.

⇒ Across treatment groups, the most predictive risk factors for SIEs were increasing age, baseline opioid use, history of chronic lung disease and time-dependent oral corticosteroid use; while those for NSIs were female sex, history of chronic lung disease/infections, past smoking and time-dependent higher Disease Activity Score in 28 joints, C-reactive protein score.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These findings from ORAL Surveillance may inform treatment decisions for patients with RA; the higher risk of infections with tofacitinib versus TNFi, and risk factors identified for infections, should be considered as part of the shared decision-making between physicians and patients.

INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory autoimmune disorder.[1] Compared with the general population, patients with RA are at a greater risk of infections, including serious infections requiring hospitalisation.[2 3] In patients with RA, infections contribute to morbidity and mortality[4 5] and may cause treatment discontinuation.[6]

The increased susceptibility to infections in patients with RA has been attributed to disease pathophysiology, comorbidities, lifestyle factors and use of immunomodulatory drugs.[3] Analyses

of real-world and clinical trial data from patients with RA have shown that the risk of serious and non-serious infections (NSIs) is increased in those receiving biologic disease-modifying antirheumatic drugs (bDMARDs) versus conventional synthetic DMARDs (csDMARDs),[7 8] and the risk of infections varies across treatments. For example, the tumour necrosis factor inhibitor (TNFi), etanercept, has been associated with reduced risk of infections versus other TNFi agents[9–11] and the Janus kinase (JAK) inhibitor, tofacitinib.[12]

ORAL Surveillance was a postauthorisation study that assessed the safety of tofacitinib versus TNFi in patients with RA aged≥50 years with ≥1 additional cardiovascular (CV) risk factor.[13] An ad hoc safety analysis of ORAL Surveillance reported the incidence of non-fatal and fatal serious infection events (SIEs) to be greater with tofacitinib versus TNFi.[14] Risk of SIEs (non-fatal/fatal) with tofacitinib was further increased in patients aged>65 years versus younger patients[14]; therefore, the European Medicines Agency recommended that patients aged>65 years should be treated with tofacitinib only when there is no suitable alternative treatment.[15] Along with increasing age, a safety analysis of randomised controlled trials/long-term extension (LTE) studies (excluding ORAL Surveillance) identified tofacitinib dose, male sex, geographical region (Asia and Australia/New Zealand/rest of the world (ROW) versus the USA/Canada), increasing Health Assessment Questionnaire-Disability Index Score, postbaseline lymphopenia, corticosteroid use, increasing body mass index (BMI) and history of diabetes and chronic lung disease as significant risk factors for SIEs in tofacitinib-treated patients.[16]

Using the final dataset from ORAL Surveillance, we sought to compare infections in patients with RA receiving tofacitinib versus TNFi, and to identify risk factors for infections in these patients.

METHODS

Study design and patients

ORAL Surveillance was a phase IIIb/IV randomised, open-label, safety endpoint study conducted from March 2014 to July 2020 in patients with active RA despite methotrexate treatment who were aged≥50 years with ≥1 additional CV risk factor.[13]

Patients with infections requiring treatment≤2 weeks prior to study start or infections requiring hospitalisation or parenteral antimicrobial therapy≤6 months prior to study start were excluded. Patients had to screen negative for active tuberculosis (TB) or inadequately treated TB (active or latent) at study entry and annually for the full study duration. Patients newly testing positive for latent TB had to receive isoniazid or other TB prophylaxis to continue in the study. Complete inclusion and exclusion criteria are published elsewhere.[13]

Patients were randomised 1:1:1 to receive oral tofacitinib 5 or 10 mg two times per day, or subcutaneous TNFi (adalimumab 40 mg once every 2 weeks (North America: the United States, Puerto Rico and Canada) or etanercept 50 mg once weekly (ROW)). Patients continued their prestudy stable dose of methotrexate unless modification was clinically indicated.

In February 2019, following a study amendment, the tofacitinib 10 mg two times per day dose was reduced to 5 mg two times per day after the Data Safety Monitory Board noted an increased frequency of pulmonary embolism in patients receiving tofacitinib 10 mg two times per day versus TNFi and an increase in overall mortality with tofacitinib 10 vs 5 mg two times per day and TNFi.

If a patient experienced an SIE, they may have had their study drug temporarily discontinued until they recovered, but they were not excluded from the study.

ORAL Surveillance was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines of the International Council on Harmonisation, and was approved by the Institutional Review Board and/or Independent Ethics Committee at each centre. Patients provided written informed consent.

Patient and public involvement

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

Outcomes

Treatment-emergent adverse events (AEs) assessed in this analysis included: all infections, SIEs (non-fatal/fatal), NSIs, herpes zoster (HZ) and adjudicated opportunistic infections (including HZ and TB). These events are defined in online supplemental material.

Statistical analysis

Safety outcomes were analysed using the safety analysis set, which included all randomised patients receiving ≥1 dose of study drug. For patients randomised to tofacitinib 10 mg two times per day who had their dose reduced to 5 mg two times per day in February 2019, the data collected after the dose switch were counted in the tofacitinib 10 mg two times per day group.

Infection events were counted within the predefined risk period, based on the 28-day on-treatment time, defined as time from the first study dose to the last study dose +28 days or to the last contact date, whichever was earliest. The last contact date was defined as the maximum of AE start date, AE stop date, last visit date, withdrawal date or telephone contact date; if a patient died, the last contact date was the death date. Patients without events were censored at the end of the risk period. For patients with multiple SIEs, NSIs and HZ, these were reported as separate events if the event start dates were different.

Crude incidence rates (IRs; for all infections, SIEs, NSIs and HZ) were expressed as the number of patients with first events per 100 patient-years, along with two-sided 95% CIs derived by exact Poisson method.[17] HR (for all infections, SIEs, NSIs and HZ) and 95% CIs for pairwise treatment comparisons (tofacitinib 5 or 10 mg two times per day versus TNFi; tofacitinib 10 vs 5 mg two times per day) were estimated using Cox proportional hazard regression models.[18]

For SIEs, the number needed to harm (NNH; number of patient-years of tofacitinib exposure needed to have one additional AE relative to TNFi) was calculated post hoc for tofacitinib 5 or 10 mg two times per day versus TNFi. The NNH for patients exposed for 5 years was calculated by dividing the number of patient-years needed to harm by 5.

The cumulative probabilities of patients experiencing a first event (SIE, NSI and HZ) at specific time intervals after initiation of each treatment were measured post hoc using Kaplan-Meier estimates of the survivor function.

Potential baseline and time-dependent risk factors (online supplemental table 1) for first SIEs, NSIs and all HZ (non-serious and serious) were evaluated post hoc, overall and for each individual treatment group; a model selection process was conducted using Cox proportional hazards (simple and multivariable) regression models (additional details are in online supplemental material).

Across all analyses, no adjustments for multiple comparisons were applied.

Table 1 Selected demographics and baseline disease characteristics in ORAL Surveillance

	Tofacitinib 5 mg two times per day (N=1455)	Tofacitinib 10 mg two times per day (N=1456)	TNFi (N=1451)
Age (years), mean (SD)	60.8 (6.8)	61.4 (7.1)	61.3 (7.5)
≥65 years, n (%)	413 (28.4)	478 (32.8)	462 (31.8)
Male sex, n (%)	286 (19.7)	332 (22.8)	334 (23.0)
RA disease duration (years), mean (SD)	10.4 (8.8)	10.2 (9.0)	10.6 (9.3)
Smoking status, n (%)			
Current smoker	411 (28.2)	402 (27.6)	353 (24.3)
Past smoker	309 (21.2)	302 (20.7)	326 (22.5)
Never smoked	735 (50.5)	752 (51.6)	772 (53.2)
Geographical region, n (%)*			
North America	402 (27.6)	409 (28.1)	432 (29.8)
ROW	1053 (72.4)	1047 (71.9)	1019 (70.2)
BMI (kg/m^2), mean (SD) (number of patients with missing values)	29.7 (6.5) (7)	29.7 (6.3) (3)	29.8 (6.6)(7)
≥30 kg/m^2, n (%)	606 (41.6)	594 (40.8)	617 (42.5)
≥35 kg/m^2, n (%)	256 (17.6)	261 (17.9)	267 (18.4)
Concomitant medication use at baseline (day 1)			
Opioids, n (%)	293 (20.1)	283 (19.4)	288 (19.8)
Oral corticosteroids, n (%)	776 (53.3)	773 (53.1)	774 (53.3)
Oral corticosteroid dose (mg/day), mean (range)†	6.0‡ (0.7–20.0)	6.1§ (0.6–20.0)	6.1¶ (0.3–20.0)
Medical history, n (%)			
Diabetes	243 (16.7)	261 (17.9)	255 (17.6)
Chronic lung disease (COPD or ILD)	178 (12.2)	173 (11.9)	172 (11.9)
Extra-articular disease	532 (36.6)	521 (35.8)	552 (38.0)
Nodules	301 (20.7)	268 (18.4)	287 (19.8)
Coronary artery disease	161 (11.1)	172 (11.8)	164 (11.3)
Heart failure	18 (1.2)	23 (1.6)	18 (1.2)
Infection	574 (39.5)	549 (37.7)	556 (38.3)
Positive for anticitrullinated protein antibodies, n (%)	1093 (75.1)	1129 (77.5)	1119 (77.1)
HAQ-DI, mean (SD) (number of patients with missing values)	1.6 (0.6) (11)	1.6 (0.6) (18)	1.6 (0.6) (25)
DAS28-4(CRP), mean (SD) (number of patients with missing values)	5.8 (0.9) (11)	5.8 (0.9) (17)	5.8 (0.9) (26)

For patients randomised to the tofacitinib 10 mg two times per day group who had their dose of tofacitinib reduced to 5 mg two times per day, the data collected after patients were switched to tofacitinib 5 mg two times per day were counted in the tofacitinib 10 mg two times per day group.
*In North America (the USA, Puerto Rico and Canada), patients randomised to TNFi received adalimumab 40 mg once every 2 weeks; in the ROW, patients randomised to TNFi received etanercept 50 mg once weekly.
†In patients taking oral corticosteroids at baseline with known dosing information.
‡n=769.
§n=771.
¶n=773.
BMI, body mass index; COPD, chronic obstructive pulmonary disease; DAS28-4(CRP), Disease Activity Score in 28 joints, C-reactive protein; HAQ-DI, Health Assessment Questionnaire-Disability Index; ILD, interstitial lung disease; n, number of patients meeting baseline criteria; N, number of evaluable patients; RA, rheumatoid arthritis; ROW, rest of the world; SD, standard deviation; TNFi, tumour necrosis factor inhibitor.

RESULTS

Patients

Overall, 4362 patients were randomised and treated (tofacitinib 5 mg two times per day: N=1455; tofacitinib 10 mg two times per day: N=1456; TNFi: N=1451); median follow-up was 4.0 years. Total exposure was 5073.5, 4773.4 and 4940.7 patient-years for tofacitinib 5 or 10 mg two times per day, or TNFi, respectively.[13] For the tofacitinib 10 mg two times per day group, approximately 79% of exposure occurred prior to the study amendment (ie, before patients randomised to tofacitinib 10 mg two times per day had their dose reduced to 5 mg two times per day); approximately 21% of exposure occurred after patients had switched to tofacitinib 5 mg two times per day. Table 1 shows selected patient demographics/baseline disease characteristics; full details are published elsewhere.[13]

Across treatments, 4.7%–5.2% of patients were reported to have received HZ vaccination (Zostavax or Shingrix) prior to study start, and 0.3%–0.8% of patients received HZ vaccination on/after study day 1. At screening, 11.5%–12.3% of patients had latent TB with a positive QuantiFERON Gold or tuberculin skin test and negative chest radiograph, and received isoniazid or other TB prophylaxis prior to the first dose of study drug. Overall, 16.8%–20.2% of patients received isoniazid or other TB prophylaxis on/after the first dose of study drug.

Incidence and risk of infections in ORAL Surveillance

Incidence and risk of all infections

Across treatments, the most frequent treatment-emergent AEs by Medical Dictionary for Regulatory Activities' System Organ Class were infections and infestations.[13] The most frequently reported infections were upper respiratory tract infections, bronchitis and urinary tract infections (table 2).

For all infections, and infections excluding HZ, IRs were higher and risk was increased for both tofacitinib doses versus TNFi and for tofacitinib 10 vs 5 mg two times per day (figure 1). Across treatments, IRs for all infections were greater in patients

Table 2 Summary of infection AEs in ORAL Surveillance

Patients with events, n (%)	Tofacitinib 5 mg two times per day (N=1455)	Tofacitinib 10 mg two times per day (N=1456)	TNFi (N=1451)
Infections and Infestations (MedDRA System Organ Class)*	1036 (71.2)	1055 (72.5)	930 (64.1)
Most frequently reported, by MedDRA Preferred Term (≥3% of patients with events in any treatment group)*			
Upper respiratory tract infection	308 (21.2)	312 (21.4)	255 (17.6)
Bronchitis	222 (15.3)	237 (16.3)	163 (11.2)
Urinary tract infection	186 (12.8)	221 (15.2)	184 (12.7)
HZ (non-serious/serious)†	176 (12.1)	167 (11.5)	55 (3.8)
Nasopharyngitis	164 (11.3)	165 (11.3)	158 (10.9)
Pneumonia	95 (6.5)	101 (6.9)	78 (5.4)
Sinusitis	92 (6.3)	79 (5.4)	91 (6.3)
Pharyngitis	86 (5.9)	79 (5.4)	75 (5.2)
Influenza	90 (6.2)	91 (6.3)	71 (4.9)
Latent TB	87 (6.0)	67 (4.6)	91 (6.3)
Gastroenteritis	64 (4.4)	79 (5.4)	53 (3.7)
Respiratory tract infection	43 (3.0)	43 (3.0)	31 (2.1)
Cellulitis	36 (2.5)	32 (2.2)	50 (3.4)
SIEs	141 (9.7)	169 (11.6)	119 (8.2)
Non-fatal	135 (9.3)	156 (10.7)	115 (7.9)
Fatal	6 (0.4)	13 (0.9)	4 (0.3)
Patients with 1 SIE	110 (7.6)	140 (9.6)	95 (6.6)
Patients with 2 SIEs‡	22 (1.5)	23 (1.6)	18 (1.2)
Patients with 3 SIEs‡	7 (0.5)	2 (0.1)	5 (0.3)
Patients with ≥4 SIEs‡	2 (0.1)	4 (0.3)	1 (0.1)
NSIs	983 (67.6)	1003 (68.9)	882 (60.8)
Patients with 1 NSI event	307 (21.1)	326 (22.4)	334 (23.0)
Patients with 2 NSI events‡	226 (15.5)	228 (15.7)	200 (13.8)
Patients with 3 NSI events‡	160 (11.0)	135 (9.3)	117 (8.1)
Patients with ≥4 NSI events‡	290 (19.9)	314 (21.6)	231 (15.9)
NSIs excluding all HZ	954 (65.6)	968 (66.5)	870 (60.0)
All HZ (non-serious/serious)§	180 (12.4)	178 (12.2)	58 (4.0)
Seriousness			
Non-serious¶	170 (94.4)	161 (90.4)	56 (96.6)
Serious¶	10 (5.6)	17 (9.6)	2 (3.4)
Severity			
Mild¶	61 (33.9)	49 (27.5)	16 (27.6)
Moderate¶	110 (61.1)	116 (65.2)	40 (69.0)
Severe¶	9 (5.0)	13 (7.3)	2 (3.4)
All HZ (non-serious/serious)§			
Patients with 1 HZ event	138 (9.5)	137 (9.4)	46 (3.2)
Patients with 2 HZ events‡	33 (2.3)	35 (2.4)	11 (0.8)
Patients with 3 HZ events‡	9 (0.6)	5 (0.3)	1 (0.1)
Patients with ≥4 HZ events‡	0 (0.0)	1 (0.1)	0 (0.0)
Adjudicated multidermatomal HZ**	29 (2.0)	24 (1.7)	12 (0.8)
Adjudicated special interest HZ††	17 (1.2)	17 (1.2)	4 (0.3)
Discontinuation from study drug due to HZ	6 (0.4)	12 (0.8)	2 (0.1)
Adjudicated opportunistic infections*	39 (2.7)	44 (3.0)	21 (1.5)
HZ adjudicated as an opportunistic infection*,‡‡	34 (2.3)	32 (2.2)	13 (0.9)
TB adjudicated as an opportunistic infection*	1 (0.1)	5 (0.3)	5 (0.3)
Adjudicated opportunistic infections excluding HZ and TB	4 (0.3)	7 (0.5)	3 (0.2)

For patients randomised to the tofacitinib 10 mg two times per day group who had their dose of tofacitinib reduced to 5 mg two times per day, the data collected after patients were switched to tofacitinib 5 mg two times per day were counted in the tofacitinib 10 mg two times per day group.
*Reported elsewhere.[13]
†Includes the Preferred Term HZ from the clinical database recorded on the AE case report forms.
‡Events were counted as separate events if the event start dates were different.
§Includes HZ adjudicated as opportunistic infections and non-adjudicated HZ events, which included preferred terms of genital HZ, HZ, HZ cutaneous disseminated, HZ disseminated, HZ infection neurological, HZ meningitis, HZ meningoencephalitis, HZ necrotising retinopathy, HZ oticus, HZ pharyngitis, ophthalmic HZ, HZ ophthalmic and HZ multidermatomal, from the clinical database recorded on the AE case report forms.
¶Percentages calculated based on number of patients with HZ adjudicated as opportunistic infections and non-adjudicated HZ events from the clinical database.
**Cases of HZ involving non-adjacent dermatomes or >2 adjacent dermatomes.
††Cases of HZ involving two adjacent dermatomes.
‡‡Cases of multidermatomal HZ and disseminated HZ (diffuse rash (>6 dermatomes)), encephalitis, pneumonia and other organ involvement) were adjudicated as opportunistic infections.
AE, adverse event; HZ, herpes zoster; MedDRA, Medical Dictionary for Regulatory Activities; n, number of patients with events; N, number of evaluable patients; NSI, non-serious infection; SIE, serious infection event; TB, tuberculosis; TNFi, tumour necrosis factor inhibitors.

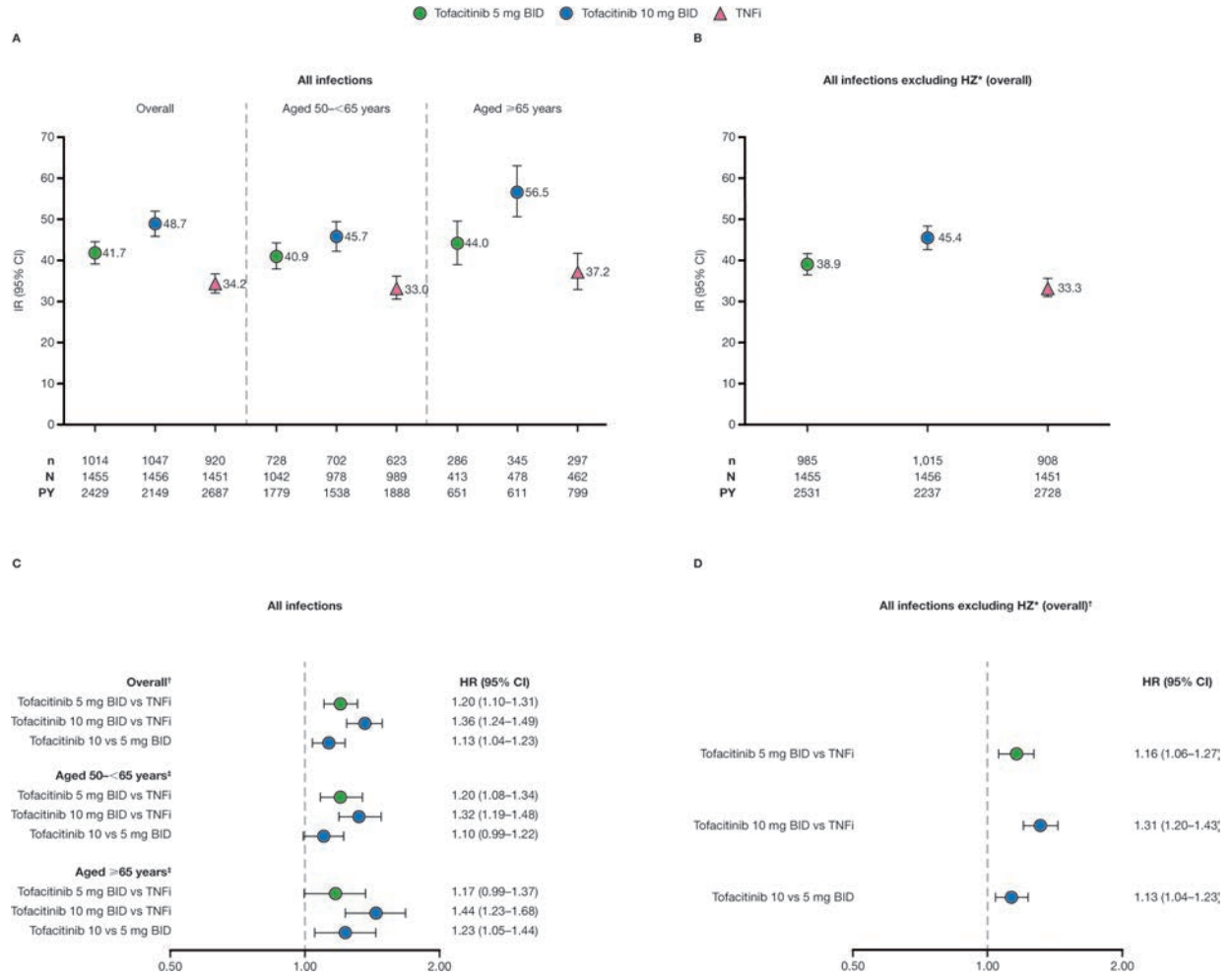

Figure 1 IRs (patients with first events/100 PY; 95% CIs) for (A) all infections, overall and stratified by age, and (B) all infections excluding HZ; and HRs (95% CIs) for (C) all infections, overall and stratified by age, and (D) all infections excluding HZ, in ORAL Surveillance. HRs are shown on a logarithmic scale. For patients randomised to the tofacitinib 10 mg two times per day group who had their dose of tofacitinib reduced to 5 mg two times per day, the data collected after patients were switched to tofacitinib 5 mg two times per day were counted in the tofacitinib 10 mg two times per day group. *Excludes HZ adjudicated as opportunistic infections and non-adjudicated HZ events from the clinical database. †HRs (95% CIs) based on a simple Cox proportional hazard model for pairwise treatment comparisons, with treatment as covariate. ‡HRs (95% CIs) based on a multivariable Cox proportional hazard model for pairwise treatment comparisons with treatment, sex, region and smoking as covariates.
BID, two times per day; HR, hazard ratio; HZ, herpes zoster; IR, incidence rate; N, number of evaluable patients; n, number of patients with events; PY, patient-years; TNFi, tumour necrosis factor inhibitors.

aged≥65 vs 50–<65 years (figure 1A). In both age groups, risk for all infections increased with tofacitinib (10>5 mg two times per day) versus TNFi (figure 1C).

HRs for the combined tofacitinib doses versus TNFi for all infections and all infections excluding HZ (as well as SIEs, NSIs, NSIs excluding HZ and all HZ) are shown in online supplemental table 2).

Incidence and risk of SIEs

Across treatments, IRs of SIEs (non-fatal/fatal) were greater in patients aged≥65 vs 50–<65 years (figure 2A). Overall, IRs of SIEs were higher with tofacitinib (10>5 mg two times per day) versus TNFi. NNH was 238 and 83 patient-years, respectively, for tofacitinib 5 and 10 mg two times per day (figure 2A), corresponding to 48 and 17 patients who would need to be treated with tofacitinib 5 and 10 mg two times per day, respectively, versus TNFi, over 5 years to have one additional event. Similar trends for IRs were observed across age groups. Risk increased with both tofacitinib doses versus TNFi and tofacitinib 10 vs 5 mg two times per day, although 95% CIs for HRs included 1 for tofacitinib 5 mg two times per day versus TNFi, overall and across age groups, and for tofacitinib 10 vs 5 mg two times per day for patients aged≥50–<65 years (figure 2B). The increased risk for SIEs with tofacitinib 10 mg two times per day versus TNFi (and tofacitinib 10 vs 5 mg two times per day) was more pronounced in patients aged≥65 vs 50–<65 years (figure 2B). Cumulative probability of a first SIE with tofacitinib 5 and 10 mg two times per day versus TNFi increased from month 18 and before month 6, respectively (figure 2C).

A total of 31 (2.1%) patients in the tofacitinib 5 mg two times per day group, 29 (2.0%) patients in the tofacitinib 10 mg two times per day group and 24 (1.7%) patients in the TNFi group experienced multiple SIEs (table 2).

Risk of fatal SIEs was greater with tofacitinib 10 mg two times per day versus TNFi (HR (95% CI), 3.34 (1.09 to 10.25)); HRs were 1.47 (0.41 to 5.21) for tofacitinib 5 mg two times per day versus TNFi and 2.27 (0.86 to 5.98) for tofacitinib 10 vs 5 mg two times per day.

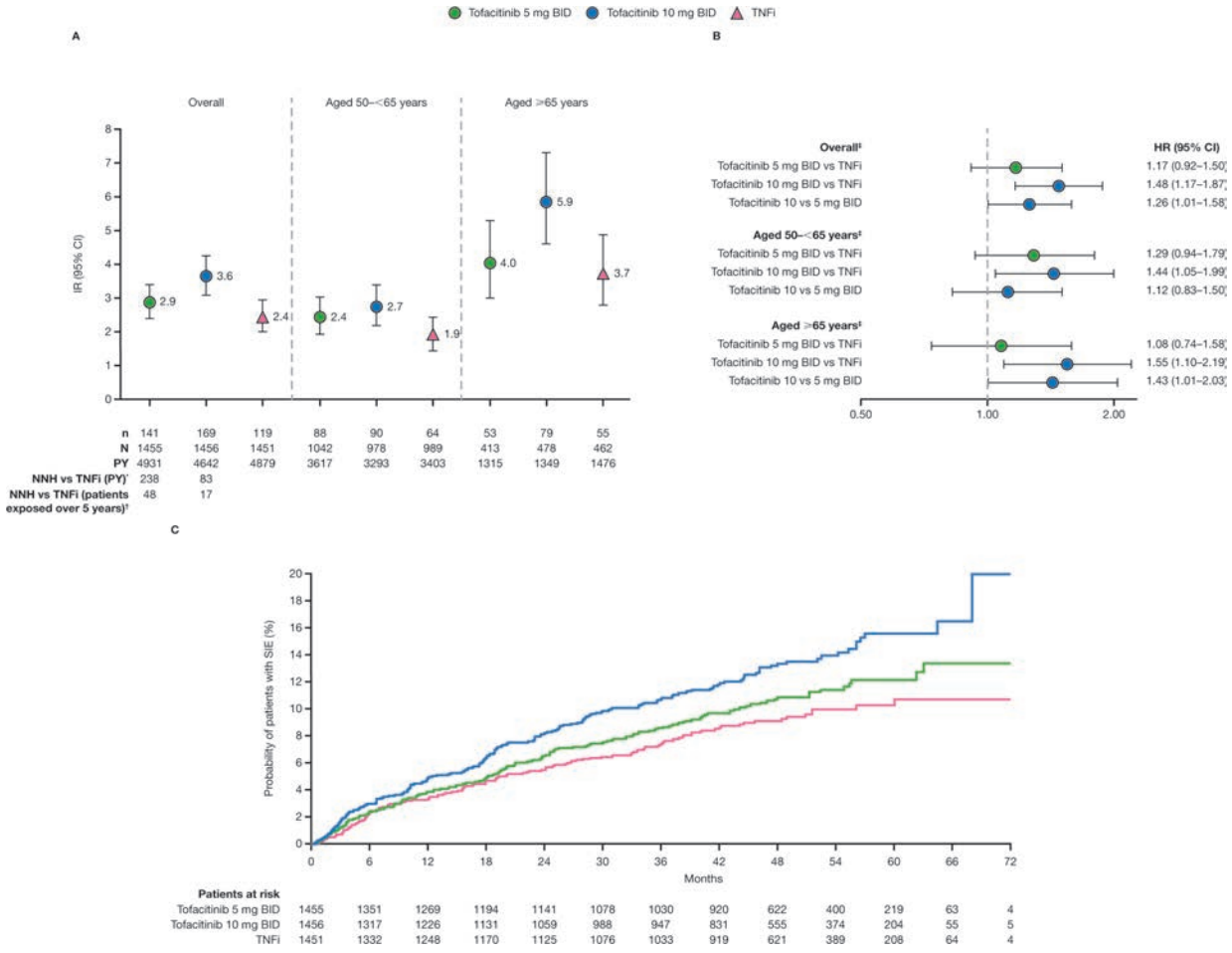

Figure 2 (A) IRs (patients with first events/100 PY; 95% CIs) and (B) HRs (95% CIs) for SIEs, overall and stratified by age; and (C) cumulative probabilities of experiencing a first SIE (Kaplan-Meier method), in ORAL Surveillance. HRs are shown on a logarithmic scale. For patients randomised to the tofacitinib 10 mg two times per day group who had their dose of tofacitinib reduced to 5 mg two times per day, the data collected after patients were switched to tofacitinib 5 mg two times per day were counted in the tofacitinib 10 mg two times per day group. IRs and HRs for SIEs overall have been reported previously.[13] *Number of PY of exposure to tofacitinib required to have one additional event, relative to a TNFi †Number of patients who would need to be treated over 5 years with tofacitinib rather than a TNFi to result in one additional event. ‡HRs (95% CIs) based on a simple Cox proportional hazard model for pairwise treatment comparisons, with treatment as covariate.
BID, two times per day; HR, hazard ratio; IR, incidence rate; N, number of evaluable patients; n, number of patients with events; PY, patient-years; SIE, serious infection event; TNFi, tumour necrosis factor inhibitors.

Incidence and risk of NSIs

For NSIs, and NSIs excluding HZ, IRs were higher and risk was increased with tofacitinib (10>5 mg two times per day) versus TNFi and tofacitinib 10 vs 5 mg two times per day (figure 3). The cumulative probability of a first NSI with tofacitinib 5 and 10 mg two times per day versus TNFi increased before month 6 (figure 3E).

Incidence and risk of HZ

IRs of all HZ (non-serious/serious) were greater in patients aged≥65 vs 50–<65 years (all treatments; figure 4A). IRs and risk for all HZ were greater with both doses of tofacitinib versus TNFi overall and across age groups (figure 4 A,B). The cumulative probability of a first HZ event with tofacitinib 5 and 10 mg two times per day versus TNFi increased before month 6 (figure 4C).

IRs (95% CIs) of adjudicated multidermatomal HZ were higher for tofacitinib 5 (0.6 (0.4 to 0.8)) and 10 mg two times per day (0.5 (0.3 to 0.7)) versus TNFi (0.2 (0.1 to 0.4)). IRs of adjudicated special interest HZ were also higher for tofacitinib 5 (0.3 (0.2 to 0.5) and 10 mg two times per day (0.4 (0.2 to 0.6)) versus TNFi (0.1 (0.0 to 0.2)).

A total of 42 (2.9%), 41 (2.8%) and 12 (0.8%) patients in the tofacitinib 5 mg two times per day, tofacitinib 10 mg two times per day and TNFi groups, respectively, reported multiple HZ events (table 2).

Risk factors for infections in ORAL Surveillance

Baseline and time-dependent risk factors across all treatments

Risk factors for infections (p<0.10) identified via simple analyses across all treatments are shown in online supplemental table 3. Figure 5 shows risk factors for infections (p<0.10) identified via multivariable analyses across all treatments. The most predictive risk factors for SIEs were increasing age, opioid use, history of chronic lung disease at baseline and time-dependent oral corticosteroid use (p<0.001; figure 5A). Patients in North America had a 22% lower risk of SIEs versus patients in the ROW (p<0.05; figure 5A). The most predictive risk factors for NSIs were female sex, history of chronic lung disease/infections, past smoking at baseline and time-dependent higher Disease Activity Score in

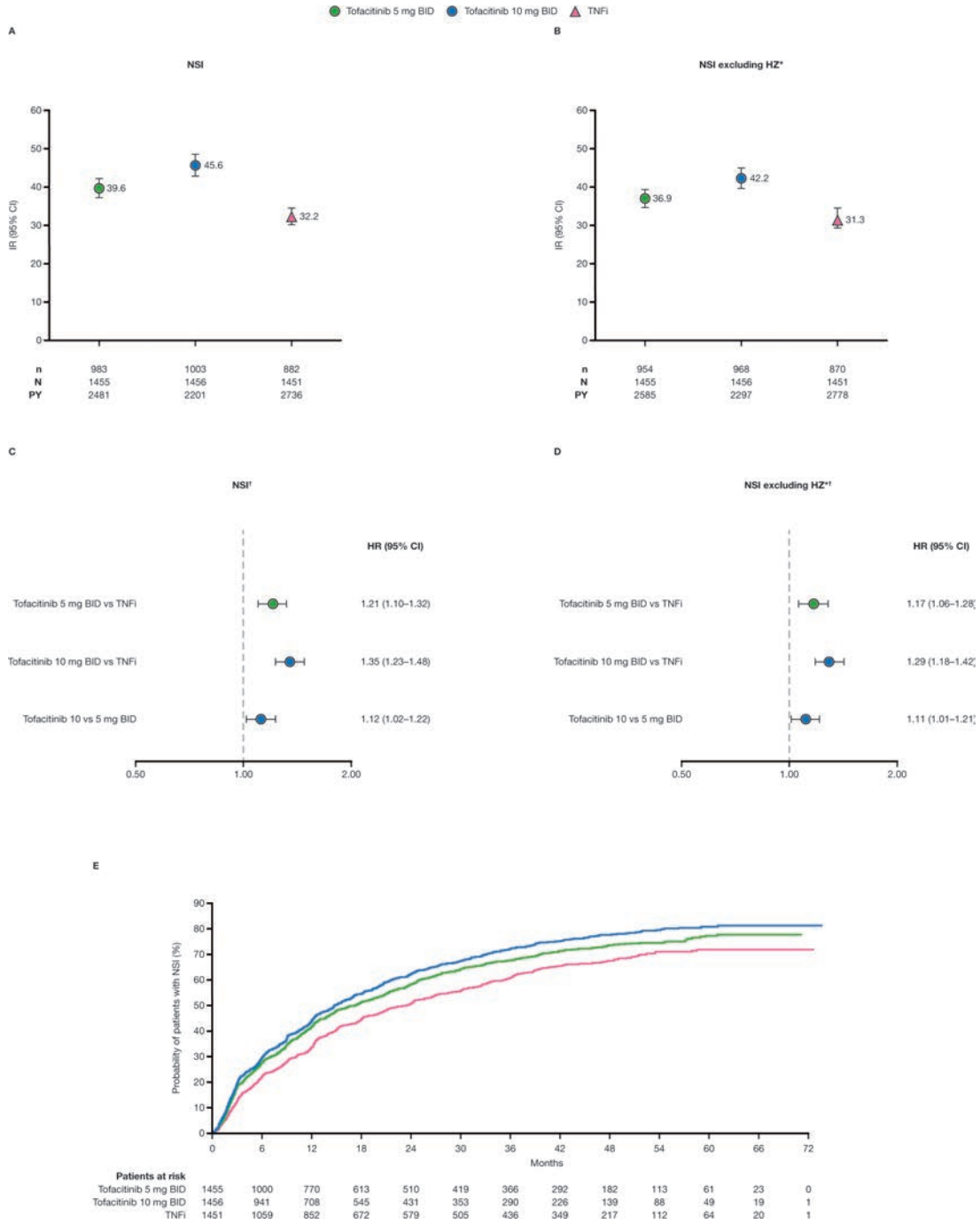

Figure 3 IRs (patients with first events/100 PY; 95% CIs) for (A) NSIs and (B) NSIs excluding HZ; HRs (95% CIs) for (C) NSIs and (D) NSIs excluding HZ; and (E) cumulative probabilities of experiencing a first NSI (Kaplan-Meier method), in ORAL Surveillance. HRs are shown on a logarithmic scale. For patients randomised to the tofacitinib 10 mg two times per day group who had their dose of tofacitinib reduced to 5 mg two times per day, the data collected after patients were switched to tofacitinib 5 mg two times per day were counted in the tofacitinib 10 mg two times per day group. *Excludes HZ adjudicated as opportunistic infections and non-adjudicated HZ events from the clinical database. †HRs (95% CIs) based on a simple Cox proportional hazard model for pairwise treatment comparisons, with treatment as covariate.
BID, two times per day; HR, hazard ratio; HZ, herpes zoster; IR, incidence rate; N, number of evaluable patients; n, number of patients with events; NSI, non-serious infection; PY, patient-years; TNFi, tumour necrosis factor inhibitors.

28 joints, C-reactive protein score ($p<0.001$; figure 5B). The most predictive risk factors for all HZ (non-serious/serious) were increasing age, history of chronic renal disease, female sex and history of coronary artery disease at baseline ($p<0.05$; figure 5C).

Baseline risk factors for individual treatments

Baseline risk factors for infections ($p<0.10$) identified using simple analyses for individual treatments are shown in online supplemental table 4. Table 3 summarises baseline risk factors for infections ($p<0.10$) identified using multivariable analyses for individual treatments. The most predictive baseline risk factors for SIEs included: increasing age and history of chronic lung disease for tofacitinib 5 mg two times per day ($p<0.001$); increasing age ($p<0.001$) and opioid use ($p<0.01$) for tofacitinib 10 mg two times per day; and increasing age ($p<0.001$), opioid use and history of chronic lung disease for TNFi ($p<0.01$; table 2). The most predictive baseline

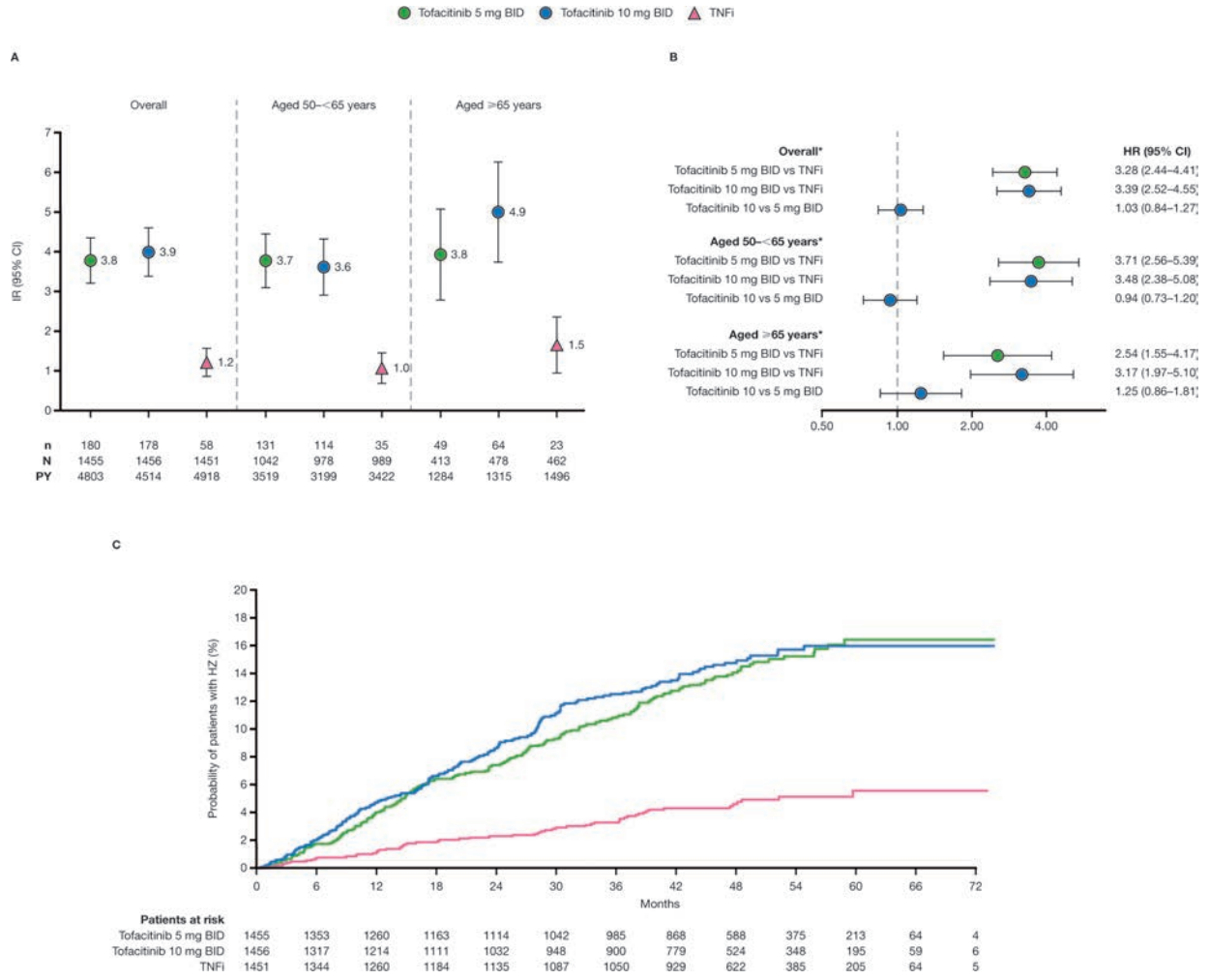

Figure 4 (A) IRs (patients with first events/100 PY; 95% CIs) and (B) HRs (95% CIs) for all HZ (non-serious/serious), overall and stratified by age; and (C) cumulative probabilities of experiencing a first HZ (non-serious/serious) event (Kaplan-Meier method), in ORAL Surveillance. HRs are shown on a logarithmic scale. For patients randomised to the tofacitinib 10 mg two times per day group who had their dose of tofacitinib reduced to 5 mg two times per day, the data collected after patients were switched to tofacitinib 5 mg two times per day were counted in the tofacitinib 10 mg two times per day group. All HZ events (non-serious/serious) include HZ adjudicated as opportunistic infections and non-adjudicated HZ events from the clinical database. *HRs based on a multivariable Cox proportional hazard model for pairwise treatment comparisons with treatment, age, region, smoking and baseline corticosteroid use as covariates.
BID, two times per day; HR, hazard ratio; HZ, herpes zoster; IR, incidence rate; N, number of evaluable patients; n, number of patients with events; PY, patient-years; TNFi, tumour necrosis factor inhibitors.

risk factors for NSIs included: female sex, past smoking and history of chronic lung disease for tofacitinib 5 mg two times per day ($p<0.001$); female sex and history of infection for tofacitinib 10 mg two times per day; and history of infection ($p<0.001$) and female sex ($p<0.01$) for TNFi (table 3).

The HRs for SIEs and NSIs comparing tofacitinib and TNFi were consistent when based on the simple Cox models (with treatment group as the only covariate; figures 2 and 3), multivariable Cox models via backward selection (figure 5) and multivariable Cox models with each of the time-dependent covariates included (data not shown).

DISCUSSION

In ORAL Surveillance, there were dose-dependent increases in the IRs/HRs for all infections, SIEs and NSIs with tofacitinib versus TNFi. For SIEs, 95% CIs for HRs included 1 for tofacitinib 5 mg two times per day versus TNFi, overall and across age groups. Kaplan-Meier plots suggested that patients were more likely to experience a first SIE with tofacitinib 5 and 10 mg two times per day versus TNFi from month 18 onwards and before month 6, respectively; and patients were more likely to experience a first NSI or HZ event with both tofacitinib doses versus TNFi before month 6. The increases in all infections and SIEs with tofacitinib 10 mg two times per day vs TNFi (and tofacitinib 10 vs 5 mg two times per day) were more pronounced in patients aged ≥65 vs 50–<65 years. While the number of patients with repeated SIEs was generally balanced across treatment groups, a greater proportion of patients had 2, 3 and ≥4 NSIs with tofacitinib (both doses) versus TNFi. Across age groups, the incidence and risk of HZ was greater with both doses of tofacitinib versus TNFi. IRs of adjudicated opportunistic infections were <1 for all treatment groups and are published elsewhere.[13]

IRs of SIEs were higher in ORAL Surveillance relative to those previously reported in a pooled analysis of data from the Phase I–IIIb/IV and LTE tofacitinib clinical trials. In ORAL Surveillance, IRs (95% CI) were 2.9 (2.4 to 3.4) and 3.6 (3.1 to 4.2) for tofacitinib 5 and 10 mg two times per day, respectively, while in the wider tofacitinib clinical programme, IRs were 2.8 (2.5 to 3.2) and 2.3 (2.1 to 2.6) for average total daily doses of

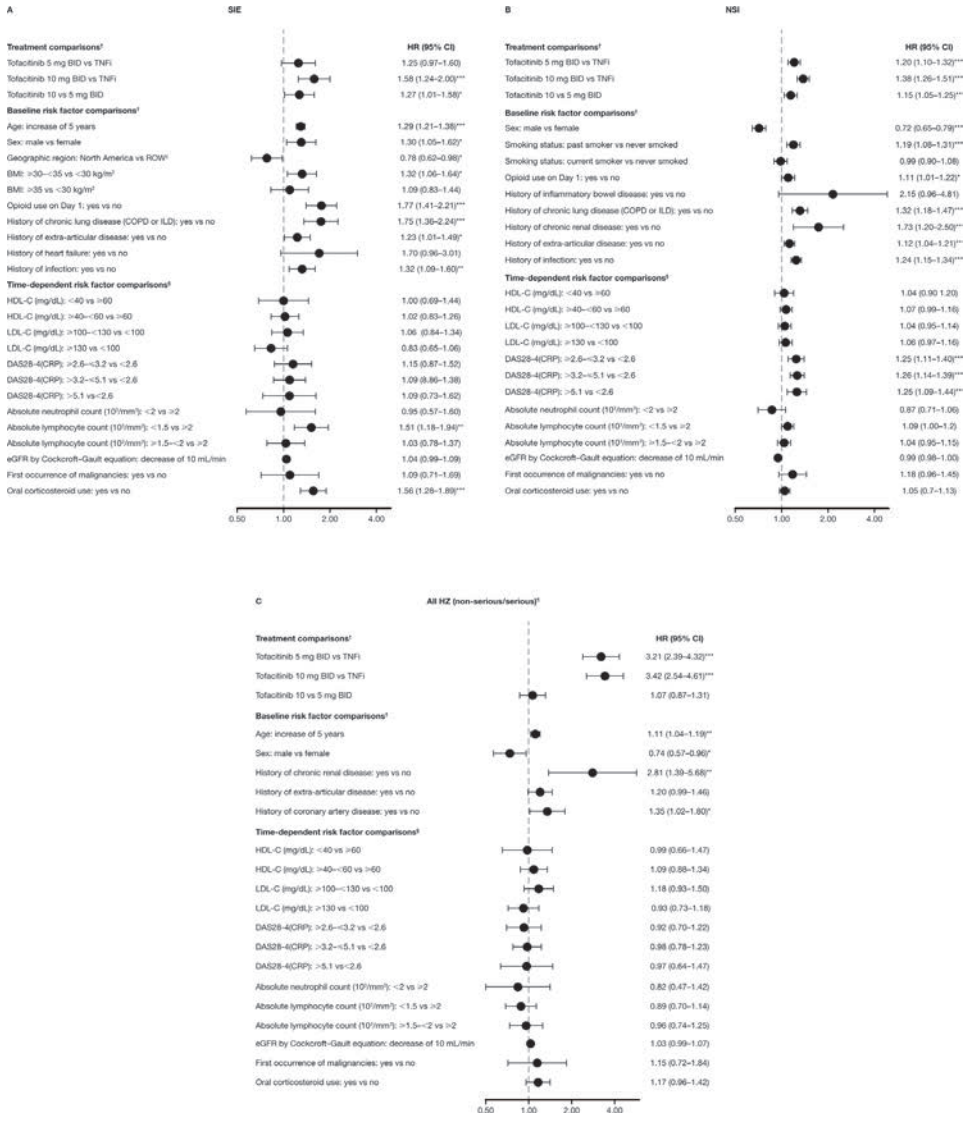

Figure 5 HRs (95% CIs) of potential baseline and time-dependent risk factors for (A) SIEs, (B) NSIs and (C) all HZ (non-serious/serious) in ORAL Surveillance (multivariable Cox analyses across treatments). For patients randomised to the tofacitinib 10 mg two times per day group who had their dose of tofacitinib reduced to 5 mg two times per day, the data collected after patients were switched to tofacitinib 5 mg two times per day were counted in the tofacitinib 10 mg two times per day group. *p<0.05, **p<0.01; ***p<0.001. HRs are shown on a logarithmic scale. †HRs were based on a backward model selection algorithm on a multivariable Cox model, including effects of treatment group (tofacitinib 5 mg two times per day, 10 mg two times per day and TNFi) and a set of candidate baseline risk factors previously selected via a simple Cox model; risk factors with p<0.10 in the simple model (see online supplemental table 3) were entered into the multivariable model, and the risk factors with p<0.10 were retained in the multivariable model, with p<0.05 interpreted as predictive. ‡In North America (the USA, Puerto Rico and Canada), patients randomised to TNFi received adalimumab 40 mg once every 2 weeks; in the ROW, patients randomised to TNFi received etanercept 50 mg once weekly. §HRs were based on a multivariable Cox time-dependent model including the fixed effects of treatment group (tofacitinib 5 mg two times per day, tofacitinib 10 mg two times per day and TNFi), the final set of baseline covariates selected from the previous multivariable Cox model, using a backward selection algorithm and a time-dependent covariate (a separate model was generated for each individual time-dependent risk factor). ¶All HZ events (non-serious/serious) include HZ adjudicated as opportunistic infections and non-adjudicated HZ events from the clinical database.
BID, two times per day; BMI, body mass index; COPD, chronic obstructive pulmonary disease; DAS28-4(CRP), Disease Activity Score in 28 joints, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; HZ, herpes zoster; ILD, interstitial lung disease; LDL-C, low-density lipoprotein cholesterol; NSI, non-serious infection; ROW, rest of the world; SIE, serious infection event; TNFi, tumour necrosis factor inhibitors.

tofacitinib 5 and 10 mg two times per day, respectively.[16] When inclusion criteria mimicking ORAL Surveillance (aged≥50 years with ≥1 additional CV risk factor (current smoker, hypertension, high-density lipoprotein cholesterol<40 mg/dL, diabetes mellitus, history of myocardial infarction or coronary heart disease at baseline)) were applied to the pooled Phase I–IIIb/IV and LTE data, IRs for SIEs increased to 3.7 (3.1 to 4.4) and 3.3 (2.8 to 3.8) for average tofacitinib 5 and 10 mg two times per day, respectively (data on file). Overall, this is in line with previous studies showing that traditional CV risk factors may contribute to an increased risk of SIEs in patients with RA.[3 19]

Increasing age is a known risk factor for infections in patients with RA and in the general population.[20 21] In ORAL Surveillance, across all treatments, the incidence of infections, including

Table 3 HRs (95% CIs) of potential baseline risk factors for SIEs and NSIs in ORAL Surveillance (multivariable Cox analyses performed for individual treatments)

Baseline risk factor comparisons, HR (95% CI)	Tofacitinib 5 mg two times per day (N=1455)	Tofacitinib 10 mg two times per day (N=1456)	TNFi (N=1451)
SIEs			
Age: increase of 5 years	1.28 (1.14 to 1.44)***	1.32 (1.19 to 1.47)***	1.26 (1.13 to 1.41)***
Positive for anticitrullinated protein antibodies: yes versus no	2.08 (1.29 to 3.36)**		
BMI: ≥30–<35 versus <30 kg/m^2	1.72 (1.18 to 2.52)**	1.37 (0.97 to 1.92)	
BMI: ≥35 versus <30 kg/m^2	1.51 (0.96 to 2.38)	0.77 (0.49 to 1.21)	
Opioid use on day 1: yes versus no	1.63 (1.13 to 2.36)**	1.67 (1.19 to 2.35)**	1.91 (1.30 to 2.81)**
History of chronic lung disease (COPD or ILD): yes versus no	2.13 (1.42 to 3.20)***	1.47 (0.98 to 2.23)	1.85 (1.18 to 2.89)**
History of extra-articular disease: yes versus no	1.36 (0.97 to 1.19)		
History of heart failure: yes versus no		2.17 (0.94 to 5.01)*	2.82 (1.03 to 7.75)*
History of infection: yes versus no		1.34 (0.98 to 1.81)	1.51 (1.05 to 2.17)*
NSIs			
Sex: male versus female	0.73 (0.62 to 0.87)***	0.69 (0.59 to 0.81)***	0.77 (0.65 to 0.91)**
Race: non-white versus white	1.19 (1.03 to 1.38)*		
Smoking status: past smoker versus never smoked	1.34 (1.14 to 1.58)***		
Smoking status: current smoker versus never smoked	1.01 (0.87 to 1.18)		
Opioid use day 1: yes versus no		1.19 (1.02 to 1.39)*	1.21 (1.03 to 1.43)*
History of chronic lung disease (COPD or ILD): yes versus no	1.38 (1.15 to 1.66)***	1.32 (1.09 to 1.59)**	1.30 (1.06 to 1.59)*
History of chronic renal disease: yes versus no	2.52 (1.39 to 4.59)**	2.16 (1.19 to 3.93)*	
History of extra-articular disease: yes versus no		1.21 (1.06 to 1.37)**	
History of infection: yes versus no	1.21 (1.06 to 1.37)**	1.31 (1.15 to 1.49)***	1.27 (1.11 to 1.45)***

For patients randomised to the tofacitinib 10 mg two times per day group who had their dose of tofacitinib reduced to 5 mg two times per day, the data collected after patients were switched to tofacitinib 5 mg two times per day were counted in the tofacitinib 10 mg two times per day group. HRs (95% CIs) were based on a backward selection algorithm used on a multivariable Cox model including candidate baseline risk factors previously selected via a simple Cox model; risk factors with p<0.10 in the simple model (see online supplemental table 4) were entered into the multivariable model, and the risk factors with p<0.10 were retained in the multivariable model, with p<0.05 interpreted as predictive. Blank cells indicate risk factors that were not included but retained in the final multivariable Cox model for that particular treatment (ie, risk factors with p≥0.10 in the final multivariable model).
*p<0.05; **p<0.01; ***p<0.001.
BMI, body mass index; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; NSI, non-serious infection; SIE, serious infection event; TNFi, tumour necrosis factor inhibitors.

SIEs, was generally greater in patients aged≥65 vs 50–<65 years; this finding aligns with previous analyses of pooled phase III and LTE studies of tofacitinib-treated patients with RA[22] and pooled phase II–IIIb/IV studies from the tofacitinib clinical programme.[23] The SIE risk was similar between age groups for tofacitinib 5 mg two times per day and adalimumab, but greater in older versus younger patients with tofacitinib 10 mg two times per day.[23] In ORAL Surveillance, an elevated risk of SIEs with tofacitinib 10 mg two times per day versus the other treatment groups was present in both age groups, but was most pronounced among those aged≥65 years. These findings could guide shared decision-making in older patients with RA.

In ORAL Surveillance, incidence of SIEs was greater with both tofacitinib doses (10>5 mg two times per day) versus TNFi. Analyses of real-world data from a 5-year postauthorisation safety study using the US CorEvitas registry reported no significant differences in SIE risk with tofacitinib versus bDMARDs (including both TNFi and non-TNFi agents).[24] Similarly, a real-world US claims database study observed no significant differences in risk of hospital admission for SIE between tofacitinib and a variety of bDMARDs, except for an increased risk with tofacitinib versus etanercept.[12] It is likely that the real-world studies mainly included patients receiving tofacitinib 5 mg two times per day (the approved dose for RA in the USA at the time), which may be why no significant differences in risk of SIEs were observed between tofacitinib and TNFi; this is similar to the results of ORAL Surveillance for tofacitinib 5 mg two times per day versus TNFi. However, ORAL Surveillance differs from these real-world studies with regard to patient selection, study design and the RA treatments compared.

Previous studies have reported variation in the risk of SIEs between individual RA treatments.[9–11] In the current multivariable analysis, patients in North America who received adalimumab had a lower risk of SIEs versus patients in the ROW who received etanercept. It is worth noting, however, that, in simple analyses, a higher crude risk of SIEs was observed for North America versus the ROW for the TNFi group but not for either tofacitinib dose (data not shown). Treatment comparisons across geographical regions are inherently biased; for example, IRs of comorbidities were generally higher in North America versus the ROW.[13]

Risk factors identified for SIEs in ORAL Surveillance were generally similar to those previously reported in an integrated safety analysis of patients with RA receiving tofacitinib[16]; common risk factors included tofacitinib dose, increasing age, male sex, geographical region (Asia and Australia/New Zealand/ROW vs the USA/Canada), corticosteroid use, increasing BMI, chronic lung disease and lymphopenia. The tofacitinib prescribing information requires the monitoring of lymphocyte counts at baseline and every 3 months.[25] Previous analysis also identified history of diabetes as a predictive risk factor for SIEs with tofacitinib in patients with RA.[16] In ORAL Surveillance, history of diabetes was identified as a predictive risk factor for SIEs in the simple but not the multivariable Cox regression analyses; it is possible that history of diabetes was strongly associated with other, more predictive baseline risk factors that were included within the final multivariable model. It should be noted

that only increasing age and baseline opioid use were identified as predictive risk factors for SIEs for both tofacitinib doses when treatment groups were analysed individually. Baseline opioid use was also a risk factor for NSIs across all treatments combined and has previously been reported to increase the risk of infections in patients with RA.[26 27] Other risk factors for NSIs, which have been previously reported in registry data analyses of patients with RA receiving bDMARDs, include female sex and comorbidities.[8]

In agreement with the current findings, real-world studies of patients with RA have consistently reported a greater risk of HZ (non-serious/serious) with JAK inhibitors versus bDMARDs.[12 24 28] For example, real-world US registry and claims database studies of patients with RA reported that HZ risk was twofold higher with tofacitinib versus bDMARDs.[12 24] Previously characterised risk factors for HZ with tofacitinib include increasing age, geographical region (Asia (particularly Japan and Korea) vs the USA/Canada), being a past smoker versus having never smoked, and corticosteroid use.[16] It is noteworthy that geographical region, smoking status and corticosteroid use were not predictive risk factors for HZ in the current study.

A post hoc analysis of phase III studies of patients with RA evaluated the safety of tofacitinib administered as monotherapy or combined with csDMARDs, with or without corticosteroids at baseline.[29] IRs of SIEs and HZ were the greatest in patients receiving tofacitinib combined with csDMARDs along with corticosteroid use at baseline. In ORAL Surveillance, oral corticosteroid use was a predictive risk factor for SIEs but not HZ. The impact of concomitant csDMARDs on IRs of infections was not evaluated in ORAL Surveillance, but it should be noted that all patients received methotrexate at the start of the trial.

A limitation of the current analyses is that ORAL Surveillance was designed to assess non-inferiority of tofacitinib versus TNFi across the primary safety endpoints of adjudicated major adverse CV events and malignancies excluding NMSC; it was not powered to compare infection events across treatment groups. Multiple SIE, NSI and HZ events were reported as separate events if the event start dates differed; it is possible that some subsequent events may have overlapped with the initial event. The Cox regression analyses of risk factors for infections were exploratory in nature; interaction terms among risk factors and between risk factors and treatments were not included in the models, and associations identified between risk factors and events do not imply causality. Backward selection, while commonly used in analysing clinical trial data,[30] may yield a biased relationship between selected covariates and the outcome, and CIs and p values may be underestimated.[31] Further, the stability of the backward selection may be affected by a small number of events in some cases.[30] Some risk factors evaluated in the Cox regression analyses, such as history of inflammatory bowel disease, chronic renal disease and heart failure, were associated with low N values; these results should be interpreted with caution. P values were reported with no adjustment for multiple comparisons, which may have increased the likelihood of false positive findings. Smoking status (eg, years smoked or years since quitting smoking) was not fully characterised. The IRs and risk of infections observed with tofacitinib and TNFi were not compared with that of placebo, csDMARDs or other bDMARDs. The tofacitinib 10 mg two times per day group included data from patients who had their dose reduced from 10 to 5 mg two times per day. Additionally, since TNFi drug (adalimumab or etanercept; not randomly assigned) was confounded by geographical region (North America or ROW), definitive conclusions cannot be made regarding risk of SIEs with tofacitinib versus etanercept or adalimumab, or for etanercept versus adalimumab.

CONCLUSIONS

Results of ORAL Surveillance showed dose-dependent increases in all infections, SIEs and NSIs with tofacitinib versus TNFi in patients aged≥50 years with ≥1 additional CV risk factor. The risk for all infections and SIEs increased with both tofacitinib doses versus TNFi, regardless of age, although an elevated risk with tofacitinib 10 mg two times per day versus 5 mg two times per day and TNFi was most pronounced in patients aged≥65 vs 50–<65 years. The NNH for tofacitinib 5 mg two times per day (recommended dosage for RA) versus TNFi for SIEs was 238 patient-years, meaning that over 5 years of treatment, 48 patients would need to be treated with tofacitinib 5 mg two times per day rather than TNFi to result in one additional SIE. ORAL Surveillance showed higher rates of MACE, malignancies (excluding NMSC) and venous thromboembolic events with tofacitinib versus TNFi (NNH (patient-years) for tofacitinib 5 mg two times per day versus TNFi: 567, 276 and 763 for MACE, malignancies and venous thromboembolic events, respectively, meaning over 5 years of treatment, 113, 55 and 153 patients, respectively, would need to be treated to have one additional event with tofacitinib 5 mg two times per day versus a TNFi).[13 32] The current post hoc analysis revealed a higher risk of NSI and HZ with tofacitinib versus TNFi, and higher risk of SIE with tofacitinib 10 mg two times per day versus TNFi, particularly in patients aged≥65 years. These results should be carefully considered as part of shared decision-making between physicians and patients.

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

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by ORAL Surveillance was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines of the International Council on Harmonisation, and was approved by the Institutional Review Board and/or Independent Ethics Committee at each centre. Participants gave informed consent to participate in the study before taking part.

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CLINICAL SCIENCE

Age-associated B cells contribute to the pathogenesis of rheumatoid arthritis by inducing activation of fibroblast-like synoviocytes via TNF-α-mediated ERK1/2 and JAK-STAT1 pathways

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ABSTRACT

Objectives Age-associated B cells (ABCs) are a recently identified B cell subset, whose expansion has been increasingly linked to the pathogenesis of autoimmune disorders. This study aimed to investigate whether ABCs are involved in the pathogenesis and underlying mechanisms of rheumatoid arthritis (RA).

Methods ABCs were assessed in collagen-induced arthritis (CIA) mice and patients with RA using flow cytometry. Transcriptomic features of RA ABCs were explored using RNA-seq. Primary fibroblast-like synoviocytes (FLS) derived from the synovial tissue of patients with RA were cocultured with ABCs or ABCs-conditioned medium (ABCsCM). IL-6, MMP-1, MMP-3 and MMP-13 levels in the coculture supernatant were detected by ELISA. Signalling pathways related to ABCs-induced FLS activation were examined using western blotting.

Results Increased ABCs levels in the blood, spleen and inflammatory joints of CIA mice were observed. Notably, ABCs were elevated in the blood, synovial fluid and synovial tissue of patients with RA and positively correlated with disease activity. RNA-seq revealed upregulated chemotaxis-related genes in RA ABCs compared with those in naive and memory B cells. Coculture of FLS with RA ABCs or ABCsCM led to an active phenotype of FLS, with increased production of IL-6, MMP-1, MMP-3 and MMP-13. Mechanistically, ABCsCM-derived TNF-α promoted the upregulation of interferon-stimulated genes in FLS, with elevated phosphorylation of ERK1/2 and STAT1. Furthermore, blockage of ERK1/2 and Janus Kinase (JAK)-STAT1 pathways inhibited the activation of FLS induced by ABCsCM.

Conclusions Our results suggest that ABCs contribute to the pathogenesis of RA by inducing the activation of FLS via TNF-α-mediated ERK1/2 and JAK-STAT1 pathways.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Age-associated B cells (ABCs) are a novel subset of B cells and its aberrant expansion has been linked to the pathogenesis of autoimmune diseases, such as systemic lupus erythematosus. However, little is known about the role of ABCs in rheumatoid arthritis (RA), despite sporadic reports on increased ABCs in RA.

WHAT THIS STUDY ADDS

⇒ The findings of this study indicate that RA ABCs display distinct transcriptomic properties that may impact their ability to migrate into the inflammatory joints of patients with RA, where they contribute to the pathogenesis of RA by inducing fibroblast-like synoviocytes activation via the TNF-α-mediated ERK1/2 and Janus Kinase-STAT1 pathways.

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

⇒ These findings demonstrate the pathogenic role of ABCs in RA, suggesting that targeting specific B cell subsets rather than pan-B cells might be a more promising strategy for the treatment of RA in the future.

INTRODUCTION

Persistent synovitis is one of the clinical features of rheumatoid arthritis (RA), the most common systemic autoimmune disease affecting approximately 1% of the world's population.[1] Although treat-to-target strategies have been intensively applied in the treatment of RA in the past decade, clinical unmet needs still exist because a substantial proportion of patients are resistant or refractory to current therapies.[2] Hence, a better understanding of the pathogenesis is crucial for the development of novel targeted therapies.

The interplay between immune cells and stromal cells in the joint, especially in the crosstalk between fibroblast-like synoviocytes (FLS) and T cells, is considered to play a central role in driving RA progression.[3 4] However, the role of B cells and their interaction with FLS has been studied less, despite the presence of rheumatoid factor (RF) and anticitrullinated protein antibodies in the serum of patients with RA implicating the involvement of B cells.[5–7] B cell-targeted therapy using anti-CD20 monoclonal antibodies has been proven to be effective in the treatment of patients with moderate to severe RA.[8] However, depletion of B cells increases the risk of severe infections.[9] Hence, targeting specific pathogenic B cell subsets rather than pan-B cells would be a more promising strategy.

In recent years, a newly identified B cell subset, named age-associated B cells (ABCs), was found to accumulate in the spleens of aged and lupus mice.[10 11] Unlike follicular and marginal zone B cells, ABCs were characterised by the expression of myeloid markers like CD11c, CD11b and transcription factor (TF) T-bet.[12] ABCs were demonstrated to produce antichromatin antibodies on stimulation, in vitro.[10] Conditional deletion of T-bet from B cells alleviated kidney damage and improved mortality in lupus mice.[13] The expansion of human ABCs has been observed in many autoimmune diseases, including systemic lupus erythematosus (SLE), Sjogren's syndrome and multiple sclerosis.[14–18] Furthermore, ABCs are the primary source of autoantibodies in SLE, and their frequency is correlated with disease activity, suggesting their pathogenic role in the development of SLE.[14 16] The role of ABCs in RA still remains largely unknown, although few studies reported increased ABCs in the blood and synovium of patients with RA.[10 16 19]

To investigate the involvement of ABCs in the pathogenesis of RA, we first examined the presence of ABCs in collagen-induced arthritis (CIA), a well-validated and widely used mouse model for RA. The development of CIA was initiated by priming of collagen-II-specific $CD4^+$ T cells and perpetuated by T follicular helper (Tfh) cells, which further stimulated B cells for antibody production. Additionally, involvement of other immune cells, including plasmacytoid dendritic cells, macrophages and synovial fibroblasts, make it a powerful tool for studying the molecular and cellular processes in the immunopathogenesis of RA.[20] We showed that ABCs were expanded in the blood, spleen and joints of CIA mice compared with wild-type unimmunised controls. In humans, ABCs were more elevated in the circulation and synovial fluid of patients with active RA, than in patients with osteoarthritis (OA), spondyloarthritis (SpA) and healthy individuals. At the transcriptome level, chemotaxis-related genes were upregulated in ABCs from patients with RA. We further delineated the function of ABCs through in vitro coculture with FLS and explored the underlying molecular mechanisms. Altogether, our findings suggest a distinct feature and role of ABCs in the pathogenesis of RA. Hence, targeting ABCs rather than pan-B cells may have therapeutic benefits in RA in the future.

METHODS

See online supplemental material.

RESULTS

Expansion of ABCs in CIA mice

ABCs have been reported to be expanded in autoimmune-prone mice, such as spontaneous lupus mice, indicating their pathogenic role in the development of disease.[10 13] To evaluate the distribution of ABCs in arthritic mice, we assessed ABCs in the blood, spleen and joints of CIA mice on day 42 by flow cytometry. Consistent with previous studies,[21 22] mouse ABCs were defined as $B220^+CD11c^+T\text{-}bet^+$ (online supplemental figure S1A). We found that the proportion and number of ABCs increased in the spleen and blood of CIA mice compared with WT controls (figure 1A,B). The number of ABCs in the joints was also higher in CIA mice than that in WT mice, although the proportion difference was not statistically significant ($p=0.09$; figure 1A,B). Thus, we showed that ABCs were expanded in arthritic mice, not only in the spleen and peripheral blood, but also in inflamed joints.

IL-21 and TLR7 promote ABCs differentiation in CIA mice

It has been reported that ABCs can be generated by stimulating IL-21 and TLRs.[11 23 24] Thus, we investigated whether these signals can drive ABCs differentiation in arthritic mice. As expected, the addition of IL-21 led to a significantly higher proportion of $CD11c^+T\text{-}bet^+$ ABCs in cultures of CIA-derived spleen $CD23^+$ B cells than in those from healthy mice (online supplemental figure S1B,C). However, addition of the TLR7 agonist imiquimod alone did not affect their differentiation (data not shown). Notably, the stimulation by the combination of IL-21 and TLR7 resulted in higher production of ABCs than that by IL-21 alone (online supplemental figure S1B,C). Consistently, the expression of CD11c and T-bet, which was assessed by MFI, was elevated in $CD23^+$ B cells from CIA mice compared with WT controls after IL-21 stimulation (online supplemental figure S1D–F). Altogether, B cells from CIA mice exhibited an increased ability to generate ABCs in vitro after exposure to IL-21 and TLR7 signalling.

ABCs present with proinflammatory phenotype in patients with RA

Next, to confirm the presence of ABCs in RA and to explore their phenotypic features, we assessed ABCs in the peripheral blood of patients with RA. As previously reported, human ABCs were defined as $CD19^+CD27^-IgD^-CD21^-CD11c^+$ (figure 2A and online supplemental figure S2A).[25] Compared with $CD11c^-$ B cells, ABCs from patients with RA displayed significantly higher expression of the TF T-bet, which has been described in both mice and humans (figure 2B).[14–16 21 24 25] Similarly, higher expression of CD86 and MHC-II, but not of CD80, was observed in RA ABCs (figure 2B), consistent with previous findings in lupus mice.[21] For plasma cell markers, there was no significant upregulation of BCMA and CD138 expression in ABCs (figure 2B). Since IL-4 signalling has been reported to downregulate ABCs formation in vitro,[23] we investigated IL-4 and IL-13 receptor expression in ABCs. Interestingly, IL-13Rα1 expression was upregulated in ABCs, compared with $CD11c^-$ B cells; whereas, IL-4Rα and IL-13Rα2 expressions were comparable between the two populations (figure 2B), which is consistent with our recent findings in mice.[26]

To understand how ABCs from healthy controls (although lower in number) compared with those present in RA, phenotypic marker expression in healthy individuals was examined. As shown in online supplemental figure S2B, the expression patterns of all markers examined by flow cytometry were comparable, suggesting similarities of these cells in healthy individuals.

To further assess the phenotypic features of ABCs related to their biological behaviours, we sorted ABCs and $CD11c^-$ B cells from patients with RA and examined the expression levels of several cytokines by qPCR. Pro-inflammatory cytokines, such as IL-21, IL-17A and TNFα, excluding IFN-γ, were elevated in ABCs (figure 2C), indicating that these cells present with a proinflammatory phenotype. Interestingly, IL-4 expression was also higher in ABCs than in $CD11c^-$ B cells (figure 2C).

Abcs increased in patients with RA and correlated with disease activity

Further inspection of ABCs in patients with RA showed that the number and percentage of ABCs in patients with RA were higher than in those with SpA, OA and healthy controls (figure 3A and online supplemental figure S3A). Of note, ABCs frequencies were higher in patients with active RA, whose Disease Activity Score-28 (DAS28) was higher than 3.2 (figure 3B and online

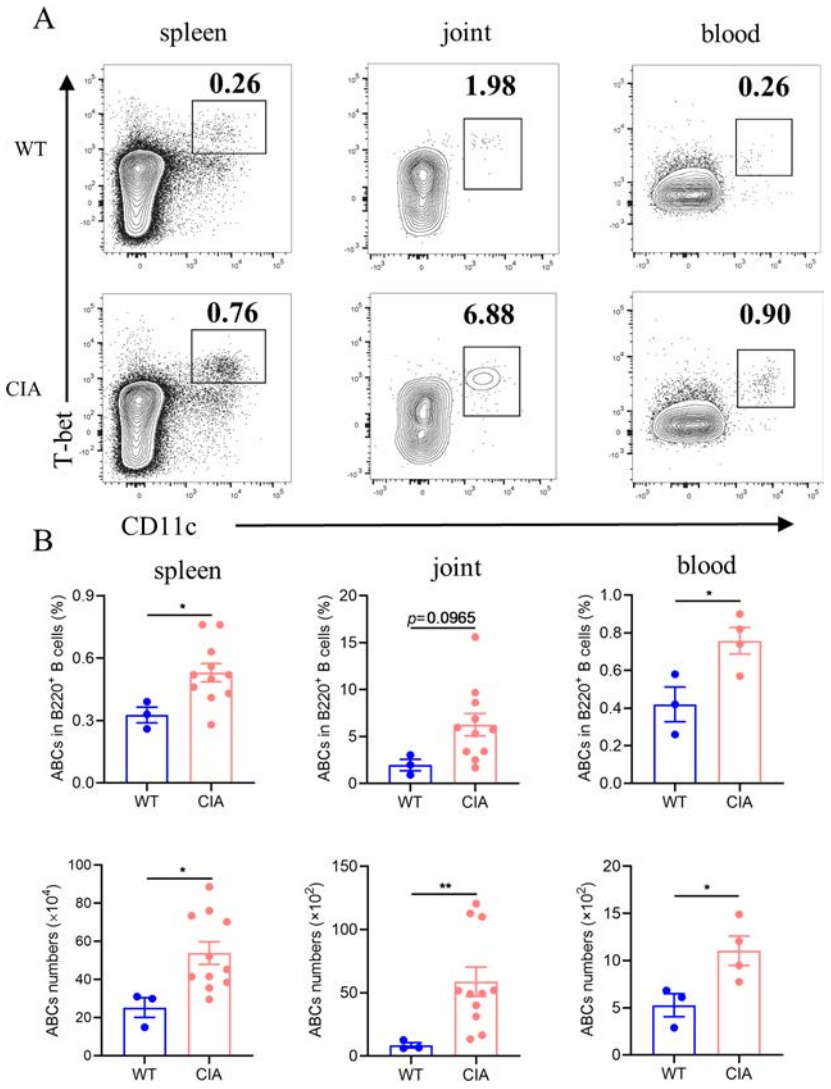

Figure 1 ABCs are expanded in CIA mice. (A, B) Representative FACS plots (A) and quantification (B) of CD11c+T-bet+ABCs in the spleen, joint and blood of WT and CIA mice on day 42. All data are representative of 2–3 independent experiments. Data are presented as mean±SEM. Statistical significances are determined by Student's t-test, *p<0.05, **p<0.01. ABCs, age-associated B cells; CIA, collagen-induced arthritis; WT, wild-type.

supplemental figure S3B). Consistently, there was a positive correlation between ABCs and clinical parameters such as tender joint count, swollen joint count and DAS28, suggesting their association with the pathogenesis of the disease (figure 3C and online supplemental figure S3C). We further stratified patients with RA according to the presence of RF and anti-cyclic citrullinated peptide (CCP) in the serum. Interestingly, anti-CCP positive patients had higher ABCs than anti-CCP negative patients, whereas comparable numbers of ABCs were observed between RF positive and negative patients (figure 3D,E and online supplemental figure S3D,E). Contrary to a previous study that showed a positive correlation between ABCs and age,[10] we did not find any correlation between the number of ABCs and age, sex or disease duration (online supplemental figure S3F–H). Next, we tested whether ABCs could also be found in the inflammatory sites of patients with RA. Impressively, the proportion of ABCs in the synovial fluid was more than 10 times higher than that in the blood of the same patients (figure 3F). Moreover, $CD20^+$C-$D11c^+$ABCs were present in the synovial tissue of patients with RA (figure 3G), indicating that these cells may have the capacity to migrate into inflammatory tissues.

To explore the potential impact of medications on ABCs, we grouped the patients based on different treatments received. However, comparable numbers of ABCs were observed among patients receiving different treatments, including corticosteroids, conventional synthetic disease-modifying antirheumatic drugs (csDMARDs,such as methotrexate, leflunomide, hydroxychloroquine, sulfasalazine) and biological agents (online supplemental figure S3I–K).

Recently, a novel peripheral helper T cell subset (T_{PH}), has been identified to be expanded in patients with RA.[19 27] Importantly, T_{PH} cells could induce the differentiation of B cells into plasma cells through the secretion of IL-21,[27 28] a cytokine that has been confirmed to be a key regulator in ABCs generation. Thus, we assessed the association between ABCs and T_{PH} cells in patients with RA. We confirmed that the frequency of T_{PH} cells was elevated in the circulation of patients with RA compared with SpA and healthy controls (figure 3H). In addition, the proportion and number of ABCs positively correlated with T_{PH} cells (figure 3I and online supplemental figure S3L). A recent study showed that synovial T_{PH} cells skewed B cell differentiation towards the ABCs phenotype in vitro by provision of IL-21

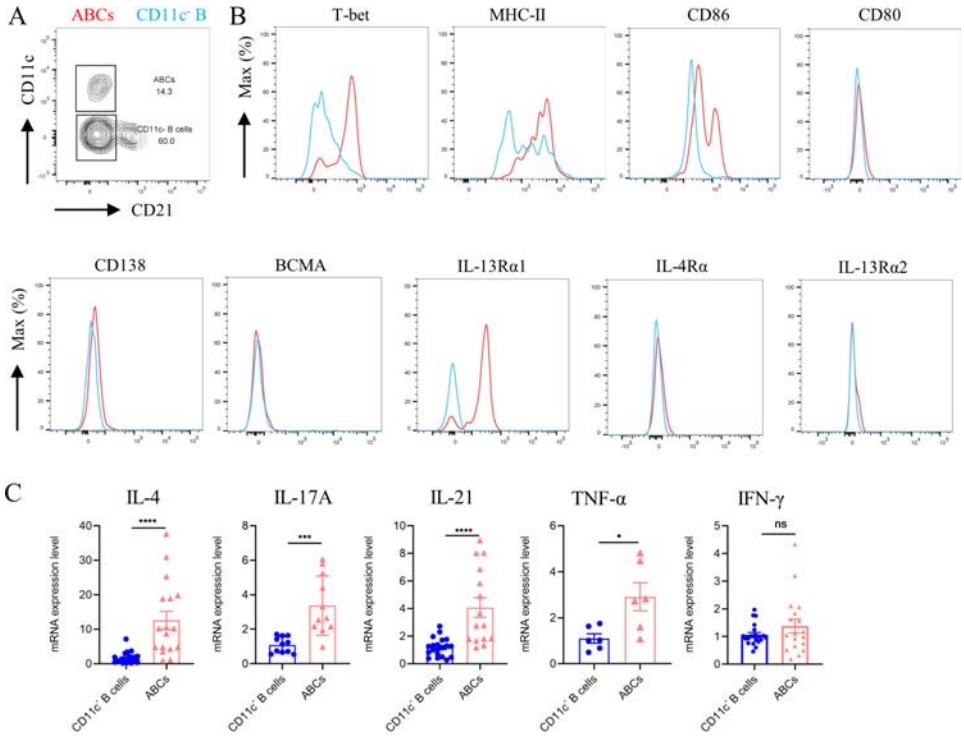

Figure 2 Phenotypic features of ABCs in patients with RA. (A) Representative FACS plot of ABCs from blood of patients with RA. (B) Representative histogram plot of T-bet, MHC-II, CD86, CD80, CD138, BCMA, IL-13Rα1, IL-4Rα and IL-13Rα2 expression on CD11c$^-$ B cells (blue line) and ABCs (red line). (C) mRNA expression of cytokines in ABCs and CD11c$^-$ B cells. Data are presented as mean±SEM. Statistical significances are determined by Student's t test, *p<0.05, ***p<0.001, ****p<0.0001. ABCs, age-associated B cells; n.s, no significance; RA, rheumatoid arthritis.

and IFN-γ,[29] it is reasonable to speculate that expanded T_{PH} cells might promote B cell differentiation into ABCs in patients with RA.

Transcriptome analyses reveal distinct features of ABCs

To better understand how RA ABCs are related to naive vs memory B cells, we sorted B cells based on the expression of CD19, CD27 and CD11c, and employed RNA-based sequencing (RNA-seq) to compare the transcriptomes of naive B cells ($CD19^+CD11c^-CD27^-$), memory B cells ($CD19^+CD11c^-CD27^+$) and ABCs ($CD19^+CD27^-CD11c^+$). As shown in the Venn diagram, ABCs showed different gene expression patterns compared with both naive and memory B cells (figure 4A). Overall, 1505 genes were upregulated and 498 genes were downregulated in ABCs compared with naive B cells. In contrast, ABCs and memory B cells displayed a similar transcriptional profile, with only 283 genes upregulated and 153 genes downregulated in ABCs compared with memory B cells (fold change>2, false discovery rate (FDR)<0.05; online supplemental figure S4A). Interestingly, most of the upregulated genes (198/283) in ABCs-vs-memory B cells were also upregulated in ABCs-vs- naive B cells, indicating that these genes are ABCs-specific. Consistent with previous findings in SLE, some key phenotypic markers of ABCs were confirmed by mRNA expression, such as elevated expression of *ITGAX* (CD11c), *TBX21* (T-bet), *FCRL5*, *IL-21R* and decreased expression of *CD27* and *CD38* (data not shown). However, we did not find upregulated expression of plasma cell markers, such as *PRDM1* and *XBP1*, although ABCs in SLE were identified as precursors of plasma cells.[16] Next, we mapped DEGs to known gene ontology biological processes via over-representation analysis. Interestingly, neutrophil activation, leucocyte migration and adhesion-related genes were significantly upregulated in ABCs compared with naive B cells (figure 4B,C). Similar results were observed when comparing ABCs with memory B cells (online supplemental figure S4B,C). We further confirmed the upregulated expression of CCR2 and CXCR3 in ABCs using qPCR (figure 4D). Considering that ABCs are present and expanded in the inflammatory joints of patients with RA, these data revealed distinct features of ABCs in RA and suggested that circulating ABCs are capable of migrating to the inflammatory joint to contribute to the pathogenesis of the disease.

ABCs induce activation of FLS through cell contact-independent way

It is well known that FLS and their interaction with immune cells play a central role in the progression of synovitis in RA.[30] For a long time, researchers have focused on the reciprocal relationship between T cells, especially $CD4^+$ T cells and FLS, whereas the crosstalk between B cells and FLS is less known. Recently, one study demonstrated that TNFα stimulation increased the ability of B cells to adhere to FLS.[31] To determine whether ABCs could interact with FLS in vitro, we sorted ABCs from patients with RA and cocultured them with FLS for 3 days. FLS derived from the synovial tissue of patients with RA were confirmed by their morphology and CD55 expression (online supplemental figure S5A,B). First, we examined the expression of ICAM-1 and VCAM-1 in FLS by flow cytometry, since the latter has been shown to contribute to B cell adhesion to FLS.[32] Similar to the findings that demonstrated the mutual effects between $CD4^+$ T cells and FLS,[33] ICAM-1 and VCAM-1 were found to be upregulated in FLS in the presence of ABCs, indicating the activation of FLS (figure 5A). Next, we measured IL-6 and MMPs levels in the supernatant, which are the hallmarks of FLS activation. Of note, the levels of IL-6, MMP-1, MMP-3 and MMP-13 were significantly elevated in the supernatant from FLS cocultured with

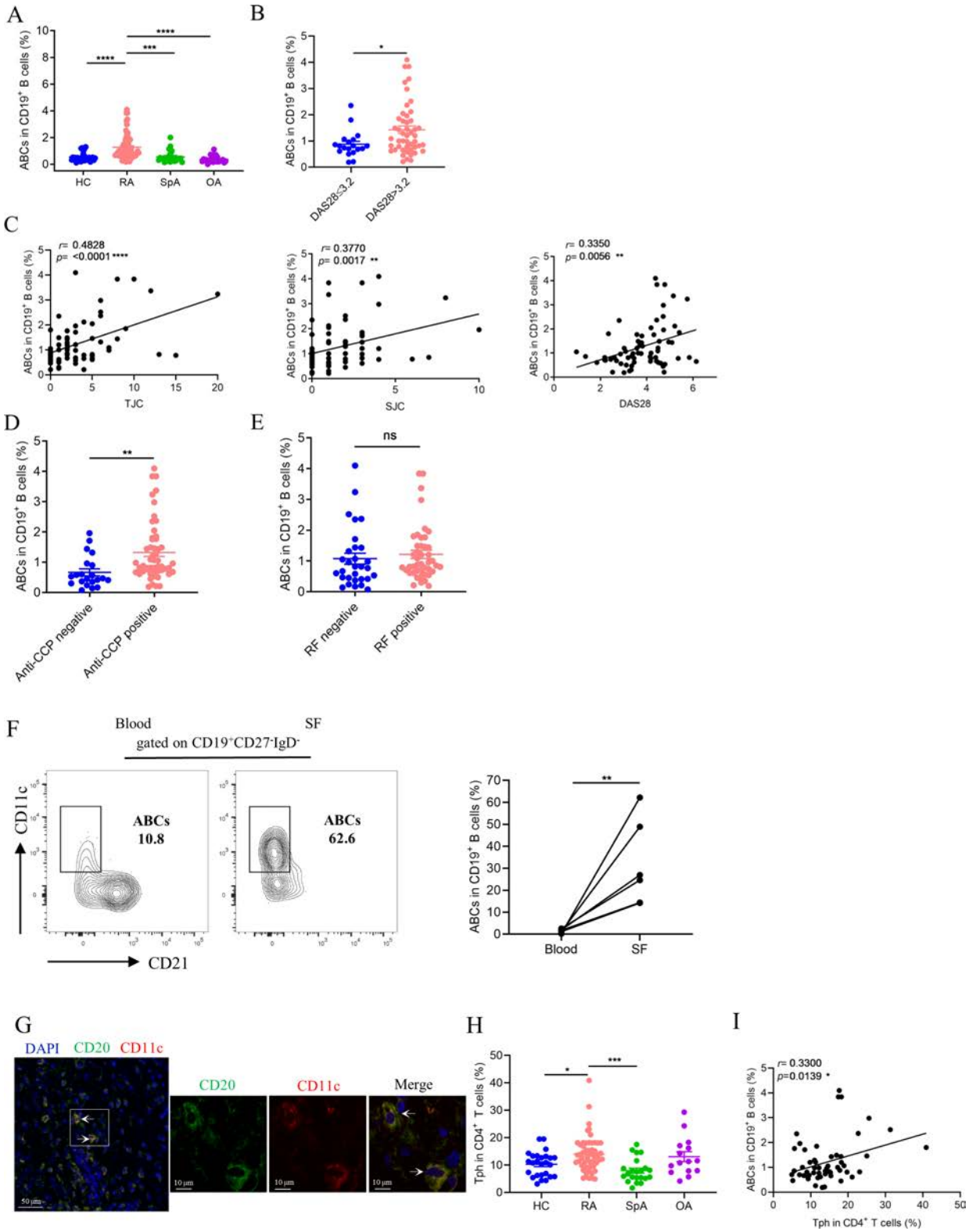

Figure 3 ABCs increased in patients with RA and correlated with disease activity. (A) Proportion of ABCs in RA (n=67), healthy control (HC, n=29), spa (n=23) and OA patients (n=25). (B) Proportion of ABCs in patients with RA grouped by DAS28. (C) Correlation between the proportion of ABCs and TJC, SJC and DAS28. (D, E) Proportion of ABCs in RA with positive or negative anti-CCP (D) and RF (E). (F) Representative FACS plots and quantification of ABCs in blood and synovial fluid of patients with RA. (G) Costaining of CD20 and CD11c in the synovial tissue of patients with RA by immunofluorescence. Green shows CD20, red shows CD11c, and yellow indicates double positive cells. (H) The proportion of Tph in patients with RA, HC, SpA and OA. (I) Correlation between the proportion of Tph among $CD4^+$ T cells and ABCs among $CD19^+$ B cells. All data are representative of 2–3 independent experiments. Data are presented as mean±SEM. Statistical significances are determined by one-way ANOVA followed by Bonferroni's multiple-comparisons test for multi-group comparisons or Student's t-test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. ABCs, age-associated B cells; ANOVA, analysis of variance; CCP, cyclic citrullinated peptide; n.s, no significance; OA, osteoarthritis; RA, rheumatoid arthritis; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count.

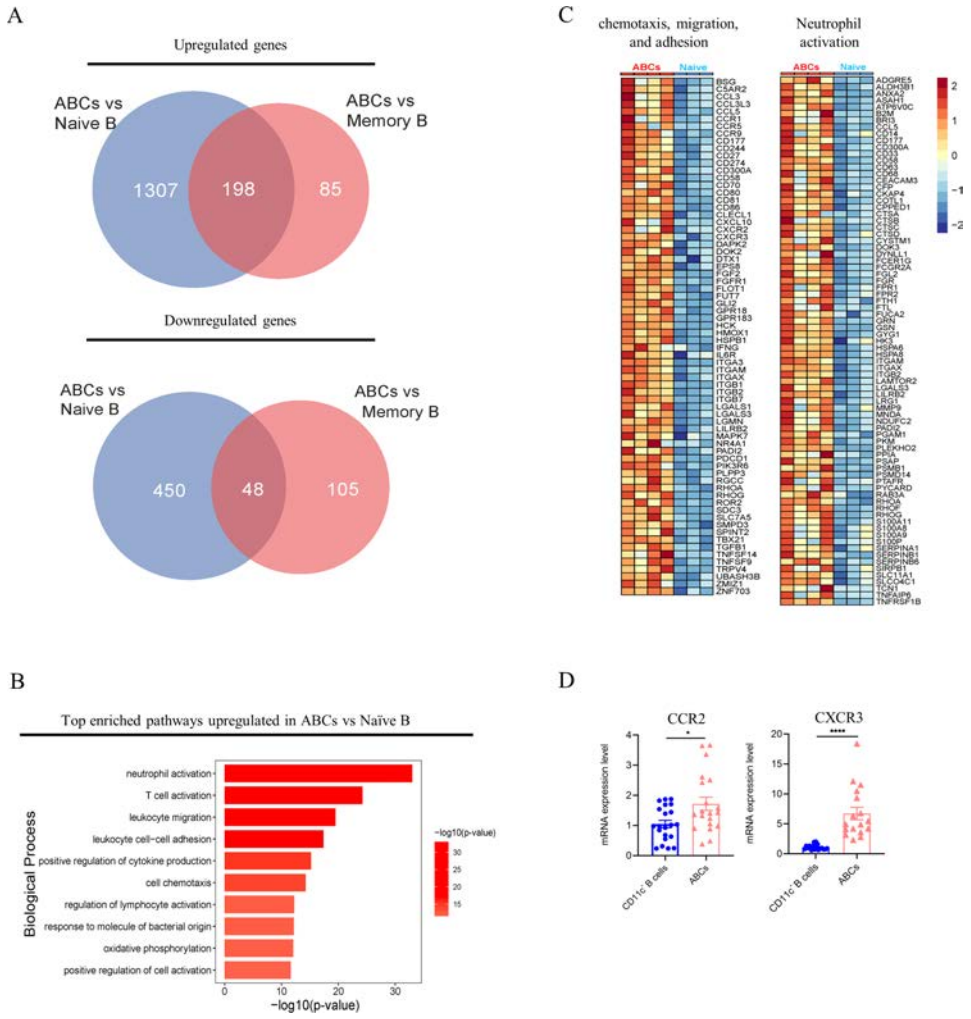

Figure 4 Transcriptome profile of ABCs in RA. (A) Venn diagram showing upregulated and downregulated genes of ABCs compared with naïve and memory B cells. (B) Bar chart showing top enriched pathways in upregulated genes of ABCs compared with naïve B cells. (C) Heatmap showing genes that are relevant to chemotaxis, migration, adhesion and neutrophil activation. (D) The mRNA expression of CCR2 and CXCR3 on ABCs compared with CD11c$^-$ B cells. Data are presented as mean±SEM. Statistical significances are determined by student's t-test, *p<0.05, ****p<0.0001. ABCs, age-associated B cells; RA, rheumatoid arthritis.

ABCs, compared with FLS alone and cocultured with CD11c$^-$ B cells (figure 5B). To further delineate whether the activation of FLS induced by ABCs was cell contact-dependent, we treated FLS with ABCs-conditioned medium (ABCsCM). Interestingly, comparable amounts of IL-6 and MMPs production were observed between ABCsCM and ABCs cocultured with FLS, suggesting that cell contact is dispensable for activation of FLS by ABCs (figure 5C). Hence, ABCsCM was used to replace ABCs in the coculture experiments for further mechanistic studies.

ABCsCM-derived TNF-α promotes the upregulation of interferon stimulated genes in FLS

The above data revealed that ABCs act on FLS in a cell contact-independent manner to induce an activated and aggressive phenotype through upregulated adhesion molecules and augmented proinflammatory cytokine production. To investigate FLS-activating mediators derived from ABCs, we quantified several inflammatory cytokines and interferons (IFNs) in ABCsCM using a cytometric bead array. Interestingly, TNF-α and IL-1β levels were significantly higher in ABCsCM, whereas IL-17A, IFN-α and IFN-γ levels were comparable between ABCsCM and naive B cell media (figure 5D). The addition of anti-TNF-α downregulated the production of IL-6, MMP-1, MMP-3 and MMP-13, while the addition of anti-IL-1β antibody did not, indicating that ABCsCM-derived TNF-α was responsible for the activation of FLS (figure 5E).

To further explore the mechanisms underlying FLS activation, we performed RNA-seq to compare the transcriptomes of FLS treated with or without ABCsCM. As seen in figure 6A, a total of 144 genes were significantly upregulated, whereas three genes were downregulated in FLS treated with ABCsCM compared with FLS alone (fold change >2, FDR<0.05). Interestingly, enriched pathways upregulated in ABCsCM-treated FLS included those related to IFN signalling (type I and type II IFN signalling) (figure 6B–D). This was consistent with a previous study which demonstrated that TNF-induced chemokine expression in FLS was dependent on type I IFN.[34]

To predict the TF that may target DEGs, we performed TF enrichment analysis based on known target genes in the MsigDB gene sets. Among the top 10 enriched TFs, *STAT1*, *IRF1* and *IRF7* were significantly upregulated in ABCsCM-treated FLS compared with FLS alone (figure 7A). Subsequently, protein–protein interaction (PPI) analysis was conducted, and the top 15 ranked genes with the highest degrees were regarded as hub

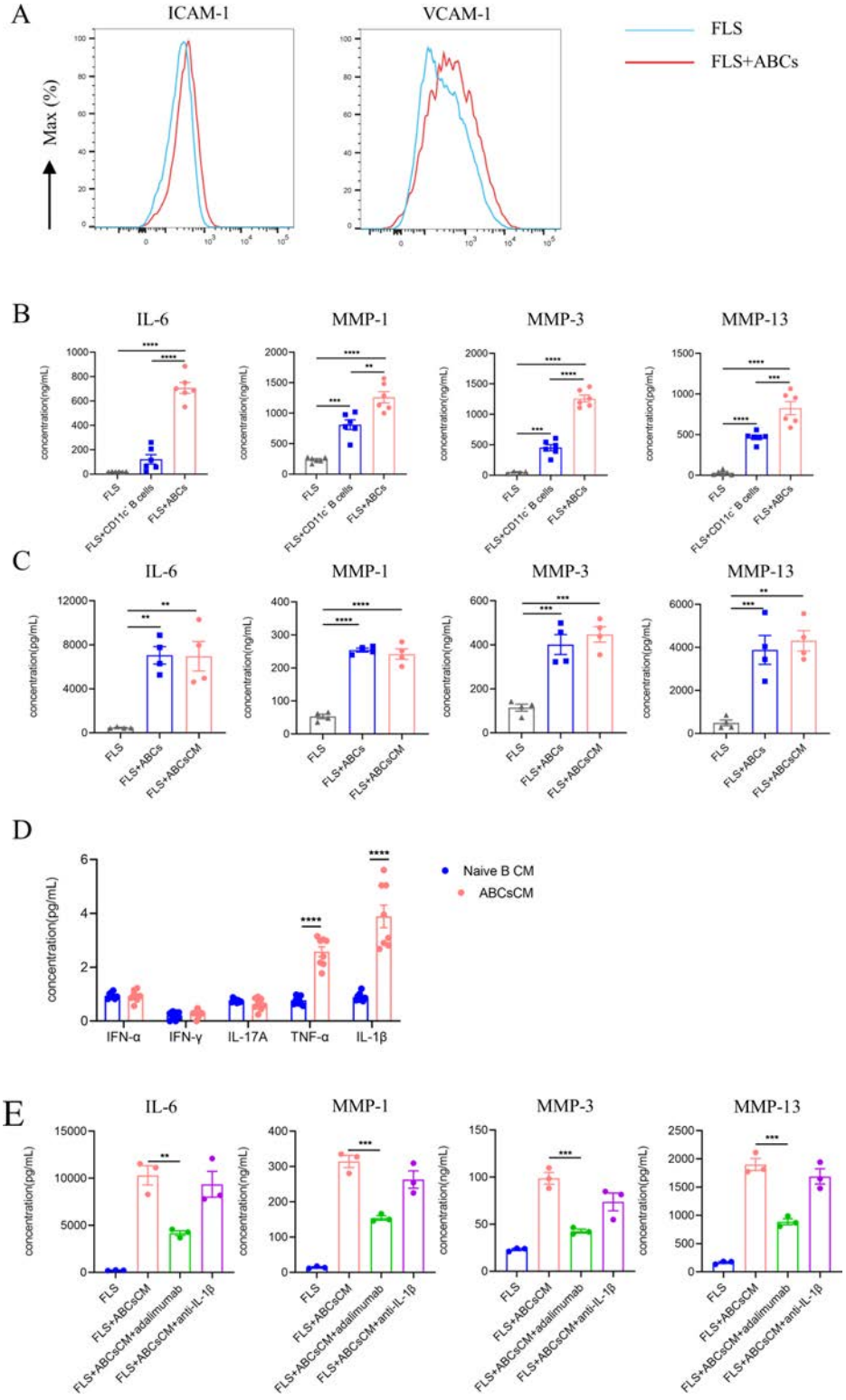

Figure 5 ABCs-derived TNF-α induce the activation of FLS. (A) Representative histogram plot of ICAM-1 and VCAM-1 expression on FLS treated with ABCsCM or FLS alone. (B) IL-6, MMP-1, MMP-3 and MMP-13 levels in the supernatant of FLS, FLS+CD11C⁻B and FLS+ABCs. (C) IL-6, MMP-1, MMP-3 and MMP-13 levels in the supernatant of FLS, FLS+ABCs and FLS+ABCsCM. (D) IFN-α, IFN-γ, TNF-α, IL-17A and IL-1β levels in the ABCsCM and naive B cells medium detected by cytometric bead array. (E) IL-6, MMP-1, MMP-3 and MMP-13 levels in the supernatant of FLS, FLS+ABCsCM, FLS+ABCsCM+adalimumab and FLS+ABCsCM+anti-IL-1β antibodies. Data are presented as mean±SEM. Statistical significances are determined by one-way ANOVA followed by Bonferroni's multiple-comparisons test for multi-group comparisons, **p<0.01, ***p<0.001, ****p<0.0001. ABCs, age-associated B cells; ABCsCM, ABCs-conditioned medium; ANOVA, analysis of variance; FLS, fibroblast-like synoviocytes.

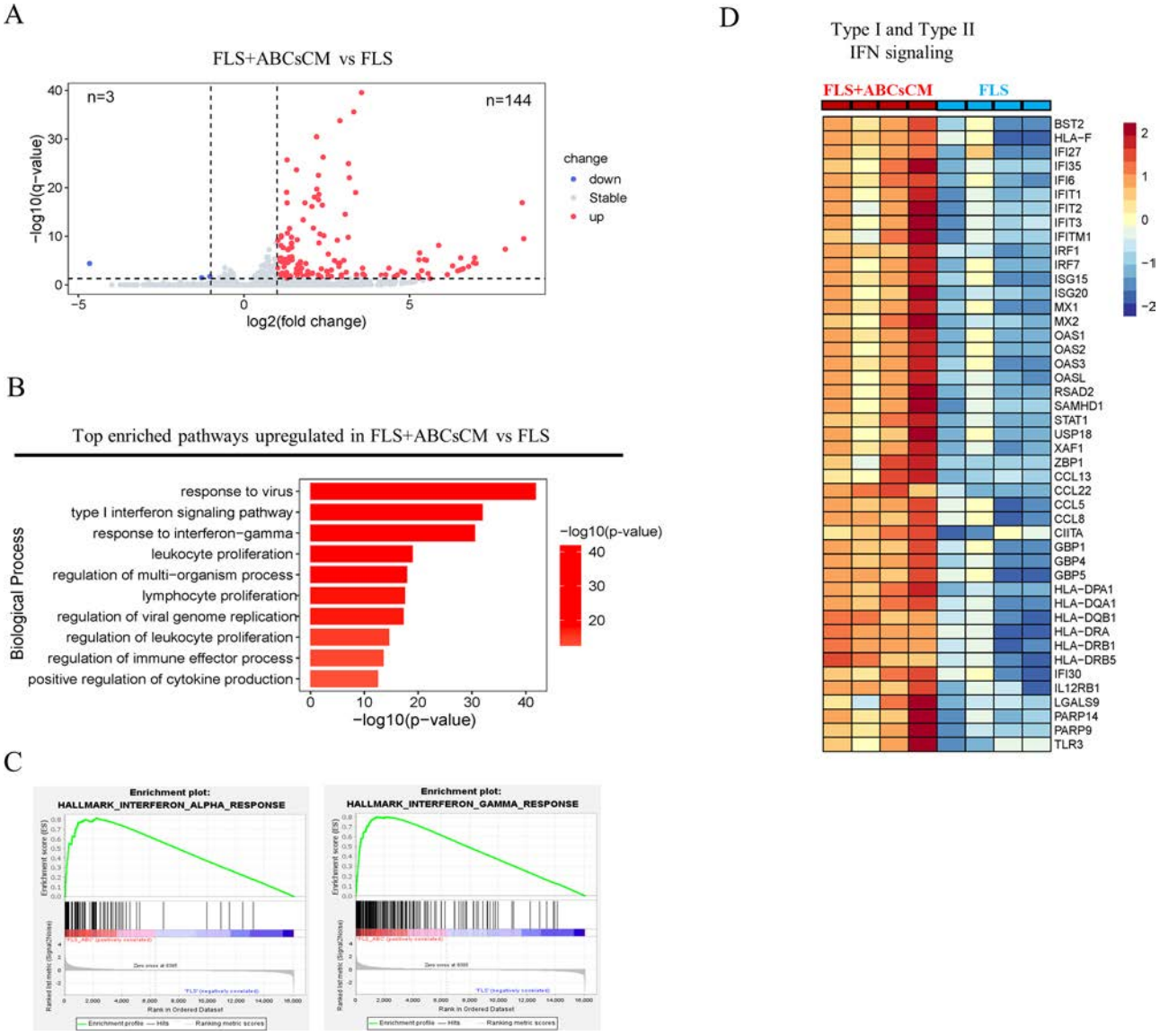

Figure 6 ABCs promote the upregulation of interferon stimulated genes in FLS. (A) volcano plot showing differentially expressed genes with significant upregulation (fold change >2; FDR<0.05) or downregulation (fold change <0.5; FDR<0.05) in cocultured FLS compared with FLS alone. (B) Bar chart showing top enriched pathways in upregulated gene of cocultured FLS compared with FLS alone. (C) Gene set enrichment analysis (GSEA) showing interferon alpha and gamma response pathways enriched in cocultured FLS compared with FLS alone. (D) Heatmap showing genes that are relevant to type I and type II IFN signalling. ABCs, age-associated B cells; ABCsCM, ABCs-conditioned medium; FLS, fibroblast-like synoviocytes.

genes, including *STAT1*, *IRF7* and *IRF1* (figure 7B). We ranked genes based on TF prediction, PPI and p value of differentially expressed analysis, and finally chose *STAT1* as the top-ranked gene for further confirmation.

Blockage of ERK1/2 and Janus kinase-STAT1 pathway prevents ABCs-induced FLS activation

Based on the above results, we futher verified whether STAT1 was induced in ABCsCM-treated FLS. As shown in online supplemental figure S6A, the expression of phosphorylated STAT1 increased 15 min after stimulation with ABCsCM, and this increase could be reduced by anti-TNF-α antibody (figure 7C and online supplemental figure S6A). Notably, inhibition of STAT1 with a specific inhibitor decreased the levels of MMP-1, MMP-3 and MMP-13 in the supernatant to half of those in the FLS +ABCsCM group, while IL-6 production was not affected, suggesting that STAT1 partially regulates MMP1, MMP3 and MMP13, but not IL-6 (figure 7D). This could be explained by previous studies demonstrating that STAT1 phosphorylation occurs in response to IL-6 signalling.[34 35] To further assess whether Janus kinase (JAK) controls the activation of FLS induced by ABCsCM, we repeated the experiment in the presence of a JAK inhibitor, Baricitinib. As expected, treatment with the JAK inhibitor robustly inhibited the expression of phosphorylated STAT1 as well as the production of IL-6, MMP-1, MMP-3 and MMP-13 (figure 7D,E).

Other pathways, such as MAPK, have been reported to regulate MMP production in FLS.[36] ABCsCM treatment induced pERK1/2 in FLS, and this induction was downregulated after adding neutralising TNF-α antibody (figure 7C and online supplemental figure S6B). Meanwhile, inhibition of pERK1/2 significantly reduced the production of MMP-1, MMP-3, MMP-13 and IL-6 (figure 7F), suggesting that ABCsCM-derived TNF-α-induced pERK1/2 regulates the production of IL-6, MMP1, MMP-3 and MMP-13.

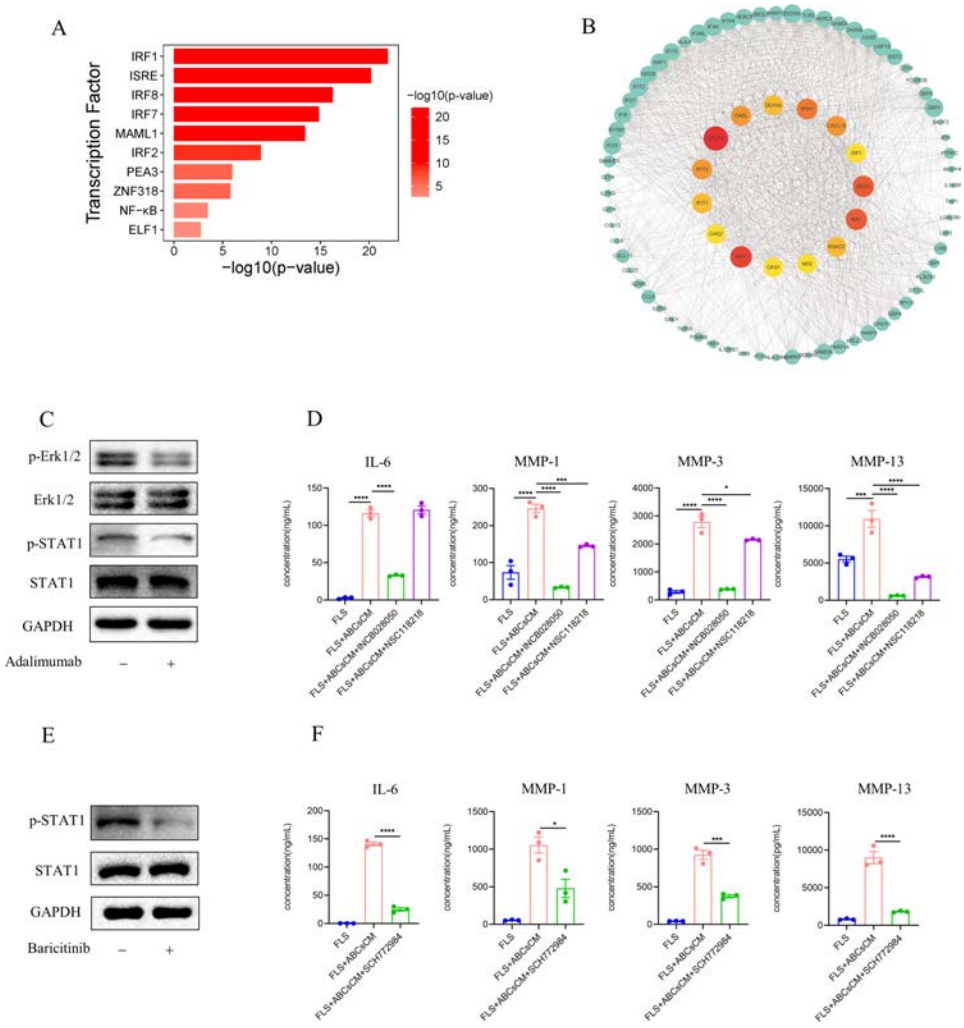

Figure 7 Blockage of ERK1/2 and JAK-STAT1 pathway prevents ABCs-induced FLS activation. (A) Transcription factor prediction based on known target genes in MsigDB gene sets. (B) Protein–protein interaction analysis of genes upregulated in FLS treated with ABCsCM compared with FLS alone. (C) Western blot analysis of STAT1, ERK1/2, STAT1 phosphorylation and ERK1/2 phosphorylation in FLS treated with ABCsCM in the presence or absence of adalimumab. (D) Level of IL-6, MMP-1, MMP-3 and MMP-13 in the supernatant of FLS alone, ABCsCM treated FLS, ABCsCM treated FLS with addition of 10 μM Baricitinib (INCB028050), and ABCsCM treated FLS with addition of 10 μM fludarabine (NSC118218). (E) Western blot analysis of STAT1 and STAT1 phosphorylation in FLS treated with ABCsCM, in the presence or absence of 10 μM Baricitinib. (F) Level of IL-6, MMP-1, MMP-3 and MMP-13 in the supernatant of FLS alone, ABCsCM treated FLS and ABCsCM treated FLS with addition of 10 μM ERK1/2 inhibitor (SCH772984). Data are presented as mean±SEM. Statistical significances are determined by one-way ANOVA followed by Bonferroni's multiple-comparisons test for multigroup comparisons, *p<0.05, ***p<0.001, ****p<0.0001. ABCs, age-associated B cells; ABCsCM; ABCs-conditioned medium; ANOVA, analysis of variance; FLS, fibroblast-like synoviocytes; JAK, Janus Kinase.

Altogether, our results suggested that ABCs induce the activation of FLS via the TNF-α-mediated ERK1/2 and JAK-STAT1 pathways.

DISCUSSION

ABCs, also known as double-negative 2 (DN2) B cells or activated naive B cells,[14 37] are an emerging B cell subset whose accumulation has been increasingly linked to viral infections and systemic autoimmune diseases like SLE.[12 38] However, not much is known about the role of ABCs in RA. Here, we showed that ABCs were increased in CIA mice, as well as in the circulation and inflammatory joints of patients with RA. Transcriptome analyses revealed a distinct transcriptional profile with upregulated chemotaxis and migration-related gene expression in RA ABCs. This was consistent with previous studies, which reported that ABCs from both mice and humans tend to upregulate specific chemokine receptor profiles and unique adhesive programmes necessary for migration into target tissues.[10 16 21 39 40] Actually, we found the frequency of ABCs was more than 10 times higher in the synovial fluid than in peripheral blood, indicating that they are recruited to the inflammatory joints from circulation. This notion is supported by a recent study which demonstrated that ABCs were expanded in the synovium of patients with RA.[19] Further, we showed that circulating ABCs were positively correlated with disease activity, similar to the findings in SLE.[16] Although comparable ABCs were noted among patients with RA who received different treatments, we could not exclude the possibility that medication might have impacted the ABCs since it is a cross-sectional study. Taken together, these data suggest that ABCs may act as major contributors in the pathogenesis of RA and may be a sensitive indicator in monitoring disease activity.

The formation of ABCs is regulated by both innate and adaptive signals.[41] Early studies in the murine system have revealed

a critical role for innate signals, TLR7 and TLR9 engagement, in promoting the differentiation and generation of ABCs.[10 11] In addition to innate stimuli, cytokines such as IL-21 and IFN-γ have been believed to play important roles in controlling ABCs generation in both mouse and human.[21 23 42–44] Very recently, one study substantiated the essential role of BCR and T cell-derived IL-21 in the in vivo expansion of ABCs by studying patients with defined inborn errors of immunity,[44] suggesting inflammatory settings in vivo are also involved in regulating ABCs differentiation. In our study, we found that costimulation with IL-21 and TLR7 agonist drives more ABCs generation in CIA mice than in non-arthritic controls. Interestingly, we found that the number of ABCs in patients is positively correlated with the number of peripheral T_{PH}, a distinct helper T cell subset that has been identified to promote B cell differentiation into plasma cells via production of IL-21.[27 28] This is consistent with a recent finding in juvenile idiopathic arthritis (JIA), which demonstrated the expansion of T_{PH} cells in the joints of JIA patients and revealed a positive correlation of synovial T_{PH} frequencies with ABCs in situ.[29] Synovial T_{PH} cells from JIA patients skewed B cell differentiation towards the ABCs phenotype in vitro by the provision of IL-21 and IFN-γ.[29] Thus, it is plausible to propose that T_{PH} cells promote the differentiation of B cells into ABCs through the secretion of cytokines such as IL-21 and IFN-γ in patients with RA.

To further delineate the effector potential of ABCs in an arthritis setting, we investigated the crosstalk between ABCs and FLS by in vitro coculture experiments. We showed that ABCs interact with FLS to induce an active phenotype by increasing the secretion of IL-6 and MMPs in a cell contact-independent manner. Further studies confirmed that ABCsCM-derived TNF-α is responsible for the activation of FLS. However, we could not exclude the possibility of cell contact between ABCs and FLS, since it has been reported that TNF-α stimulation enhances the adhesion of B cells to FLS.[31] Next, RNA-seq was performed to gain deeper insights into how FLS were activated by ABCsCM. Interestingly, ABCsCM promoted the upregulation of interferon-stimulated genes in FLS. Additionally, we tested ERK1/2 and STAT1 phosphorylation in activated FLS and validated the blockage of ERK1/2 and JAK-STAT1 pathways in preventing ABCsCM-induced FLS activation. This has clinical significance since JAK inhibitors like Baricitinib have been approved for the clinical treatment of active RA.[45]

In conclusion, our results demonstrated that ABCs may contribute to the chronicity of synovitis by inducing the activation of FLS via the TNF-α-mediated ERK1/2 and JAK-STAT1 pathways.

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Contributors YQ and M-LC performed the experiments, analysed the data and developed the figures. H-ZJ, WH and CZ collected the samples and analyzed the data. AB analysed the data and revised the manuscript. JH and ZC designed the study, analysed the data, wrote the manuscript and funded the study. ZC is responsible for the overall content as the guarantor.

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Competing interests None declared.

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

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Ethical committee of the First Affiliated Hospital of USTC. ID:2021-N(H)-007. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The data supporting the results of this study are available from the corresponding author on reasonable request. The annotated genome and raw sequence reads were deposited at NCBI under Bioproject accession number PRJNA822860.

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CLINICAL SCIENCE

Efficacy and safety of upadacitinib for active ankylosing spondylitis refractory to biological therapy: a double-blind, randomised, placebo-controlled phase 3 trial

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ABSTRACT

Objectives To evaluate the efficacy and safety of upadacitinib, a Janus kinase inhibitor, in patients with active ankylosing spondylitis (AS) with an inadequate response (IR) to biological disease-modifying antirheumatic drugs (bDMARDs).

Methods Adults with active AS who met modified New York criteria and had an IR to one or two bDMARDs (tumour necrosis factor or interleukin-17 inhibitors) were randomised 1:1 to oral upadacitinib 15 mg once daily or placebo. The primary endpoint was Assessment of SpondyloArthritis international Society 40 (ASAS40) response at week 14. Sequentially tested secondary endpoints included Ankylosing Spondylitis Disease Activity score, Spondyloarthritis Research Consortium of Canada MRI spine inflammation score, total back pain, nocturnal back pain, Bath Ankylosing Spondylitis Functional Index, Bath Ankylosing Spondylitis Metrology Index and Maastricht Ankylosing Spondylitis Enthesitis Score. Results are reported from the 14-week double-blind treatment period.

Results A total of 420 patients with active AS were randomised (upadacitinib 15 mg, n=211; placebo, n=209). Significantly more patients achieved the primary endpoint of ASAS40 at week 14 with upadacitinib vs placebo (45% vs 18%; p<0.0001). Statistically significant improvements were observed with upadacitinib vs placebo for all multiplicity-controlled secondary endpoints (p<0.0001). Adverse events were reported for 41% of upadacitinib-treated and 37% of placebo-treated patients through week 14. No events of malignancy, major adverse cardiovascular events, venous thromboembolism or deaths were reported with upadacitinib.

Conclusion Upadacitinib 15 mg was significantly more effective than placebo over 14 weeks of treatment in bDMARD-IR patients with active AS. No new safety risks were identified with upadacitinib.

Trial registration number NCT04169373.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Advanced treatment options for ankylosing spondylitis (AS) are mainly limited to biological disease-modifying antirheumatic drugs (bDMARDs), such as tumour necrosis factor inhibitors (TNFi) and interleukin-17 inhibitors (IL-17i).

⇒ Janus kinase inhibitors (JAKi-) have recently emerged as alternative, oral treatment options for active AS based on clinical trials conducted in AS bDMARD-naïve patients.

WHAT THIS STUDY ADDS

⇒ The SELECT-AXIS 2 AS bDMARD-inadequate response (IR) study is the first clinical trial to evaluate the efficacy and safety of a JAKi in an active AS bDMARD-IR population, including patients with an IR to IL-17i.

⇒ Upadacitinib 15 mg significantly improved the signs and symptoms of active AS and was well tolerated for 14 weeks of treatment in bDMARD-IR patients, consistent with results observed in the upadacitinib AS bDMARD-naïve study.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Upadacitinib 15 mg offers an effective treatment option for bDMARD-naïve and bDMARD-IR patients with active AS.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory condition that encompasses non-radiographic axSpA and radiographic axSpA, also known as ankylosing spondylitis (AS).[1–3] AxSpA is characterised by inflammatory back pain[4–6] and other symptoms including spinal mobility or functional impairments, peripheral and extra-musculoskeletal manifestations, diminished quality of life and loss of work productivity.[1 6–9]

Non-steroidal anti-inflammatory drugs (NSAIDs) are the first-line pharmacological therapy for axSpA.[10 11] Treatment with a biological disease-modifying antirheumatic drug (bDMARD), such as a tumour necrosis factor inhibitor (TNFi) or an interleukin-17 inhibitor (IL-17i), is recommended in patients who do not sufficiently respond to NSAIDs. However, many patients do not achieve desired treatment goals, including low disease activity, with bDMARD therapy.[12–15] Overall,

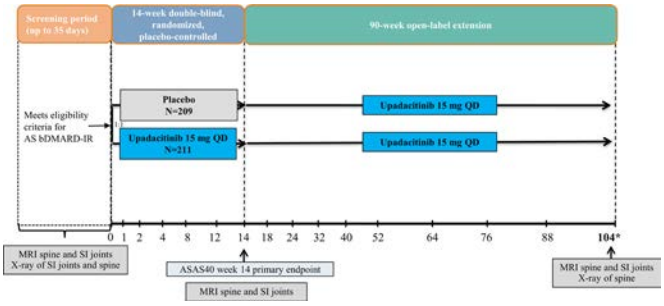

Figure 1 Study design. Study design of the AS bDMARD-IR study of the SELECT-AXIS 2 master protocol is illustrated. *Patients in remission at week 104 could enter a remission-withdrawal period until flare or week 152. AS, ankylosing spondylitis; ASAS40, Assessment of SpondyloArthritis international Society 40 response; bDMARD, biological disease-modifying antirheumatic drug; IR, inadequate response; QD, once daily; SI, sacroiliac.

treatment options for axSpA remain limited compared with other rheumatic diseases such as rheumatoid arthritis (RA) or psoriatic arthritis (PsA), also given that conventional synthetic DMARDs or long-term corticosteroids are ineffective for treating axial symptoms.[10 11] Growing evidence supports the benefit of Janus kinase inhibitors (JAKi) as an effective oral therapy for the treatment of active AS.[16–20]

Upadacitinib 15 mg once daily, an oral JAKi, demonstrated sustained efficacy and was well tolerated for up to 2 years in bDMARD-naïve patients with AS in the SELECT-AXIS 1 trial.[21–23] To date, no dedicated studies of JAKi treatment in an AS population with an inadequate response (IR) to bDMARD therapy have been conducted. SELECT-AXIS 2 was designed to evaluate the efficacy and safety of upadacitinib 15 mg once daily vs placebo in a bDMARD-IR AS population, including patients with an IR to IL-17i.

METHODS

Study design

SELECT-AXIS 2 (NCT04169373) was conducted using a master protocol (details provided in online supplemental methods). The AS bDMARD-IR study includes a 35-day screening period followed by a 14-week, randomised, double-blind, parallel-group, placebo-controlled treatment period and a 90-week open-label extension period (figure 1). Here, we present the primary 14-week results from the AS bDMARD-IR study.

Patient and public involvement

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

Patients

Eligible patients were adults (aged ≥18 years) who had an AS diagnosis and fulfilled modified New York criteria based on central reading of sacroiliac joint radiographs. Patients had active disease at the screening and baseline visits defined as a Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) score and a patient's assessment of total back pain score of ≥4 on a 0–10 scale, an IR to ≥2 NSAIDs or intolerance to or contraindication for NSAIDs, and an IR to bDMARD therapy. In this study, an IR to bDMARD therapy was defined as patients who discontinued bDMARD therapy (TNFi or IL-17i) due to lack of efficacy (after ≥12 weeks of treatment at an adequate dose) based on the investigators' assessment or intolerance (irrespective of treatment duration). Prior exposure to two bDMARDs was allowed for no more than 30% of patients; among patients with prior exposure to two bDMARDs, a lack of efficacy to one bDMARD and intolerance to another was permitted, but a patient could not have a lack of efficacy to two bDMARDs. Patients receiving concomitant oral corticosteroids or NSAIDs must have been on a stable dose for at least 14 days prior to the baseline visit, while those receiving concomitant conventional synthetic DMARDs were required to be on a stable dose for at least 28 days prior to the baseline visit. Patients who were previously exposed to a JAKi or had total spinal ankylosis, which for the purpose of this study was defined as bridging syndesmophytes (fusion) in a total sum of ≥5 C2–T1 or T12–S1 spine segments, were excluded.

Randomisation and masking

Patients were randomised (1:1) to receive either blinded oral upadacitinib 15 mg once daily or placebo for 14 weeks using interactive response technology. Dose selection for upadacitinib 15 mg once daily was based on favourable results from the SELECT-AXIS 1 AS bDMARD-naïve study, including exposure-response analyses.[21 24] Randomisation was stratified by screening high-sensitivity C-reactive protein (hsCRP; ≤ or > upper limit of normal of 2.87 mg/L), class of prior bDMARD use (one TNFi, one IL-17i or two bDMARDs) and geographical region. The sponsor, investigators, study site personnel and the patients were blinded to the treatment assignments.

Procedures

Study visits occurred at baseline and weeks 1, 2, 4, 8, 12 and 14. MRI of the spine and sacroiliac joints was performed during the screening period prior to or at the baseline visit and week 14 visit. MRIs were independently assessed by two readers blinded to treatment allocation and imaging time points. Discrepancies between the readers were resolved through adjudication by a third reader if scoring differences exceeded a certain mean absolute difference threshold (details provided in online supplemental methods).[21] The average scores of the two readers or the average of the two closest scores of the three readers in adjudicated cases were used to calculate MRI spine and sacroiliac joint inflammation scores. Radiographs of the sacroiliac joints were obtained during the screening period and centrally read (modified New York criteria) for eligibility purposes by two readers and an adjudicator in case of discrepancy; additionally, radiographs of the spine were obtained.

Outcomes

The primary endpoint was Assessment in SpondyloArthritis international Society 40 (ASAS40) response at week 14.[25] Multiplicity-controlled secondary endpoints assessed at week 14 included changes from baseline in Ankylosing Spondylitis Disease Activity Score based on CRP (ASDAS (CRP))[26] and Spondyloarthritis Research Consortium of Canada (SPARCC) MRI spine inflammation score,[27] BASDAI50, ASAS20, ASDAS inactive disease (ID; score <1.3), ASDAS low disease activity (LDA; score <2.1),[26] ASAS partial remission (absolute score of ≤2 units for each of the four domains of ASAS40), and changes from baseline in the following outcomes: patient's assessment of total back pain, patient's assessment of nocturnal back pain, Bath Ankylosing Spondylitis Functional Index (BASFI), Ankylosing Spondylitis Quality of Life (ASQoL), ASAS Health Index, Linear Bath Ankylosing Spondylitis Metrology Index (BASMI) and Maastricht Ankylosing Spondylitis Enthesitis Score (MASES) (online supplemental figure 1). Other efficacy endpoints

included ASDAS major improvement (≥2 point-decrease from baseline), ASDAS clinically important improvement (≥1.1 point-decrease from baseline), and changes from baseline in ASAS and ASDAS components,[25] SPARCC MRI sacroiliac joint inflammation score,[28] tender/swollen joint counts and the six questions of the BASDAI.

Safety outcomes were reported with an onset of up to week 14 and included treatment-emergent adverse events (TEAEs) and laboratory assessments. TEAEs were defined as adverse events (AEs) with an onset after the first dose of study drug and prior to the week 14 dose date or up to 30 days after the last dose of study drug if discontinued prematurely before week 14.

Statistical analysis

A planned sample size of 386 patients was estimated to provide ≥90% power for testing the superiority of upadacitinib to placebo for the primary endpoint of ASAS40 at week 14. The assumed response rates were 24% for upadacitinib and 6% for placebo.[12 21 29] Power and sample size estimations were calculated using a two-sided significance level of 0.05 based on a 10% dropout rate. Efficacy analyses were performed based on randomised treatment using the full analysis set, which included all randomised patients who received at least one dose of study drug. The primary endpoint was also analysed in the per-protocol population. Safety analyses were conducted using the safety analysis set based on actual treatment received in patients who had at least one dose of study drug. For binary efficacy endpoints, response rates were compared between treatment groups using the Cochran-Mantel-Haenszel test, adjusting for the stratification factor of screening hsCRP level. Non-responder imputation incorporating multiple imputation (NRI-MI) was used to handle missing data and intercurrent events. Patients who prematurely discontinued the study drug were treated as non-responders. Missing data due to COVID-19 infection or logistical restriction were handled by MI. Additional missing data due to other reasons were categorised as non-responders for study visits. For continuous efficacy endpoints, mean changes from baseline were compared between treatment groups using a mixed-effect model for repeated measures or the analysis of covariance method. A sequential multiple testing procedure was conducted for all primary and multiplicity-controlled secondary endpoints, controlling the overall type I error rate at the two-sided significance level of 0.05 (online supplemental figure 1). Post hoc subgroup analyses were performed for the primary endpoint by the number (one or two) and type of previous bDMARDs (TNFi vs IL-17i) used.

RESULTS

Patient disposition and baseline characteristics

A total of 420 patients from 119 sites in 22 countries were enrolled in the AS bDMARD-IR study and randomly assigned to receive upadacitinib 15 mg once daily (n=211) or placebo (n=209) (figure 2, online supplemental table 1). Of these 420 patients, 206 (98%) on upadacitinib and 203 (97%) on placebo completed the 14-week double-blind treatment period. The most common primary reasons for premature discontinuation of study drug were AEs in the placebo group (n=3; 1%) and other reasons in the upadacitinib group (n=2; 1%).

Demographic and baseline disease characteristics were generally balanced between treatment groups and reflective of an active AS bDMARD-IR population (table 1). Most patients had prior exposure to one TNFi (74%) followed by one IL-17i (13%), two TNFi (8%), one TNFi and one IL-17i (5%) and two IL-17i (0.5%); 77% of patients discontinued prior bDMARD therapy because of lack of efficacy and 30% because of intolerance.

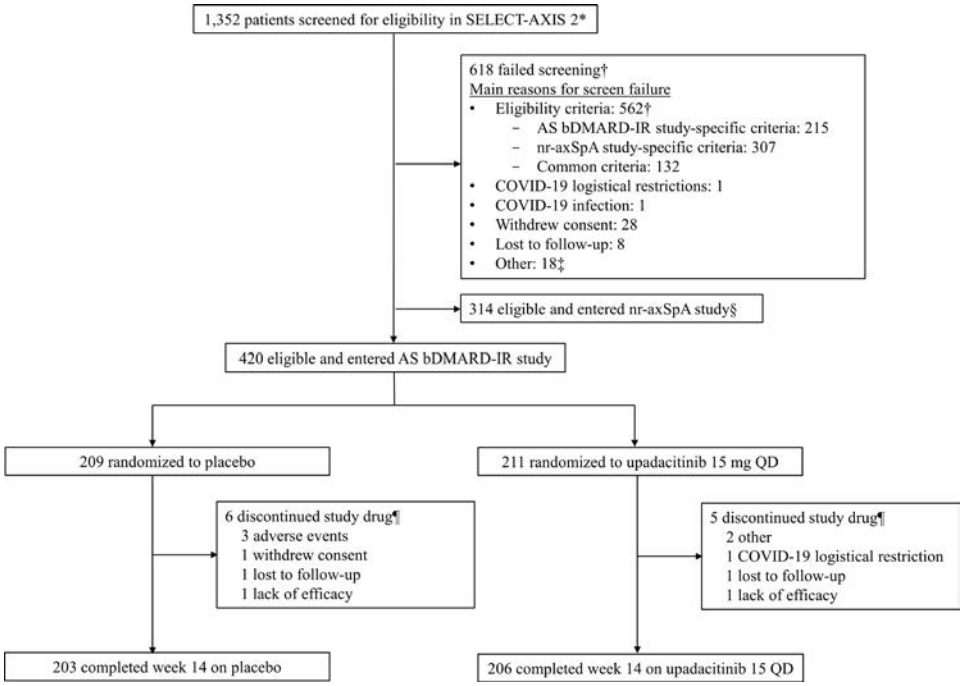

Figure 2 Patient disposition. *Patients were screened between 26 November 2019 and 20 May 2021, for the SELECT-AXIS 2 master protocol, which used a common screening platform to assign patients either to the AS bDMARD-IR study or nr-axSpA study. †Patients could have multiple criteria or multiple reasons for screening failure. Details of screen failure due to study eligibility criteria are presented in online supplemental table 1). ‡Other reasons included imaging, site, or system issues. §Patients did not fail screening (master protocol details provided in online supplemental methods). ¶Primary reason for discontinuation provided. AS, ankylosing spondylitis; bDMARD, biological disease-modifying antirheumatic drug; IR, inadequate response; nr-axSpA, non-radiographic axial spondyloarthritis; QD, once daily.

Table 1 Demographic and baseline disease characteristics

Characteristic	Placebo (n=209)	Upadacitinib 15 mg once daily (n=211)
Sex		
Male	158 (76%)	153 (73%)
Female	51 (24%)	58 (27%)
Age, years	42.2 (11.8)	42.6 (12.4)
Body mass index, kg/m^2	26.4 (5.0)	27.2 (5.7)
Race		
White	169 (81%)	168 (80%)
Asian	37 (18%)	42 (20%)
African American	3 (1%)	1 (0.5%)
Region		
North America	25 (12%)	25 (12%)
South/Central America	14 (7%)	13 (6%)
Western Europe	25 (12%)	16 (8%)
Eastern Europe	98 (47%)	109 (52%)
Asia*	34 (16%)	41 (19%)
Other†	13 (6%)	7 (3%)
HLA-B27 positive	168 (81%)	180 (85%)
Time since AS diagnosis, years	7.5 (7.5)	7.9 (7.5)
Time of AS symptoms, years	12.6 (9.3)	12.9 (9.1)
Baseline medication use		
NSAIDs	163 (78%)	163 (77%)
Oral corticosteroids	18 (9%)	27 (13%)
csDMARDs	62 (30%)	68 (32%)
Prior bDMARD use‡		
One TNFi	158 (76%)	154 (73%)
Two TNFi	14 (7%)	19 (9%)
One IL-17i	24 (11%)	29 (14%)
Two IL-17i	1 (0.5%)	1 (0.5%)
One TNFi and one IL-17i	11 (5%)	8 (4%)
Total back pain (0–10 NRS)§	7.4 (1.4)	7.5 (1.5)
Nocturnal back pain (0–10 NRS)¶	7.2 (1.5)	7.1 (1.8)
Patient Global Assessment of Disease Activity (0–10 NRS)	7.2 (1.4)	7.4 (1.5)
Morning stiffness (0–10 NRS)**	6.8 (1.6)	6.9 (1.8)
ASDAS (CRP)	3.9 (0.8)	3.9 (0.8)
BASDAI score	6.8 (1.3)	6.8 (1.3)
BASFI score	6.2 (1.9)	6.3 (2.0)
BASMI score	3.9 (1.6)	3.9 (1.6)
Enthesitis	162 (78%)	148 (70%)
MASES score††	4.2 (3.1)	4.9 (3.0)
SPARCC MRI spine score‡‡	8.8 (12.5)	10.7 (15.4)
SPARCC MRI sacroiliac joint score‡‡	5.6 (10.6)	5.0 (10.8)
hsCRP at screening, mg/L	14.5 (17.8)	15.8 (17.7)
hsCRP >ULN (2.87 mg/L) at screening	163 (78%)	165 (78%)
ASQoL§§	11.5 (4.4)	11.6 (4.4)
ASAS Health Index¶	8.9 (3.7)	9.4 (3.5)
History of uveitis	15 (7%)	21 (10%)
History of IBD	5 (2%)	7 (3%)
History of psoriasis¶¶	7 (3%)	7 (3%)

Data are n (%) or mean (SD) unless noted otherwise.
*Patients were from China (n=32), Taiwan (n=21), Japan (n=12) and South Korea (n=10).
†Patients were from New Zealand (n=10), Australia (n=7) and Israel (n=3).
‡Categories for prior bDMARD use were mutually exclusive. One patient on placebo did not have prior bDMARD exposure.
§Total back pain was defined on a numerical rating scale (0–10) based on the question, 'What is the amount of back pain that you experienced at any time during the last week?'.
¶Assessed n=208 in the placebo group.
**Morning stiffness was defined as the mean of questions 5 (severity of morning stiffness) and 6 (duration of morning stiffness) of the BASDAI.
††Assessed n=162 in the placebo group; and n=148 in the upadacitinib group with MASES >0 at baseline.
‡‡Assessed n=202 in the placebo group; and n=199 in the upadacitinib group with available baseline MRI data up to 3 days after the first dose of study drug.
§§Assessed n=208 in the placebo group; and n=210 in the upadacitinib group.
¶¶History of psoriasis was obtained based on 12 psoriasis-related preferred terms, including 'psoriasis'.
AS, ankylosing spondylitis; ASDAS, Ankylosing Spondylitis Disease Activity Score; ASQoL, Ankylosing Spondylitis Quality of Life Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; bDMARDs, biological disease-modifying antirheumatic drugs; CRP, C-reactive protein; csDMARDs, conventional synthetic DMARDs; HLA-B27, human leucocyte antigen B27; hsCRP, high-sensitivity CRP; IBD, inflammatory bowel disease; IL-17i, interleukin-17 inhibitor; MASES, Maastricht Ankylosing Spondylitis Enthesitis Score; NRS, Numeric Rating Scale; NSAIDs, non-steroidal anti-inflammatory drugs; SPARCC, Spondyloarthritis Research Consortium of Canada; TNFi, tumour necrosis factor inhibitor; ULN, upper limit of normal.

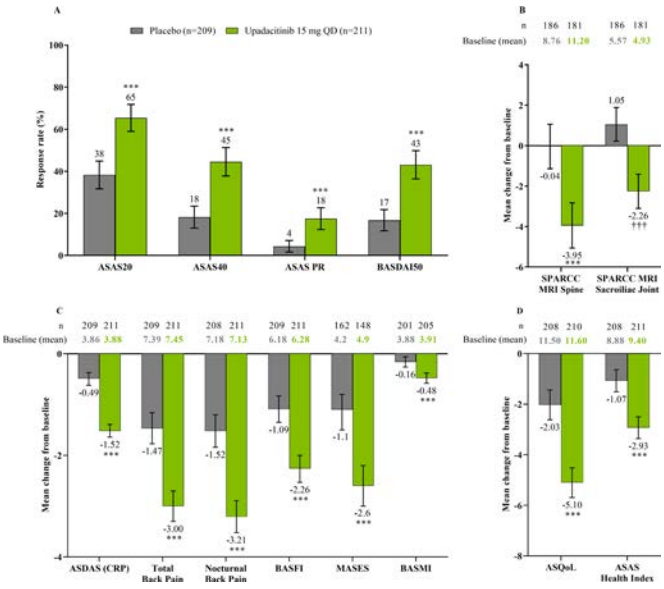

Figure 3 Multiplicity-controlled and key secondary endpoints at week 14. (A) ASAS20, ASAS40, ASAS PR and BASDAI50 responses at week 14 based on NRI-MI analysis. (B) Change from baseline in SPARCC MRI spine and sacroiliac joint scores at week 14 based on ANCOVA analysis. SPARCC MRI was assessed in patients with available baseline MRI data up to 3 days after the first dose of study drug and available week 14 MRI data up to the first dose of study drug in the open-label period. (C) Additional multiplicity-controlled key secondary efficacy endpoints at week 14; ANCOVA analysis for BASMI and MMRM analysis for other endpoints. MASES was assessed in patients with baseline enthesitis. (D) Change from baseline in ASQoL and ASAS Health Index at week 14 based on MMRM analysis. ANCOVA/MMRM analyses are based on as observed data. All endpoints were multiplicity controlled and tested sequentially (online supplemental figure 1), except for SPARCC MRI sacroiliac joint score. Error bars show 95% CI. Significant in multiplicity-controlled analysis: ***p<0.0001. Without adjustment for multiplicity (nominal): ††† p<0.0001. ANCOVA, analysis of covariance; ASAS, Assessment of SpondyloArthritis international Society; ASAS20, Assessment of SpondyloArthritis international Society 20 response; ASAS40, Assessment of SpondyloArthritis international Society 40 response; ASAS PR, Assessment of SpondyloArthritis international Society partial remission; ASDAS, Ankylosing Spondylitis Disease Activity Score; ASQoL, Ankylosing Spondylitis Quality of Life Score; BASDAI50, at least 50% improvement from baseline in Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; CRP, C-reactive protein; MASES, Maastricht Ankylosing Spondylitis Enthesitis Score; MMRM, mixed-effect model for repeated measures; NRI-MI, non-responder imputation incorporating multiple-imputation; QD, once daily; SPARCC, Spondyloarthritis Research Consortium of Canada.

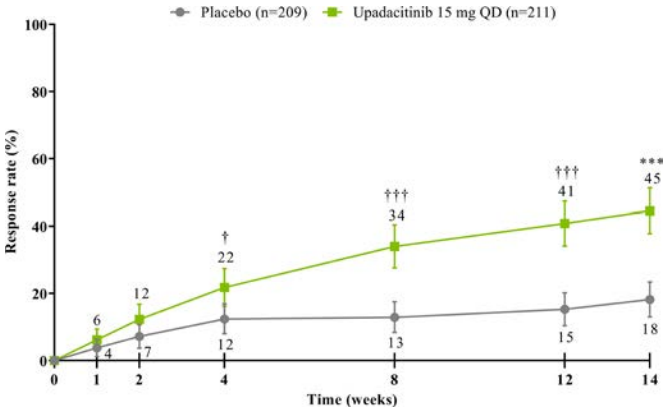

Figure 4 ASAS40 response through week 14. NRI-MI analysis was used. Error bars show 95% CI. Significant in multiplicity-controlled analysis: ***p<0.0001. Without adjustment for multiplicity (nominal): †p<0.05; †††p<0.0001. ASAS40, Assessment of SpondyloArthritis international Society 40 response; NRI-MI, non-responder imputation incorporating multiple imputation; QD, once daily.

Approximately one-third of patients (31%) used conventional synthetic DMARDs at baseline.

Efficacy

The study met its primary and all multiplicity-controlled secondary endpoints at week 14 (figure 3; online supplemental table 2). A significantly higher proportion of patients achieved the primary endpoint of ASAS40 at week 14 in the upadacitinib group vs the placebo group (45% vs 18%; p<0.0001) with a treatment difference of 26% (95% CI 18% to 35%). A clear separation between treatment groups was observed for ASAS40 starting at week 4 (nominal p≤0.05; figure 4). Consistent improvements were observed for the four ASAS components with greater improvement in the upadacitinib than the placebo group (nominal p≤0.05) from week 1 onwards for three of the four components and from week 4 onwards for BASFI (online supplemental figure 2). ASAS40 responses at week 14 were similar in the per-protocol analysis set (online supplemental figure 3). Greater ASAS40 treatment effects were also seen with upadacitinib vs placebo in the subgroups of patients treated with one (46% vs 20%) or two (36% vs 4%) prior bDMARDs; with previous exposure to TNFi (47% vs 22%) or IL-17i (37% vs 4%; online supplemental figure 4); and with baseline hsCRP of ≤ or > 2.78 mg/L (52% vs 15% and 42% vs 19%, respectively) and ≤ or > 5 mg/L (47% vs 15% and 44% vs 20%, respectively; online supplemental figure 5). ASAS40 response rates were consistent between Eastern European (50% vs 19%) and non-Eastern European patients (39% vs 17%) treated with upadacitinib vs placebo (online supplemental figure 6). Statistically significant improvements in disease activity, function and pain were achieved among upadacitinib-treated vs placebo-treated patients at week 14, as measured by change from baseline in ASDAS, total and nocturnal back pain, and BASFI, and achievement of ASDAS ID, ASDAS LDA, BASDAI50, ASAS20 and ASAS partial remission (p<0.0001; figure 3 A, C, figure 5, online supplemental figures 2B, 7, 8A). Consistent responses were observed for other patient-reported pain, ASDAS-related measures and BASDAI (figure 5A and online supplemental figures 8B-10). Upadacitinib also improved objective signs of inflammation as measured by hsCRP and SPARCC MRI spine and sacroiliac joint inflammation scores (p<0.0001 vs placebo; figure 3B, online supplemental figures 10B and 11). Other clinically relevant domains significantly improved with upadacitinib treatment vs placebo at week 14, including quality of life (ASQoL and ASAS Health Index), spinal mobility (BASMI) and enthesitis (MASES) (p<0.0001; figure 3C, D). Additional efficacy endpoints, including change from baseline in tender/swollen joint counts at week 14, are presented in online supplemental table 3.

Safety

The rate of AEs during the 14-week double-blind treatment period was similar between the two treatment groups (41% with upadacitinib and 37% with placebo; table 2). Serious AEs were reported more frequently with upadacitinib (2.8%) than placebo

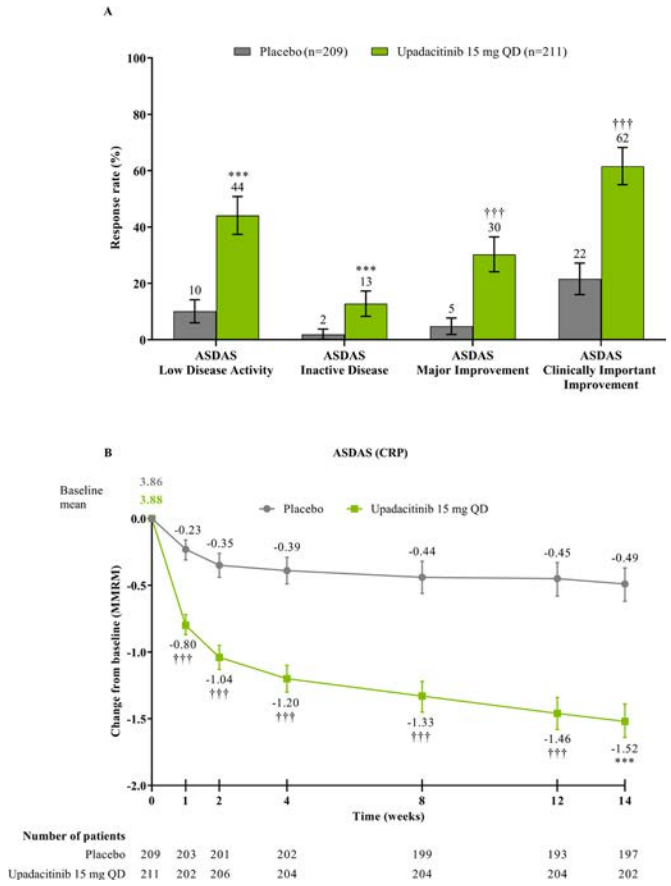

Figure 5 ASDAS responses at and through week 14. (A) Proportion of patients with ASDAS responses at week 14 was based on NRI-MI analysis. ASDAS low disease activity was defined as ASDAS (CRP) <2.1 and ASDAS inactive disease as ASDAS (CRP) <1.3. (B) Mean change from baseline in ASDAS (CRP) through week 14 was based on MMRM analysis, and the numbers of patients were as observed at each visit. Error bars show 95% CI. Significant in multiplicity-controlled analysis: ***p<0.0001. Without adjustment for multiplicity (nominal): †††p<0.0001. ASDAS, Ankylosing Spondylitis Disease Activity Score; CRP, C-reactive protein; MMRM, mixed-effect model for repeated measures; NRI-MI, non-responder imputation incorporating multiple-imputation; QD, once daily.

Table 2 Safety outcomes through week 14

	Placebo (n=209)	Upadacitinib 15 mg once daily (n=211)
Any AE	77 (37%)	86 (41%)
Serious AE	1 (0.5%)*	6 (2.8%)†
Discontinuation of study drug due to AE	3 (1.4%)‡	0
COVID-19-related AE§	6 (2.9%)	12 (5.7%)
Death	0	0
Infection	27 (12.9%)	31 (14.7%)
Serious infection	0	5 (2.4%)¶
Opportunistic infection	0	0
Active tuberculosis	0	0
Herpes zoster	0	2 (0.9%)**
Malignancy	1 (0.5%)	0
Malignancy other than NMSC	1 (0.5%)*	0
NMSC	0	0
Lymphoma	0	0
Hepatic disorder	2 (1.0%)	6 (2.8%)††
Anaemia	1 (0.5%)	3 (1.4%)‡‡
Neutropenia	2 (1.0%)	6 (2.8%)§§
Lymphopenia	2 (1.0%)	1 (0.5%)¶¶
Renal dysfunction	0	0
Gastrointestinal perforation (adjudicated)	0	0
Major adverse cardiovascular events (adjudicated)	0	0
Venous thromboembolic events (adjudicated)	0	0
Uveitis	3 (1.4%)***	1 (0.5%)†††
Inflammatory bowel disease	0	1 (0.5%)‡‡‡
Psoriasis§§§	1 (0.5%)	0

Data are n (%).
*Tonsil cancer.
†COVID-19 (n=4), cholangitis (n=1) and uveitis (n=1).
‡One patient each with tonsil cancer, hip and back pain, inguinal hernia.
§As collected in the AE electronic case report form. An AE with the preferred term 'urinary tract infection' was incorrectly attributed to COVID-19 by the site. Therefore, five subjects in the placebo group had COVID-19-related AEs.
¶COVID-19 (n=4) and uveitis (n=1). All events resolved and were deemed by the investigators as having no reasonable possibility of being related to study drug.
**Two patients from Japan had non-serious herpes zoster confined to a single dermatome.
††ALT/AST elevations were transient, and study drug was not interrupted for patients receiving upadacitinib.
‡‡All anaemia events were non-serious and mild or moderate. Treatment with upadacitinib was interrupted in two patients in which events resolved with no treatment discontinuation.
§§All neutropenia events were non-serious: one was severe and five were mild or moderate. One patient interrupted upadacitinib but neutropenia resolved without study drug discontinuation.
¶¶Lymphopenia event was non-serious, mild, and did not lead to study drug interruption or discontinuation.
***Two patients had a history of uveitis.
†††One patient with a history of uveitis had recurrent uveitis that was considered as having no reasonable possibility of being related to study drug by the investigator.
‡‡‡One patient without a history of inflammatory bowel disease had a non-serious event of Crohn's disease.
§§§AE of psoriasis was based on 12 psoriasis-related preferred terms, including 'psoriasis'.
AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; NMSC, nonmelanoma skin cancer.

(0.5%): one patient (0.5%) had acute cholangitis, and five (2.4%) patients had serious infections on upadacitinib (table 2). Four of the five serious infections on upadacitinib were COVID-19-related infections; all patients had risk factors for more severe disease[30] including older age, male sex, hypertension or obesity, and all events resolved. Overall, COVID-19-related AEs, including the serious infections reported above, occurred in 17 patients (5.7% on upadacitinib vs 2.4% on placebo; online supplemental table 4). None of the 17 affected patients had to discontinue study drug treatment prematurely, and none were vaccinated against COVID-19 except one patient on upadacitinib with a non-serious asymptomatic COVID-19 AE. A numerically higher proportion of patients from Eastern Europe (5.3%) than non-Eastern Europe (3.3%) had a COVID-19-related AE; the four serious COVID-19-related AEs were reported in patients from Eastern Europe (online supplemental table 5). No deaths, opportunistic infections, non-melanoma skin cancer, lymphoma, adjudicated gastrointestinal perforation, renal dysfunction, active tuberculosis or adjudicated major adverse cardiovascular or venous thromboembolic events were reported through week 14 in either treatment group. Two non-serious events of herpes zoster (0.9%) on upadacitinib occurred in patients from Japan, were confined to a single dermatome, and did not lead to treatment discontinuation. One event of tonsil cancer (0.5%) was reported in a patient receiving placebo who was a former smoker, leading to discontinuation of study drug. No malignancy was reported with upadacitinib. Uveitis occurred in four patients (one (0.5%) patient on upadacitinib with a history of uveitis; three (1.4%) patients on placebo with two with a history of uveitis). One AE of Crohn's disease was reported in a patient (0.5%) without a history of inflammatory bowel disease (IBD) in the upadacitinib group; no events of IBD were reported in

the placebo group. An AE of psoriasis occurred in one patient (0.5%) without a history of psoriasis in the placebo group; no events were reported in the upadacitinib group.

Hepatic disorders in the upadacitinib group were mild or moderate transaminase elevations; none resulted in treatment discontinuation. Three patients had a grade 3 elevation in alanine aminotransferase (ALT; one patient (0.5%)) or aspartate aminotransferase (AST; two patients (0.9%)) levels with upadacitinib treatment. The patient with elevated ALT also experienced acute cholangitis as described above, concurrent with increased AST. No cases met the criteria of Hy's law. During the 14-week double-blind treatment period, four patients temporarily interrupted study drug per the study protocol (three due to ALT/AST elevations and one due to a decrease of haemoglobin). Adverse events of anaemia, neutropenia and lymphopenia were generally mild or moderate, non-serious and did not lead to discontinuation of the study drug. Mean haemoglobin concentrations remained stable for both treatment groups, and changes in other laboratory values were generally transient (online supplemental figure 12). Five patients treated with upadacitinib had a grade three decrease in lymphocyte (0.5%) or neutrophil (1.9%) counts, which were not associated with serious infections.

DISCUSSION

SELECT-AXIS 2 is the first clinical trial dedicated to evaluating the efficacy and safety of a JAKi in an AS population that had a lack of efficacy or were intolerant to bDMARDs, including TNFi or IL-17i. The study met its primary endpoint of ASAS40 response, and all ranked secondary endpoints at week 14, demonstrating the consistent benefit of upadacitinib 15 mg once daily relative to placebo for treating multiple clinically relevant domains and components of AS, including improvements in objective signs of axial inflammation. In addition, upadacitinib provided quick symptom relief as early as week 1.

Results of this study in a treatment-refractory AS patient population were consistent with and complementary to those of SELECT-AXIS 1, which evaluated upadacitinib in AS bDMARD-naïve patients.[21] The responses in our study were also overall in line with those reported for other compounds, including IL-17i.[12 13 18 31] However, few placebo-controlled studies in bDMARD-IR AS patients are available. In addition, subgroup analyses showed consistent improvements in ASAS40 responses with upadacitinib treatment irrespective of CRP elevation at baseline and the number or type of previous bDMARDs used, although the number of patients exposed to IL-17i and two bDMARDs were small.

Overall, upadacitinib was well tolerated. As the study was conducted during the initial phase of the COVID-19 pandemic, the observed events reflect the prevalence of COVID-19 and the associated hospitalisation rate at the time the study was conducted.[32] In this study, all COVID-19 events resolved and were considered to have no reasonable possibility of being related to upadacitinib as assessed by the investigators. Only one patient who experienced a COVID-19-related AE was vaccinated. Longer-term data from this trial will help to inform about the impact of upadacitinib treatment and vaccination status in the development of COVID-19-related AEs in the AS patient population. Available data from other inflammatory arthritic conditions such as RA and PsA suggest that the rates of COVID-19 infection were lower or similar in patients treated with upadacitinib than adalimumab.[33] Notably, JAK inhibition has been recognised as an option to treat severe COVID-19.[34] The safety profile of upadacitinib in this bDMARD-IR AS population was generally consistent with that observed in SELECT-AXIS 1[21–23] and the RA[35] and PsA[36 37] programmes. Herpes zoster occurrence has been reported with JAKi therapy with a particularly increased rate in patients of Asian descent,[35 38 39] which is aligned with the findings of this study. No deaths, opportunistic infections, malignancy and adjudicated major adverse cardiovascular or venous thromboembolic events were reported with upadacitinib.

A few limitations of our study should be acknowledged. In the absence of an active comparator, data comparison with a similar AS bDMARD-IR population treated with another therapy should be made in the appropriate context. The decision to define a patient as having an IR due to a lack of efficacy or intolerance to a bDMARD was based solely on the discretion of the study investigators, which is also in line with the approach used in other studies.[12 13] A lack of an established definition of an IR to therapy may explain potential patient selection variability, which may have influenced the magnitude of treatment responses.[14] Rates of extra-musculoskeletal manifestations including uveitis or IBD in this study and SELECT-AXIS 1 were low overall,[22] and upadacitinib has been shown to be effective in phase 3 IBD trials.[40–43] However, few patients had a history of uveitis and IBD at baseline, and case report forms documenting efficacy in uveitis and IBD were not used.[44] Therefore, additional data are needed to derive definitive conclusions about the efficacy of upadacitinib treatment on uveitis. Lastly, the ongoing long-term extension study will provide data on when upadacitinib treatment reaches a therapeutic plateau in this treatment-refractory AS population and whether there is similar efficacy in terms of maintenance of response through 2 years as observed in SELECT-AXIS 1.[23]

In summary, upadacitinib 15 mg once daily significantly improved the signs and symptoms of active AS in bDMARD-IR patients after 14 weeks of treatment compared with placebo. Treatment with upadacitinib was generally safe and well tolerated. No new safety risks were identified compared with the known safety profile of upadacitinib. These findings show that upadacitinib, which offers the convenience of an oral therapy,[45] may be an effective treatment option for patients with active AS, including those with treatment-refractory AS who have shown an IR based on lack of efficacy or intolerance to bDMARD therapy.

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Competing interests DvdH has received consulting fees from AbbVie, Bayer, BMS, Cyxone, Eisai, Galapagos, Gilead, GSK, Janssen, Lilly, Novartis, Pfizer, and UCB Pharma; is the director of Imaging Rheumatology BV; is an Associate Editor of the Annals of Rheumatic Diseases; is an editorial board member of the Journal of Rheumatology; and is an advisor for the Assessment in SpondyloArthritis international Society. XB has received grant/research support from AbbVie and Novartis; consulting fees from AbbVie, BMS, Chugai, MSD, Novartis, Pfizer, and UCB; speakers' bureau fees from AbbVie, BMS, Celgene, Chugai, Merck, Novartis, Pfizer, and UCB; and is an editorial board member of the Annals of Rheumatic Diseases. JS has received grant/research support from AbbVie, Merck, and UCB; has been a consultant for AbbVie, Merck, Novartis, and UCB; and has served on the speakers' bureau for AbbVie, Merck, and Novartis. AD has received grant/research support from AbbVie, BMS, Celgene, GSK, Lilly, Novartis, Pfizer, and UCB; and honoraria or consultation fees from AbbVie, Amgen, Aurinia, BMS, Celgene, GSK, Janssen, Lilly, MoonLake, Novartis, Pfizer, and UCB. RDI has received grant/research support from AbbVie, Amgen, Janssen, and Novartis; and has been a consultant for AbbVie, Amgen, Janssen, Lilly, Novartis, Pfizer, and Sandoz. HK has received grant/research support from AbbVie, Asahi-Kasei, Boehringer Ingelheim, Chugai, Eisai, and Mitsubishi-Tanabe; consulting fees from AbbVie, Janssen, Lilly, Novartis, Sanofi, and UCB; and received speakers' bureau fees from AbbVie, Asahi-Kasei, BMS, Chugai, Eisai, Janssen, Lilly, Mitsubishi-Tanabe, Novartis, and Pfizer. XZ has none declared. YS, XB, PW and I-HS are employees of AbbVie and may own stock or options. ALP is a former employee of AbbVie and may own stock or options.

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

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Ethics approval This study was conducted according to the International Council for Harmonisation guidelines, local regulations and guidelines governing clinical study conduct, and the Declaration of Helsinki. The protocol and consent forms were approved by an institutional review board or independent ethics committee at each study site. All patients provided written informed consent.

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Data availability statement Data are available on reasonable request. AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymized, individual, and trial-level data (analysis data sets), as well as other information (eg, protocols, clinical study reports, or analysis plans), 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. These 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, Statistical Analysis Plan (SAP), and execution of a Data Sharing Agreement (DSA). Data requests can be submitted at any time after approval in the USA and Europe and after acceptance of this manuscript for publication. 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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EPIDEMIOLOGICAL SCIENCE

Pregnancy and neonatal outcomes in women with axial spondyloarthritis: pooled data analysis from the European Network of Pregnancy Registries in Rheumatology (EuNeP)

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ABSTRACT

Objective To investigate outcome and course of pregnancies in women with axial spondyloarthritis (axSpA) in a pooled data analysis of pregnancy registries in rheumatology.

Methods Prospectively followed women with axSpA, fulfilling ASAS classification criteria and for whom a pregnancy outcome was reported, were eligible for the analysis. Anonymised data of four registries was pooled. Rates of adverse pregnancy outcomes were calculated. Systemic inflammation, disease activity and treatment patterns with tumour necrosis factor inhibitor (TNFi) before, during and after pregnancy were analysed.

Results In a total of 332 pregnancies from 304 axSpA women, 98.8% of the pregnancies resulted in live birth. Mean maternal age was 31 years and disease duration 5 years. Most of these patients received pre-conception counselling (78.4%). Before pregnancy, 53% received TNFi treatment, 27.5% in first and 21.4% in third trimester. Pregnancy and neonatal outcomes were favourable with rates of 2.2% for pre-eclampsia, 4.9% for preterm birth, 3.1% for low birth weight and 9.5% for small for gestational age. Neonates were delivered by caesarean section in 27.7% of pregnancies, of which 47.4% were emergencies. Pooled mean CRP was 4 mg/L before conception peaking in the second trimester at 9.4 mg/L. Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) was below 4 at all time-points.

Conclusions Pooled rates of most outcomes were better than what had been reported in the literature and within expected rates of those reported for the general population. Pre-conception counselling, planned pregnancies and a tight management in expert centres applying a tailored treatment approach may have contributed to the favourable pregnancy outcomes.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Recent meta-analyses showed higher risks of adverse pregnancy outcomes in women with axial spondyloarthritis (axSpA) compared with healthy controls, especially for caesarean section and small for gestational age born neonates.

WHAT THIS STUDY ADDS

⇒ In this first pooled analysis of observational data from four European pregnancy registries in rheumatology, we showed favourable pregnancy outcomes in women with axSpA that were comparable with the general population and lower than rates reported from other axSpA populations.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Most of the patients received pre-conception counselling and a tight management of pregnancies with a tailored treatment approach in centres with an expertise on pregnancy management of patients with rheumatic diseases. This may have contributed to the very good outcomes of our study.

⇒ Our findings can reassure women with axSpA in the phase of family planning.

INTRODUCTION

Spondyloarthritis is a chronic rheumatic inflammatory disease that can present with different clinical features, including axial involvement, peripheral signs (enthesitis, arthritis and dactylitis), but also extra-articular manifestations like inflammatory bowel disease, psoriasis and uveitis.[1] When the disease is predominantly axial, patients are diagnosed with axial spondyloarthritis (axSpA).[2]

AxSpA has been historically seen as a predominantly male disease, but recent data show a more balanced sex prevalence.[3] The disease starts in the third decade of life, thus women can be affected in their reproductive years. It is therefore important to understand the influence of axSpA on pregnancy and on the health condition of the mother and the fetus.

Recent meta-analyses showed higher risks of adverse pregnancy outcomes (APOs) in women with axSpA compared with healthy controls.[4–6] They had a greater chance of having a caesarean section (C-section), especially elective C-section,[4 5] and for delivering neonates born small for gestational age (SGA).[4 6] Pooled results of other APOs and foetal complications, for example, pre-eclampsia, preterm birth (PTB), low birth weight (LBW) or congenital abnormalities, were less conclusive.[4–6]

There is only limited information on disease activity levels during pregnancy in patients with axSpA. A review of six studies reported a disease activity increase in almost half of the axSpA pregnancies with a peak in second trimester.[5] In an analysis of 61 prospectively followed women with axSpA, flares occurred in 25% of pregnancies. Stopping treatment with tumour necrosis factor inhibitor (TNFi) at the time of the positive pregnancy test was associated with a three-fold higher flare risk during pregnancy.[7]

Data from the above-mentioned studies mainly derive from claims data analysis, Nordic registries or single-centre (hospital) cohorts. Data from prospectively followed patients with axSpA before, during and after pregnancy are however scarce and were mainly reported from the Norwegian pregnancy registry in rheumatology (RevNatus)[8 9] and the Bern cohort.[7 10]

This study presents results of a pooled analysis using data from four European pregnancy registries with prospectively collected information on women with axSpA before, during and after pregnancy. We focused on the investigation of pregnancy outcomes, on the health of live-born neonates, disease activity and treatment patterns with TNFi.

PATIENTS AND METHODS

Data sources

This cohort study is based on the secondary use of observational data that was initially collected by four European pregnancy registries. Since 2017, the registries EGR2 (France, FR), RePreg (Switzerland, CH), RevNatus (Norway, NO) and Rhekiss (Germany, DE) collaborate in the European Network of Pregnancy Registries in Rheumatology (EuNeP). All registries are multi-centre and enrol women with a rheumatic disease diagnosis, either when they wish to become pregnant or during (early) pregnancy. Data is collected prospectively and nationwide. After enrolment, rheumatologists (in FR also internists, in NO also rheumatology nurses) and patients report information regularly at pre-defined time-points before, during (once per trimester) and after pregnancy, which was described elsewhere.[11]

Relevant variables and their definition were specified by all collaborators in a protocol. Data was extracted by each registry, transferred in an anonymised format via the file-sharing software Seafile (encrypted data via HTTPS/TLS) and pooled into one single dataset after being quality checked.

Study population

Pregnancies were eligible for the analysis if the woman (1) was enrolled and observed in one of the registries, (2) was diagnosed with axSpA before conception and fulfilled the ASAS classification criteria for axSpA,[12] and (3) had a reported pregnancy outcome until the database closing date. Pregnancies with an early pregnancy loss before or at 12 weeks of gestation (WG) were excluded to account for the variation of inclusion criteria regarding WG in the four registries.

Assessments

Selected variables included maternal (age, weight and height, smoking status) and axSpA disease characteristics (disease duration, HLA-B27, extra-articular manifestations, C reactive protein (CRP), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), TNFi exposure), pregnancy (gravidity, multiple pregnancy, pre-eclampsia, delivery mode) and neonatal information (sex, weight, malformations).

Outcomes

The primary endpoints of this analysis were several pregnancy and neonatal outcomes. In pregnancies with a live birth, we investigated rates of pre-eclampsia, PTB (birth before 37 WG) and mode of delivery (ie, vaginal delivery or C-section, which was then further stratified into elective and emergency C-section). Neonatal outcomes were analysed for all live-born neonates and comprised rates of LBW (birth weight<2500 g), macrosomia (birth weight>4000 g), SGA (weight <10th percentile in the according WG) and large for gestational age (LGA; weight >90th percentile in the according WG). The growth curves provided by Voigt *et al*[13–15] were used for the calculation of SGA and LGA in all registries, except for RevNatus.[16]

As secondary outcomes, systemic inflammation and disease activity were investigated 6 months before pregnancy, in every trimester and 6 months postpartum. Analyses of the data 6 months before pregnancy were only performed if the patient was enrolled prior to conception. Elevated inflammation and activity were defined as CRP>5 mg/L and BASDAI≥4. Furthermore, TNFi treatment was addressed. A patient was considered to be exposed to TNFi 6 months before pregnancy, in first, second and third trimester, or 6 months after delivery if she has received at least one dosage in the respective time period. Four different mutually exclusive treatment patterns during pregnancy were defined: (1) no TNFi in any of the trimesters, (2) TNFi in every trimester, (3) TNFi solely in first or in first and second trimester, (4) all patterns which are not covered by (1) to (3).

Statistical analyses

Data was descriptively analysed and is presented per registry and as a pooled estimate of all pregnancies across registries. Descriptive statistics include means (SD) or numbers (percentage) as appropriate. Rates of the primary endpoints were calculated by dividing the number of events by the number of pregnancies with live birth (applies to pre-eclampsia, PTB, delivery mode) or the number of live born neonates (LBW, macrosomia, SGA, LGA) and multiplying by 100 to obtain a percentage.

Missing data were not imputed. Pregnancies/neonates with missing data on the respective outcome were not included in the calculation. The tables indicate the number of pregnancies with missing information, the figures pregnancies with available information. Data was analysed with the software package SAS, V.9.4 (SAS Institute, Cary, USA).

Subgroup and sensitivity analyses

To investigate whether the results are affected by the number, order or characteristics of pregnancies or by disease severity, primary and secondary outcomes were investigated in four different subgroups comprising singleton pregnancies, the first reported pregnancy in a patient, the first ever pregnancy in a patient (primigravida) and pregnancies in patients fulfilling the New York classification criteria.[17]

To investigate the influence of medical treatment on the primary outcomes as well as on systemic inflammation and

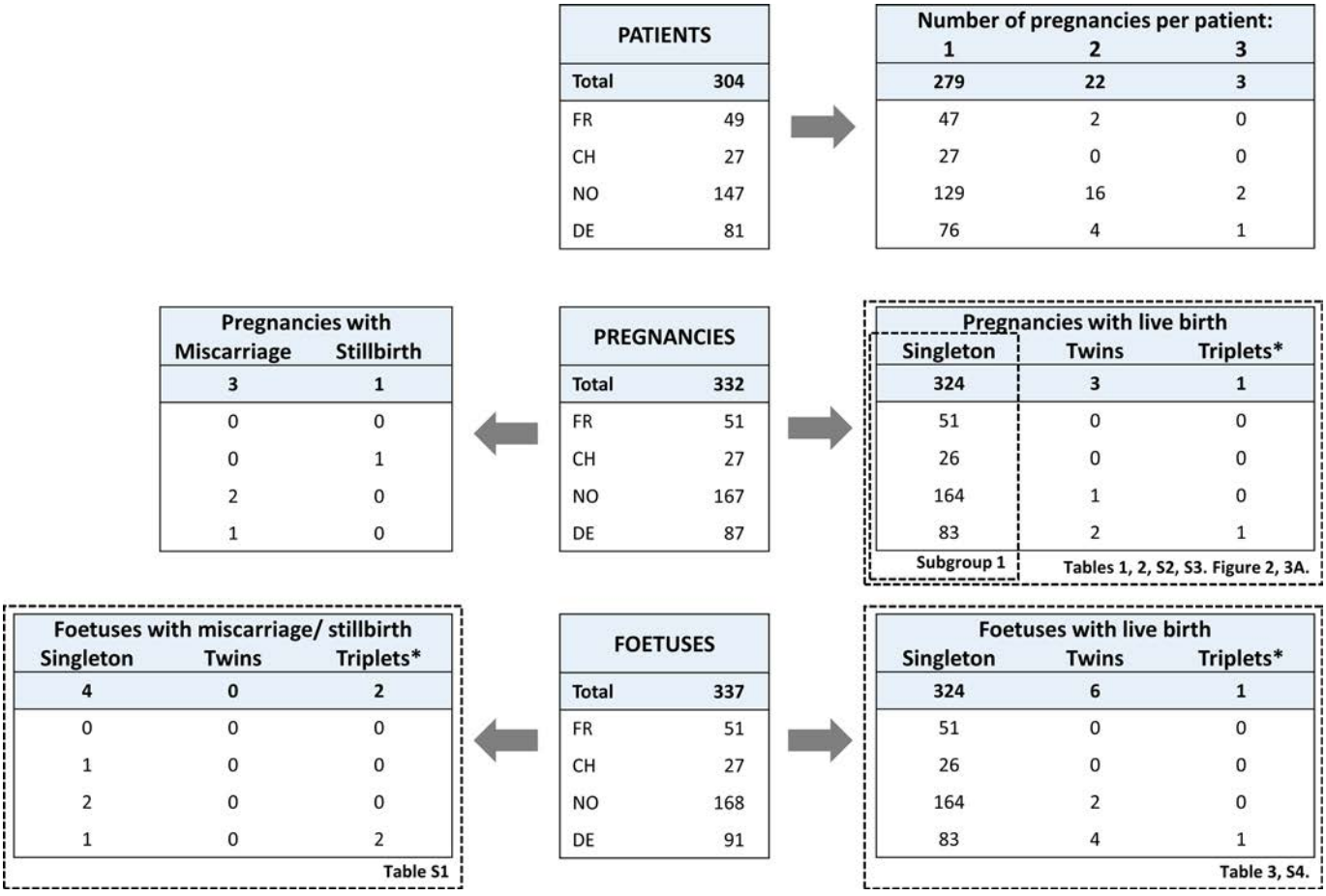


Figure 1 Illustration of number of pregnancies, related number of patients and fetuses, and of pregnancy outcomes for single registries and in combination. *Triplet pregnancy resulted in two aborted foetuses and one live born neonate. The pregnancy itself was counted as live-born pregnancy. CH, Switzerland; DE, Germany; FR, France; NO, Norway.

disease activity, these were further stratified in a sensitivity analysis according to the treatment patterns described above.

RESULTS

A total of 332 pregnancies from 304 women fulfilling the ASAS classification criteria for axSpA were reported (figure 1). Pregnancies were documented between 2008 and 2020, the majority of them occurred from 2015 onwards (93.6%). The Norwegian registry contributed to half of the pregnancies (50.3%), followed by Germany (26.2%), France (15.4%) and Switzerland (8.1%). Except for three twin and one triplet pregnancies, all pregnancies were singletons. The majority of pregnancies resulted in live births (98.8%). A miscarriage after week 12 was reported for three pregnancies and stillbirth for one pregnancy (online supplemental table 1). Of note, two of the fetuses of the triplet pregnancy died, but the pregnancy itself was counted as pregnancy with a live birth. The following analyses refer to 328 pregnancies with live births in 300 patients. The majority was enrolled in early pregnancy (69.9%). For 99 pregnancies (30.1%), information was also available for the period prior to conception.

Maternal and disease characteristics

At the time of conception, mean maternal age was 31.4 years, the average time between axSpA diagnosis and conception was 5.0 years. Almost half of the patients fulfilled the New York criteria (48%; information was not available from NO). Three-quarter of the pregnancies were HLA-B27 positive (77%), and in one out of ten pregnancies at least one extra-articular manifestation was reported (table 1). Pregnancies in each registry did not differ in maternal age, but in other maternal and axSpA characteristics (online supplemental table 2).

Pregnancy and neonatal outcomes

The great majority of pregnancies were planned (86.5%) and 78.4% received rheumatology counselling prior to conception (table 2). Overall, pre-eclampsia occurred in 2.2% of pregnancies, mean WG at delivery was 39.0% and 4.9% of pregnancies were premature. Almost three-quarters of the infants were delivered vaginally (72.3%). Delivery by C-section ranged between 16.7% in France and 56.5% in Switzerland (online supplemental table 3). Of the pooled data, 47.4% were emergency C-sections (table 2).

Reduced birth weight, namely LBW and SGA, occurred in 3.1% and 9.5%, increased birth weight, namely macrosomia and LGA, in 10.7% of the neonates, respectively (table 3). Rates of these outcomes were comparable between registries (online supplemental table 4). For five neonates, malformations were reported: one neonate was suspected of having a genetic syndrome (intrauterine growth restriction, hypertelorism, hypertyrosinemia), three had minor malformations (cleft lip, hypospadias, hip dysplasia) and no details were available for the last one.

Table 1 Maternal and disease characteristics for pregnancies with live birth as pooled results of the main and subgroup analysis

	Main analysis	Subgroup analysis			
	Pooled total pregnancies	Singleton pregnancies	First pregnancy per registry	First ever pregnancy (primigravida)	NY criteria fulfilled‡
No of pregnancies	328	324	300	132	70
No of patients	300	296	300	132	67
Age in years*	31.4±4.5	31.4±4.5	31.4±4.5	30.3±4.1	32.7±4.4
Weight in kg before WG 20	67.5±14.2	67.3±13.8	67.8±14.3	65.9±11.1	68.6±16.0
BMI in kg/m^2	24.4±5.0	24.3±4.8	24.5±5.0	23.6±3.5	24.5±5.7
BMI≥30 kg/m^2	28 (12.6)	27 (12.3)	27 (13.4)	4 (4.5)	6 (12.8)
Smoking*	18 (7.2)	18 (7.3)	18 (7.9)	6 (6.3)	4 (10.5)
Years since diagnosis*	5.0±4.0	5.0±4.0	4.9±4.1	5.0±3.6	7.1±4.5
Fulfilment of NY criteria	70 (47.6)	69 (47.9)	67 (48.2)	35 (53.0)	70 (100)
HLA-B27 positive	203 (76.6)	201 (76.4)	188 (75.8)	95 (84.1)	51 (77.3)
Extra-articular manifestations†	31 (9.8)	29 (9.3)	27 (9.3)	12 (9.6)	4 (6.2)
Thereof IBD	19 (6.0)	19 (6.1)	16 (5.5)	9 (7.2)	3 (4.6)
Thereof psoriasis	7 (2.2)	6 (1.9)	6 (2.1)	2 (1.6)	0
Thereof uveitis	7 (2.2)	6 (1.9)	7 (2.4)	3 (2.4)	1 (1.5)

Results are given as number (percentage) or mean±SD.
*At the time of conception.
†History of inflammatory bowel disease, psoriasis and/or uveitis.
‡Information was not available from the Norwegian registry.
BMI, body mass index; IBD, inflammatory bowel disease; NY, New York; WG, week of gestation.

Systemic inflammation, disease activity and treatment

Pooled CRP ranged between 4.0 mg/L before conception and 9.4 mg/L in second trimester. Mean postpartum CRP did not reach the low pre-conceptional level (figure 2A). Changes in BASDAI were not as pronounced as for CRP with a pooled mean of 3.0 before conception (figure 2B), and values during pregnancy and postpartum between 3.4 and 3.5. The proportion of patients with elevated inflammation level (CRP>5 mg/L) was highest in second and third trimester with 49% and 46%, respectively (figure 3A). The same pattern was observed for BASDAI≥4 (figure 3B).

In more than half of the pregnancies, patients were treated with TNFi before conception (52.6%, figure 4). During pregnancy, the proportions were 27.5%, 21.7% and 21.4% in first, second and third trimester, respectively, and raised to 42.3% postpartum. In one-third of pregnancies (32.7%), patients received TNFi at any time between conception and delivery. In 17.8% of pregnancies, TNFi was given in all three trimesters,

Table 2 Pregnancy characteristics, adverse pregnancy outcome and mode of delivery for pregnancies with live birth as pooled results of the main and subgroup analysis

	Main analysis	Subgroup analysis			
	Pooled total pregnancies	Singleton pregnancies	First pregnancy per registry	First ever pregnancy (primigravida)	NY criteria fulfilled*
No of pregnancies	328	324	300	132	70
Pregnancy was planned	218 (86.5)	214 (86.3)	202 (86.3)	101 (93.5)	47 (90.4)
Rheumatologic counselling	196 (78.4)	168 (76.0)	158 (75.6)	72 (77.4)	34 (63.0)
Primigravida	132 (41.0)	131 (41.2)	131 (44.6)	132 (100)	35 (52.2)
Number of fetuses					
Singleton pregnancy	324 (98.8)	324 (100)	296 (98.7)	131 (99.2)	69 (98.6)
Twin pregnancy	3 (0.9)	0	3 (1.0)	1 (0.8)	1 (1.4)
Triplet pregnancy	1 (0.3)	0	1 (0.3)	0	0
Pre-eclampsia	7 (2.2)	7 (2.2)	7 (2.4)	3 (2.3)	0
Gestational week at delivery	39±1.9	39±1.9	39±1.9	39.3±1.8	38.7±2.4
Preterm birth	16 (4.9)	16 (4.9)	15 (5.0)	8 (6.1)	7 (10.0)
Mode of delivery					
Vaginal delivery	224 (72.3)	222 (72.5)	206 (72.8)	91 (75.8)	43 (66.2)
Caesarean section (C-section)	86 (27.7)	84 (27.5)	77 (27.2)	29 (24.2)	22 (33.8)
Thereof elective C-sections	41 (52.6)	40 (52.6)	36 (50.7)	6 (23.1)	13 (68.4)
Thereof emergency C-sections	37 (47.4)	36 (47.4)	35 (49.3)	20 (76.9)	6 (31.6)

Results are given as number (percentage) or mean±SD.
*Information was not available from the Norwegian registry.
NY, New York.

Table 3 Characteristics of live-born neonates (n=331) as pooled results of the main and subgroup analysis

	Main analysis	Subgroup analysis			
	Pooled total pregnancies	Singleton pregnancies	First pregnancy per registry	First ever pregnancy (primigravida)	NY criteria fulfilled*
No of neonates	331	324	303	133	71
Female sex	159 (49.1)	155 (48.9)	140 (47.1)	67 (51.1)	35 (50.7)
Birth weight in g	3370.5±551.9	3382.4±545.4	3378±546.8	3347.4±525.9	3276.9±609.7
Low birth weight (<2500 g)	10 (3.1)	9 (2.9)	8 (2.7)	4 (3.1)	3 (4.3)
Small for gestational age	30 (9.5)	28 (9.0)	29 (10.0)	15 (11.7)	7 (10.1)
Macrosomia (>4000 g)	34 (10.7)	33 (10.6)	32 (11.0)	11 (8.6)	7 (10.1)
Large for gestational age	34 (10.7)	33 (10.6)	32 (11.0)	12 (9.4)	4 (5.8)
Malformations	5 (3.2)	5 (3.3)	5 (3.4)	2 (2.8)	2 (2.9)

Results are given as number (percentage) or mean±SD.
*Information was not available from the Norwegian registry.
NY, New York.

and in 8.9%, TNFi was only given in first or in first and second trimester. In two-thirds of all pregnancies, the patients did not receive any TNFi (67.3%, figure 5). Substantial differences were observed between countries, for example, exposure to TNFi in first trimester was reported for 58% and 37% in Switzerland and France, and for 22% and 20% in Norway and Germany, respectively. Besides TNFi, no other anti-rheumatic therapies were investigated.

Subgroup and sensitivity analyses

The results of the subgroup analyses are presented in tables 1–3 and online supplementary table S5. Primary outcomes were comparable among pooled data and subgroups comprising singleton pregnancies, first reported pregnancy per registry and first ever pregnancy. However, pregnancies of patients fulfilling New York criteria were twice as likely to result in PTB compared with pooled data, and LGA rate was lower.

Whether the patient received no TNFi during pregnancy, or received TNFi throughout pregnancy or in the first trimesters, respectively, did not result in changes of delivery mode and SGA rates. Yet, higher rates of pre-eclampsia, PTB, macrosomia and SGA and lower LBW rates were observed in patients not treated with TNFi during pregnancy than in those who received TNFi. Furthermore, treatment with TNFi during pregnancy resulted in lower rates of patients with elevated inflammation/ disease activity in third trimester.

DISCUSSION

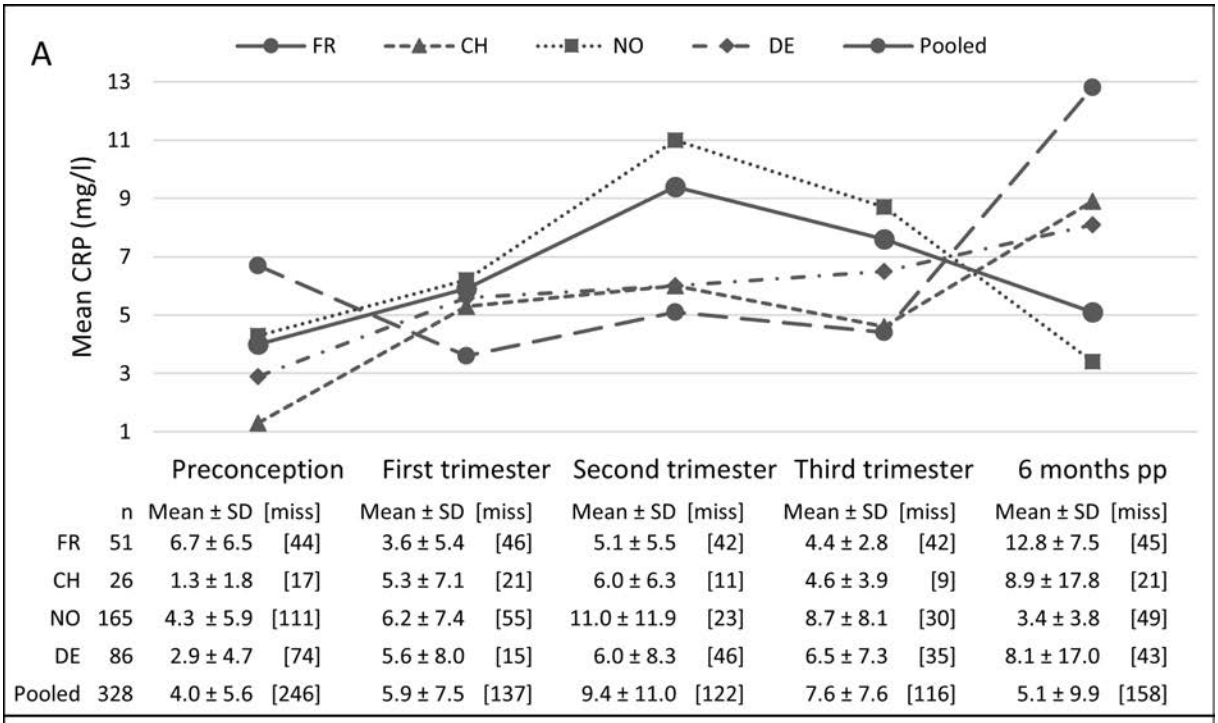
In this pooled analysis of pregnancies in patients with axSpA using observational data from four European pregnancy registries in rheumatology, overall APO rates were very low. This especially refers to pre-eclampsia, PTB, LBW and SGA. Secondary outcomes of this analysis were inflammation/disease activity and treatment with TNFi. Systemic inflammation measured by CRP showed higher levels in second trimester compared with the time before pregnancy and after delivery. These patterns were not as pronounced for disease activity indicated by BASDAI, whose mean values were below 4 throughout pregnancy. With regard to treatment, the majority of patients did not receive TNFi during pregnancy. Before conception, treatment rate was at 53%.

This study investigated rather recent pregnancies, with most deliveries occurring from 2015 onwards, which might reflect both the wider use of very effective treatments (eg, biologics) and also the increased knowledge about pregnancies in this patient groups and therefore changed rheumatology and obstetric routines. The great majority of patients underwent preconception counselling and had a planned pregnancy. Presumably, these women received tight rheumatologic management of their disease at centres specialised in pregnancies of patients with rheumatic conditions and that participate in special pregnancy registries. The low APO rates found herein might therefore not be comparable with older studies or those with retrospective data collection.[4–6] In our study, rates of APO were within the expected rates of the general population despite including singleton and multiple pregnancies in the main analysis as well as more than one pregnancy per patient—both of which can contribute to poorer outcomes.[18 19] These populations were addressed by subgroup analyses, which revealed comparable results.

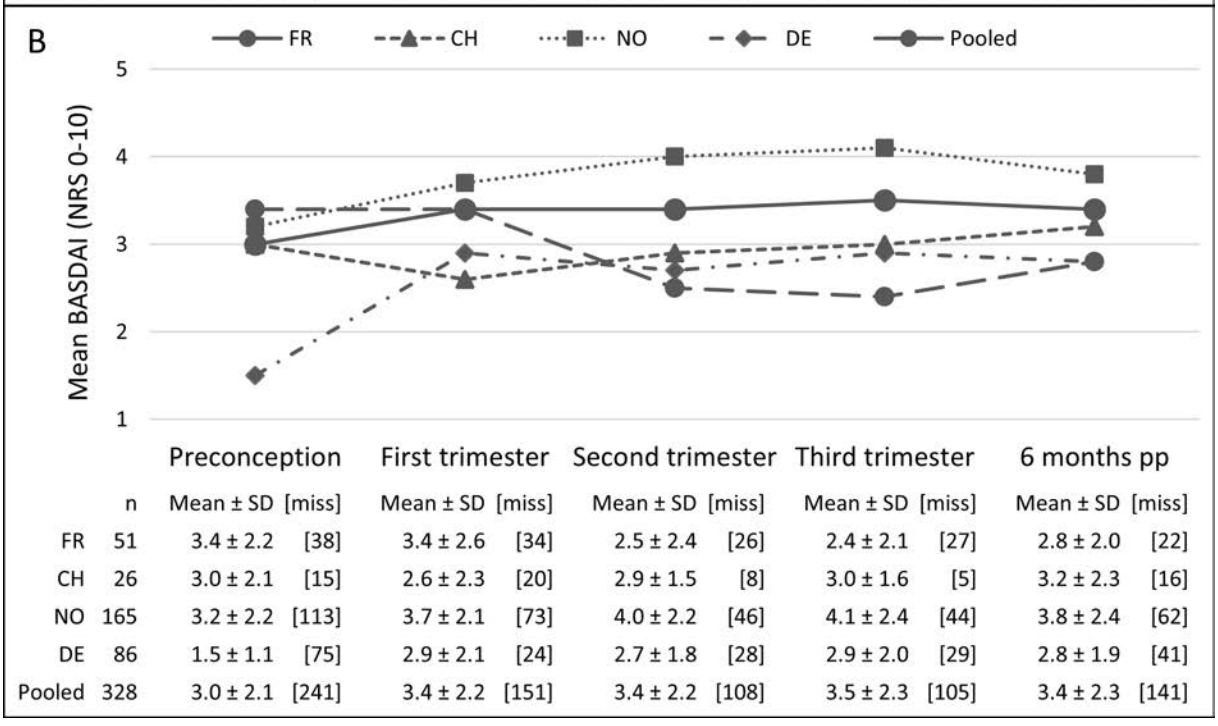
The pooled pre-eclampsia rate in our analysis was 2.2% and varied between 0% and 3.8% depending on country. Rates reported for the general population range from 2.2% to 4.0%,[20] and for patients with axSpA from 1.3% to 7.7%.[21–24] While one meta-analysis of axSpA pregnancies showed no significant association for pre-eclampsia (overall OR 1.3 (95% CI 0.92 to 1.82)) compared with the general population,[5] another showed a risk increase of 59%.[4]

In our data, PTB was reported in 4.9% of the live birth pregnancies (range 0% to 8.1%). This rate is lower than the rate reported for the European general population (8.7% (uncertainty interval 6.3–13.3)).[25] In most of the published data for axSpA, 6.8% to 11.4% of pregnancies were preterm.[10 21–23 26–28] One study reported a rate of only 1.4%[22] and another of 17.3%.[24] Meta-analyses of two, seven and nine studies found controversial outcomes with, on the one hand, significant risk increases for PTB of 64% and 99%[4 6] and, on the other hand, a non-significant result (OR 0.84, 95% CI 0.39 to 1.81),[5] respectively.

In our analysis, the pooled rate of neonates with LBW was 3.1% (range 2.1% to 8.7%), which is quite low compared with the prevalence of LBW in Europe of 7.0 (uncertainty range 6.8–7.1) per 100 live births.[29] Rates of LBW reported by other axSpA studies from different countries vary widely between 3.9% and 22.0%.[22 23 28] One out of ten neonates in our analysis was born SGA. In the French and Swiss data, the SGA prevalence was higher than the expected 10% (17.0% and 21.7%, respectively), which may be caused by the reference cohort as German national growth curves were used.[14 15] Other axSpA cohorts reported rates between 3.1% and 16.4%.[10 21 22 26 28] Two meta-analyses estimated a pooled 2-fold and 2.4-fold increased risk of SGA born infants to women with axSpA in comparison to healthy pregnant women, respectively.[4 6]



		Preconception		First trimester		Second trimester		Third trimester		6 months pp	
	n	Mean ± SD	[miss]	Mean ± SD	[miss]	Mean ± SD	[miss]	Mean ± SD	[miss]	Mean ± SD	[miss]
FR	51	6.7 ± 6.5	[44]	3.6 ± 5.4	[46]	5.1 ± 5.5	[42]	4.4 ± 2.8	[42]	12.8 ± 7.5	[45]
CH	26	1.3 ± 1.8	[17]	5.3 ± 7.1	[21]	6.0 ± 6.3	[11]	4.6 ± 3.9	[9]	8.9 ± 17.8	[21]
NO	165	4.3 ± 5.9	[111]	6.2 ± 7.4	[55]	11.0 ± 11.9	[23]	8.7 ± 8.1	[30]	3.4 ± 3.8	[49]
DE	86	2.9 ± 4.7	[74]	5.6 ± 8.0	[15]	6.0 ± 8.3	[46]	6.5 ± 7.3	[35]	8.1 ± 17.0	[43]
Pooled	328	4.0 ± 5.6	[246]	5.9 ± 7.5	[137]	9.4 ± 11.0	[122]	7.6 ± 7.6	[116]	5.1 ± 9.9	[158]

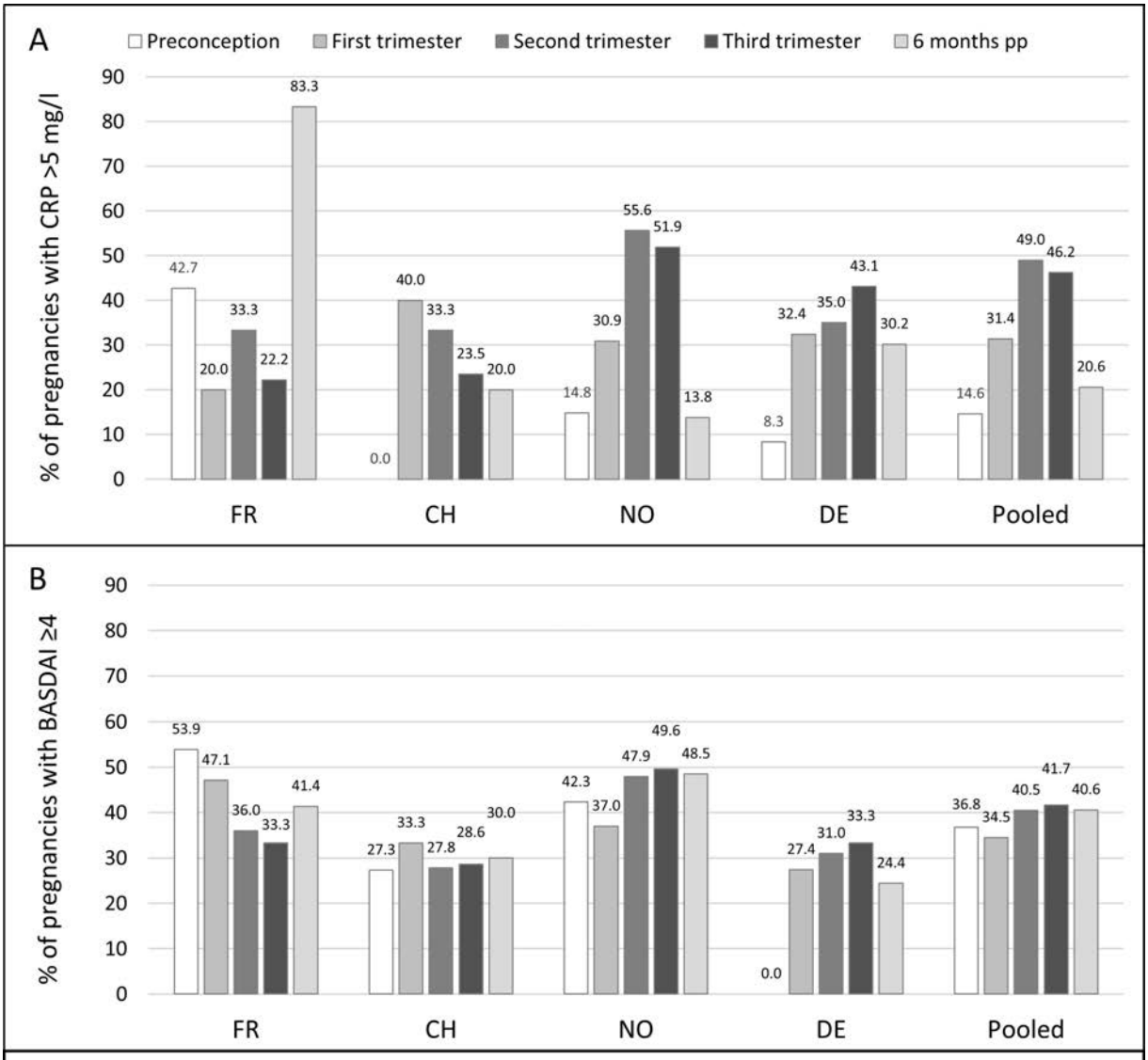


		Preconception		First trimester		Second trimester		Third trimester		6 months pp	
	n	Mean ± SD	[miss]	Mean ± SD	[miss]	Mean ± SD	[miss]	Mean ± SD	[miss]	Mean ± SD	[miss]
FR	51	3.4 ± 2.2	[38]	3.4 ± 2.6	[34]	2.5 ± 2.4	[26]	2.4 ± 2.1	[27]	2.8 ± 2.0	[22]
CH	26	3.0 ± 2.1	[15]	2.6 ± 2.3	[20]	2.9 ± 1.5	[8]	3.0 ± 1.6	[5]	3.2 ± 2.3	[16]
NO	165	3.2 ± 2.2	[113]	3.7 ± 2.1	[73]	4.0 ± 2.2	[46]	4.1 ± 2.4	[44]	3.8 ± 2.4	[62]
DE	86	1.5 ± 1.1	[75]	2.9 ± 2.1	[24]	2.7 ± 1.8	[28]	2.9 ± 2.0	[29]	2.8 ± 1.9	[41]
Pooled	328	3.0 ± 2.1	[241]	3.4 ± 2.2	[151]	3.4 ± 2.2	[108]	3.5 ± 2.3	[105]	3.4 ± 2.3	[141]

Figure 2 Systemic inflammation and disease activity before, during and after pregnancy in pregnancies with live births. Mean values±SD deviation (number of pregnancies with missing information) of CRP (A) and BASDAI (B) are shown. Means are given for pregnancies with available information as pooled results for all pregnancies with live birth (n=328) and stratified by registry. BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CH, Switzerland; CRP, C reactive protein; DE, Germany; FR, France; miss, number of pregnancies with missing information on CRP (figure A) and BASDAI (figure B); N, number of pregnancies with live birth; NO, Norway; pp, postpartum.

In this pooled study, 27.7% of the neonates were delivered by C-section (range 16.7%–56.5%), and 47.4% of the procedures were due to emergency reasons (range 20.0%–61.5%). Studies in other axSpA populations similarly reported widely varying values ranging from 23.4% to 55%.[10 21–23 26 30] Differences in delivery mode may be caused by a variety of reasons such as disease activity and treatment modalities, appear to be strongly affected by country-specific or even hospital-specific factors and are ultimately at the discretion of the physician and patient. However, most previous studies that have compared delivery mode in women with and without axSpA, found a significantly increased risk of C-section in axSpA, which was confirmed by two meta-analyses.[4 5]

For systemic inflammation, pooled levels were low with the highest peak of CRP in second trimester. Means were mainly triggered by the Norwegian registry, which contributed to about

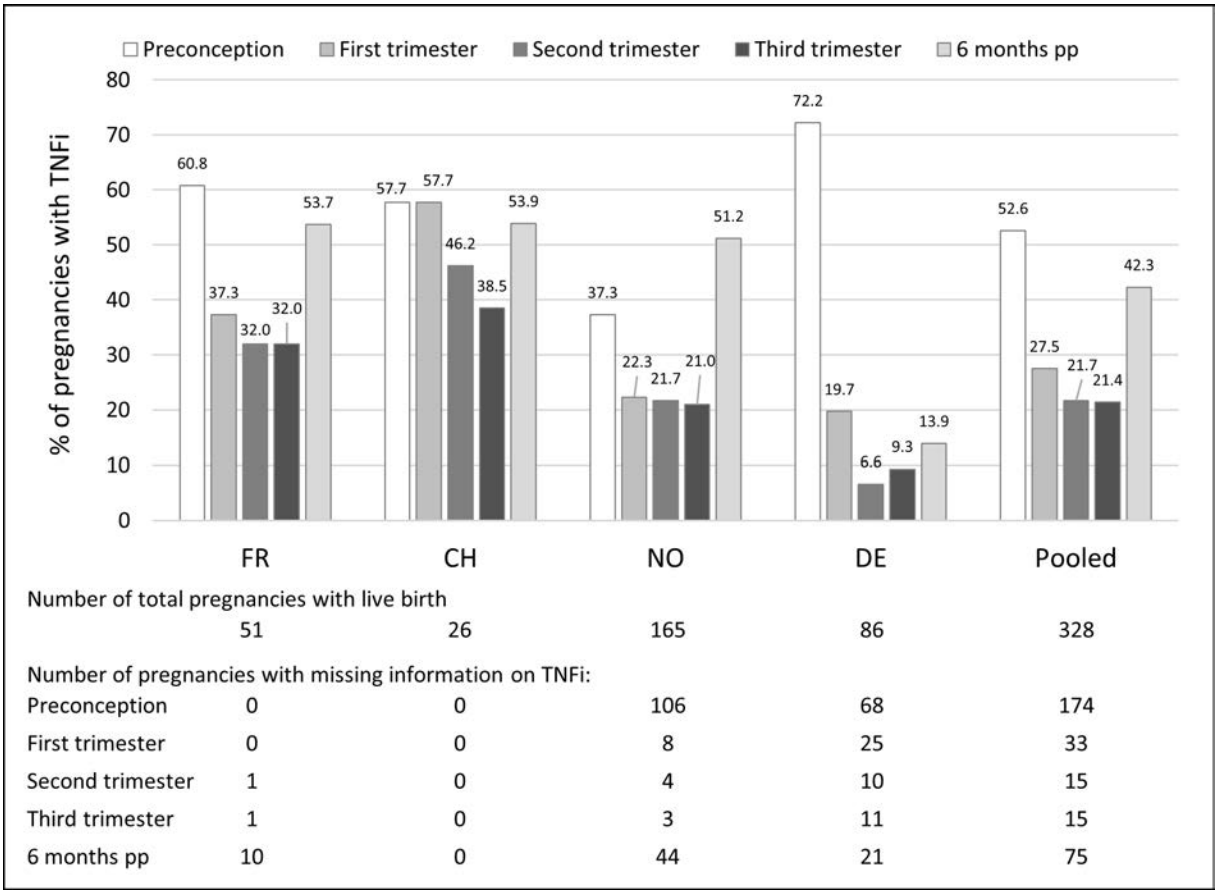


Number of total pregnancies with live birth	51	26	165	86	328
Number of pregnancies with missing information on CRP | BASDAI					
Preconception	44 | 38	46 | 34	42 | 26	42 | 27	45 | 22
First trimester	17 | 15	21 | 20	11 | 8	9 | 5	21 | 16
Second trimester	111 | 113	55 | 73	23 | 46	30 | 44	49 | 62
Third trimester	74 | 75	15 | 24	46 | 28	35 | 29	43 | 41
6 months pp	246 | 241	137 | 151	122 | 108	116 | 105	158 | 141

Figure 3 Percentages of patients with elevated systemic inflammation and disease activity before, during and after pregnancy in pregnancies with live births. Percentages of pregnancies with elevated CRP>5 mg/L (A) and BASDAI≥4 (B) are shown. Percentages are given for pregnancies with available information as pooled results for all pregnancies with live births (n=328) and stratified by registry. BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CH, Switzerland; CRP, C reactive protein; DE, Germany; FR, France; NO, Norway; pp, postpartum.

50% of the available data. However, almost half of the patients had elevated CRP levels in second and third trimester. This pattern was not found for the disease activity measured with the BASDAI. This could be explained by the fact that pregnancy is a state of low-grade inflammation with elevation of CRP in the ultra-high sensitivity range.[31] In normal pregnancy, CRP slowly increases reaching levels in the range of 1000 ng/mL around term. However, usually, these pregnancy-related ultra-low CRP levels are not captured by normal tests. In a previous prospective analysis of patients with axSpA, 44% had elevated CRP levels in second trimester which were related to disease activity and not to changes due to pregnancy.[10] We assume that this is also the case in this study. Of note, unlike systemic lupus erythematosus, there is no pregnancy-specific disease activity instrument for axSpA.

While half of the patients were treated with TNFi before conception, the rate declined to 28% in first and 21% in third trimester. The lower proportion of patients using TNFi during



	FR	CH	NO	DE	Pooled
Number of total pregnancies with live birth	51	26	165	86	328
Number of pregnancies with missing information on TNFi:					
Preconception	0	0	106	68	174
First trimester	0	0	8	25	33
Second trimester	1	0	4	10	15
Third trimester	1	0	3	11	15
6 months pp	10	0	44	21	75

Figure 4 Treatment with TNFi before, during and after pregnancy for pregnancies with live birth. Percentages are given for pregnancies with available information as pooled results for all pregnancies with live birth (n=328) and stratified by registry. CH, Switzerland; DE, Germany; FR, France; NO, Norway; pp, postpartum; TNFi, tumour necrosis factor inhibitor.

third trimester reflects treatment recommendations that advise TNFi discontinuation in the last trimester of pregnancy, except for Fc-free TNFi.[32 33] After birth, TNFi use increased again, but we cannot conclude from our data whether the drug was initiated because of an increase in disease activity or prophylactically to prevent disease flares. Stratifying patients by TNFi treatment,

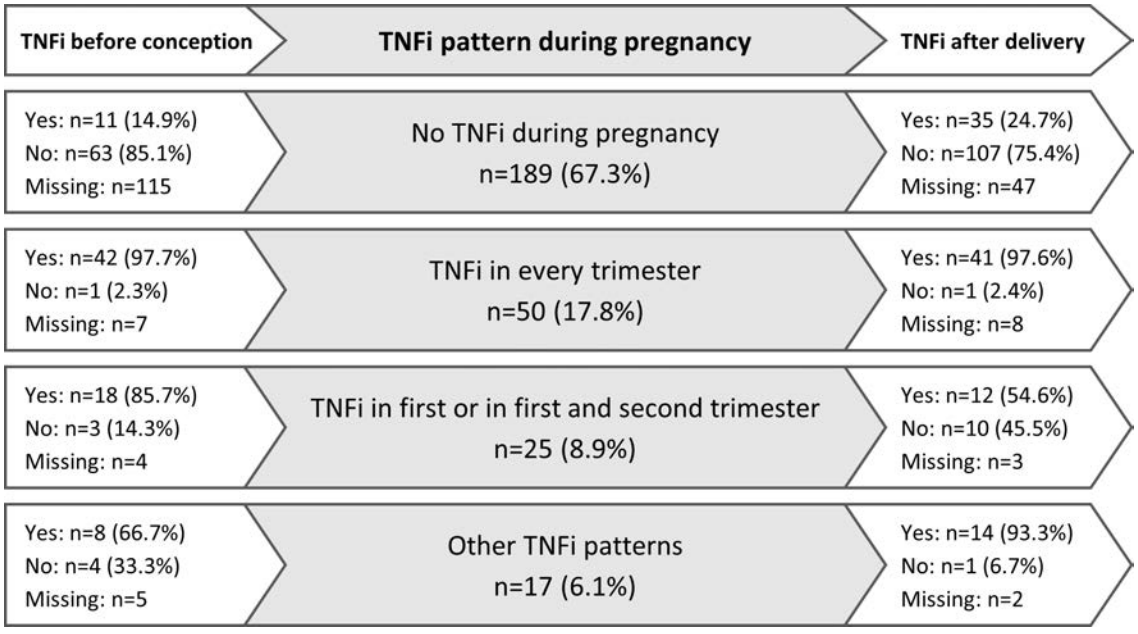


Figure 5 Treatment patterns with TNFi during pregnancy for pregnancies with available information in all three trimesters (n=281). Treatment with TNFi during pregnancy was categorised into different patterns. The figure additionally shows if patients of each pattern received TNFi before conception and after birth. TNFi, tumour necrosis factor inhibitor.

we saw lower pre-eclampsia, PTB and LGA rates in women receiving TNFi during pregnancy and a lower percentage of patients with elevated inflammation/disease activity in third trimester.

This study has several strengths and limitations. Strengths are that we investigated recent pregnancies. Even though, data collection started in 2008, most of the pregnancies were reported between 2015 and 2020. The pregnancies in women with axSpA were followed prospectively in the four participating registries. Despite using different data sources, a homogeneous group of patients was achieved by applying stringent inclusion criteria and selecting only women who fulfilled the ASAS classification criteria for axSpA. As a limitation, it can be considered that these different data sources also introduce a certain level of heterogeneity even though all four registries are comparable in their study design.[11] Different social and healthcare structures, varying prescription and reimbursement patterns in the different countries can be the causes. Only the variables defined in the protocol were available for this analysis. Due to heterogeneity of the registries and differences in data collection, some results could not be investigated in more detail, for example, indications for C-section, treatments besides TNFi, reasons for stopping TNFi treatment or comorbidities such as hypertension and diabetes. Although a relatively large cohort of pregnancies in patients with axSpA was available, we were not able to investigate risk factors for adverse outcomes by regression models. In particular, the interplay of treatment, disease activity and APOs should be deciphered by adjusted analyses. Several reasons hindered such an approach, for example, low number of outcomes and uneven distribution within registries, missing information or unavailability of covariates. Finally, a selection bias of rather planned and well-controlled pregnancies followed mainly in centres with a wide experience and particular interest on the management of pregnancies in patients with rheumatic diseases cannot be ruled out and the positive outcomes observed here may not be generalisable to all women with axSpA.

CONCLUSION

This is the first collaborative analysis of four European pregnancy registries in rheumatology with reassuring results for women with axSpA who want to become pregnant. We found favourable outcomes of pregnancies in women with underlying axSpA who were observed in rheumatologic centres with an expertise on pregnancies in women with rheumatic diseases. The pooled rates of pre-eclampsia, PTB and SGA in these patients were within expected rates in the general population. Our findings underline the importance of pre-conception counselling, pregnancy planning and tight monitoring aiming at low disease activity or remission and assume that they contribute to achieve good pregnancy outcomes in women with axSpA.

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Correction notice This article has been corrected since it published Online First. The collaborator group has been added to the author list.

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EPIDEMIOLOGICAL SCIENCE

Early identification of axial psoriatic arthritis among patients with psoriasis: a prospective multicentre study

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ABSTRACT

Objectives To evaluate a dermatologist-centred screening tool followed by a structured rheumatological examination including MRI of sacroiliac joints and spine for the recognition of psoriatic arthritis with axial involvement (axPsA).

Methods This was a prospective multicentre study. Adult patients with a confirmed diagnosis of psoriasis who had chronic back pain (≥3 months), onset <45 years and had not been treated with any biologic or targeted synthetic disease-modifying antirheumatic drug in the 12 weeks before screening were referred to a specialised rheumatology clinic. A rheumatological investigation including clinical, laboratory and genetic assessments as well as imaging with conventional radiography and MRI of sacroiliac joints and spine was performed. The primary outcome of the study was the proportion of patients diagnosed with axPsA among all referred patients with PsO.

Results Rheumatologists examined 100 patients of those who qualified for referral. 14 patients (including 3 with both axial and peripheral involvement) were diagnosed with axPsA and 5 were diagnosed with peripheral PsA solely. All patients diagnosed with axPsA had active inflammatory and/or structural (post) inflammatory changes in the sacroiliac joints and/or spine on imaging. In five patients, MRI changes indicative of axial involvement were found only in the spine. All but one patient with PsA (13/14 with axPsA and 5/5 with pPsA) fulfilled the Classification Criteria for Psoriatic Arthritis criteria for PsA. The Assessment of SpondyloArthritis International Society criteria for axSpA were fulfilled in 9 (64.3%) patients diagnosed with axPsA.

Conclusions Applying a dermatologist-centred screening tool may be useful for the early detection of axPsA in at-risk patients with psoriasis .

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Early diagnosis of psoriatic arthritis (PsA) (and psoriatic arthritis with axial involvement (axPsA) in particular) is essential and dermatologists are in a strategic position to screen at-risk patients with psoriasis before advanced structural damage of the joints and spine appears.

⇒ While different validated screening/referral tools focusing on peripheral manifestations of PsA exist, validated referral algorithms for axPsA are missing.

WHAT THIS STUDY ADDS

⇒ Our study revealed that application of a dermatologist-centred screening tool focusing on identifying signs of axial involvement among patients with psoriasis may be useful for the detection of PsA (and specifically axPsA) in these patients.

⇒ MRI of the spine in addition to MRI of sacroiliac joints is required to recognise patients presenting with spinal involvement only.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings provide insights into the possibility of diagnosing axPsA early with the ultimate goal of improving the care and quality of life of patients living with this disease.

INTRODUCTION

Spondyloarthritis (SpA) encompasses a group of overlapping disorders, namely ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive arthritis, undifferentiated SpA and non-radiographic axial SpA.[1] Psoriatic arthritis (PsA) is a chronic, inflammatory musculoskeletal disease[2–4] that affects up to 30% of patients with psoriasis[5 6] and typically manifests as peripheral arthritis, enthesitis, dactylitis and skin and nail changes.[7 8] Between 20% and 75% of patients with PsA have axial involvement (axPsA) and present with additional symptoms, such as back pain that might have inflammatory characteristics including morning stiffness.[3 9]

Back pain in patients with axPsA is caused by inflammation in sacroiliac joints and/or spine that over time might result into development of structural damage including radiographic sacroiliitis, syndesmophytes and ankylosis. AxPsA is associated with more severe disease and patients with axial involvement often experience worse pain, significantly impaired physical function and overall activity and reduced quality of life compared with patients without axial involvement.[8 9]

Because a delayed diagnosis of PsA (and axPsA in particular) may lead to irreversible joint and spinal

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damage and poor long-term outcomes,[2] early diagnosis and treatment of patients with PsA is essential.[3 5–7] However, PsA is a heterogeneous disease with a very variable clinical manifestation, which makes early identification very challenging.[2 4]

In the absence of reliable serological and/or imaging biomarkers for early PsA[2] and an existing diagnostic delay, there is a need for screening tools for detection of early PsA. Skin lesions associated with psoriasis typically precede symptoms of PsA, which places dermatologists in a strategic position to screen at-risk patients before advanced structural damage of the joints and spine appears.[3] However, despite awareness of the disease, prevalence of undiagnosed PsA among patients with psoriasis at risk remains high[5 6] with up to one-third of patients with psoriasis who regularly attend dermatology clinics being undiagnosed for PsA.[6]

Moreover, while different validated screening/referral tools focusing on peripheral manifestations of PsA exist,[10] validated referral algorithms for PsA with axial involvement (axPsA) are missing. To address this gap, we conducted a prospective, multicentre study in which we applied a dermatologist-centred, easy and not time-consuming screening tool followed by a structured rheumatological examination including MRI of sacroiliac joints and spine to identify patients with axPsA among patients with psoriasis attending dermatology clinics.

METHODS

Study design and patient eligibility

This prospective, multicentre study was conducted in coordination with the Charité—Universitätsmedizin Berlin specialised rheumatology clinic and 14 dermatology sites in the area of Berlin, Germany between October 2019 and January 2020. Consecutive patients with psoriasis who consented to participating in the study were screened by their treating dermatologist for eligibility for referral to Charité specialised rheumatology clinic. Patients eligible for referral were adults (18 years or older) with a confirmed diagnosis of psoriasis who reported having chronic back (defined as back pain lasting ≥3 months) with onset prior to 45 years of age and who had not been treated with any biologic or targeted synthetic disease-modifying antirheumatic drug (DMARD) within 12 weeks prior to screening (online supplemental Annex 1).

Patients who qualified for referral were contacted to schedule an appointment at the rheumatology clinic, where they confirmed their interest participating in the study and signed a second informed consent form. For all patients who attended the rheumatology clinic, a complete rheumatological investigation, including clinical, laboratory and genetic assessments namely the HLA-B27 as well as imaging with conventional radiography of sacroiliac joints and MRI of sacroiliac joints (short tau inversion recovery—STIR and T1-weighted sequences, semicoronal planes) and spine (STIR and T1-weighted sequences, sagittal planes) were performed. Plaque-type psoriasis severity was evaluated by Psoriasis Area and Severity Index.[11]

Images were evaluated by a panel consisting of at least two rheumatologists and a musculoskeletal radiologist; the presence or absence of radiographic sacroiliitis and the sacroiliitis grade on radiographs according to the modified New York (mNY) criteria[12] and the presence or absence of active inflammatory and structural changes on MRI compatible with axial involvement was recorded by consensus. The diagnosis of axPsA (or pPsA) was performed clinically by the treating rheumatologist after performing the clinical examination of patients and receiving all the imaging, genetic and laboratory results.

Patient and public involvement

Patients and/or public were not involved in any steps of the design, conduct, analysis and results dissemination of this study.

Outcomes

The primary outcome of the study was the proportion of patients diagnosed with axPsA with or without peripheral involvement, among all referred psoriasis patients seen at the rheumatology clinic. Secondary outcomes included the proportion of patients diagnosed with peripheral PsA (pPsA) without axial involvement and the proportion of patients fulfilling the Assessment of SpondyloArthritis International Society (ASAS) classification criteria for axial spondyloarthritis (axSpA) and/or the Classification Criteria for Psoriatic Arthritis (CASPAR) for PsA criteria.

Statistical analysis

The proportion of patients with psoriasis diagnosed with axPsA or pPsA was calculated out of the total number of psoriasis patients referred and seen at the rheumatology clinic. The same approach was applied for the calculation of the proportion of patients fulfilling the ASAS classification criteria for axSpA and the CASPAR classification criteria for PsA.

Patient demographic, clinical, laboratory and imaging characteristics were tabulated and summarised by means, medians, SD, IQR (Q3–Q1), minimum and maximum for continuous variables and by number and percentages for categorical variables. All patients with psoriasis seen at the rheumatology clinic had fully completed screening questionnaires and underwent a complete rheumatological investigation. As only patients with PsO with fully completed screening questionnaires and complete data of the rheumatological assessment including imaging were included into this analysis, there were no missing data in the dataset.

Statistically significant differences between the psoriasis patients diagnosed with axPsA and patients with psoriasis diagnosed with neither axPsA nor pPsA were determined by using Mann-Whitney U test for continuous variables and χ^2 test for categorical variables. Significance tests were conducted at significance level $\alpha=0.05$. All statistical analyses were conducted in SAS Studio V.9.4 (SAS Institute).

RESULTS

Patient disposition and diagnosis of axPsA and pPsA

In total, 355 patients were screened at 14 dermatology sites, of whom 151 (42.5%) qualified for referral to Charité specialised rheumatology clinic. Rheumatologists ultimately examined 100 (28.2%) consecutively referred patients to reduce the risk of bias. The diagnosis of axPsA was made in 14 patients (14%), and 3 of these patients presented with both axial and peripheral involvement. The diagnosis of pPsA without axial involvement was made in five patients (5%). Finally, 81 (81%) patients were diagnosed with neither axPsA nor pPsA (figure 1).

The ASAS classification criteria for axSpA were fulfilled in nine (64.3%) of the patients diagnosed with axPsA. All but one patient diagnosed with PsA (13/14 with axPsA and 5/5 with pPsA) fulfilled the CASPAR for PsA as illustrated in figure 1.

Demographic and clinical characteristics

Demographic and clinical characteristics of all patients are presented in table 1. The mean (SD) age was similar among patients diagnosed with axPsA (46.2 (13.6) years) and patients diagnosed with neither axPsA nor pPsA (45.7 (13.3) years), while patients diagnosed with pPsA were slightly younger (42.8 (9.0) years). Fifty-six per cent of all patients were female; the

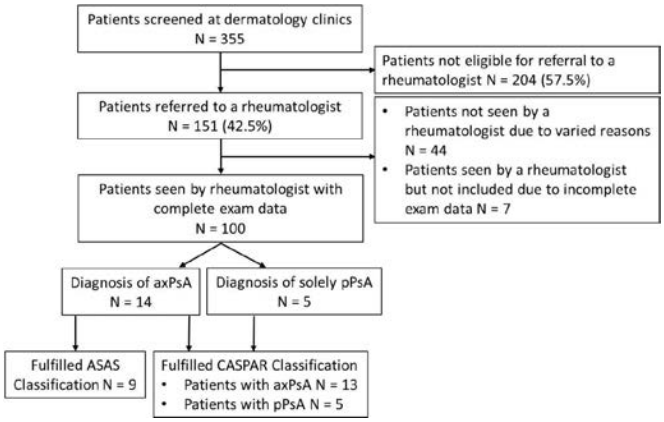

Figure 1 Patient disposition, total number of patients screened, referred and seen by a rheumatologist. ASAS, Assessment of SpondyloArthritis International Society; axPsA, axial psoriatic arthritis; CASPAR, Classification Criteria for Psoriatic Arthritis; pPsA, peripheral psoriatic arthritis.

proportion of females was higher among patients diagnosed with axPsA (64.3%) and lower among patients diagnosed with pPsA (40.0%).

Patients with axPsA had a lower mean (SD) psoriasis duration with 13.6 (9.2) years than those patients not diagnosed with PsA (20.3 (16.7) years); nevertheless, this difference did not reach statistical significance. Mean (SD) duration of back pain was lower as well among patient with axPsA (12.2 (15.2) years) compared with patients not diagnosed with PsA (18.6 (14.8) years). A larger proportion of patients with axPsA experienced inflammatory back pain compared with patients not diagnosed with PsA (57.1% vs 44.4%).

Compared with patients not diagnosed with PsA, patients with axPsA presented with a significantly higher disease activity as assessed by the Ankylosing Spondylitis Disease Activity (ASDAS) score; the mean (SD) ASDAS score was 2.9 (0.8) for patients with axPsA and 2.3 (0.7) for patients not diagnosed with PsA (p=0.017). Patients with axPsA also presented with higher disease activity as assessed by the Disease Activity in Psoriatic Arthritis (DAPSA) score; the mean (SD) DAPSA score was 17.5 (14.3) for patients with axPsA and 11.2 (7.4) for patients not diagnosed with PsA.

Laboratory and imaging characteristics

Laboratory and imaging characteristics of all patients are presented in table 2. A higher proportion of patients with axPsA had HLA-B27 positive compared with patients not diagnosed with PsA (28.6% vs 14.8%). Significant differences were noted on CRP (mg/L) levels among patients with axPsA and patients not diagnosed with PsA. The mean (SD) CRP level was 8.0 (10.8) in patients with axPsA and 2.5 (3.1) in patients not diagnosed with PsA (p=0.039). Moreover, patients with axPsA tended to present with elevated CRP, defined as CRP higher than 5 mg/L. 35.7% of patients with axPsA presented with elevated CRP compared with 13.6% of patients not diagnosed with PsA (p=0.041).

All patients diagnosed with axPsA had active inflammatory and/or structural (post)inflammatory changes in the sacroiliac joints and/or spine on imaging (table 2). In five (35.7%) patients, MRI changes indicative of axial involvement were found only in the spine (figure 2). Five (35.7%) patients with axPsA presented with radiographic sacroiliitis ≥2 unilaterally and four (28.6%) patients in this group presented with radiographic sacroiliitis fulfilling the mNY criteria.

Table 1 Demographic and clinical characteristics of patients diagnosed with psoriasis with pPsA, axPsA and patients not diagnosed with PsA

	Patient group				
Patient characteristic	All patients seen at rheumatology (N=100)	pPsA (N=5)	axPsA (N=14)	No PsA (N=81)	P value*
Age (years)—mean (SD)	45.6 (13.0)	42.8 (9.0)	46.2 (13.6)	45.7 (13.3)	0.883
Female—n (%)	56 (56.0)	2 (40.0)	9 (64.3)	45 (55.6)	0.543
BMI (kg/m^2)—mean (SD)	27.4 (5.5)	23.6 (1.2)	27.8 (6.6)	27.5 (5.4)	0.933
Positive family history of SpA—n (%)	48 (48.0)	3 (60.0)	7 (50.0)	39 (48.1)	0.511
Psoriasis, duration (years)—mean (SD)	19.2 (16.0)	16.6 (19.4)	13.6 (9.2)	20.3 (16.7)	0.291
PASI—mean (SD)	4.0 (4.4)	3.3 (2.1)	4.3 (4.9)	4.0 (4.5)	0.971
Inflammatory back pain—n (%)	49 (49.0)	5 (100.0)	8 (57.1)	36 (44.4)	0.379
Duration of back pain (years)—mean (SD)	17.3 (14.8)	10.8 (11.7)	12.2 (15.2)	18.6 (14.8)	0.058
Enthesitis, current (last 7 days)—n (%)	8 (8.0)	0	0	8 (9.9)	0.219
Dactylitis, current (last 7 days)—n (%)	1 (1.0)	0	1 (7.1)	0	0.016
Uveitis, ever—n (%)	1 (1.0)	1 (20.0)	0	0	NA
ASDAS (0–10)—mean (SD)†	–	3.1 (1.2)	2.9 (0.8)	–	–
BASDAI (0–10)—mean (SD) †	–	5.6 (2.1)	4.8 (1.5)	–	–
DAPSA—mean (SD) †	–	23.2 (14.2)	17.5 (14.3)	–	–

*Statistically significant differences between the axPsA and noPsA groups of patients were determined by using Mann–Whitney U test for continuous data and χ^2 test for categorical data
†Since these scores (ASDAS, BASDAI and DAPSA) are intended to assess disease activity in patients with inflammatory axial disease, values are only presented in the pPsA and axPsA groups. In addition, given the low number of patients with pPsA, no statistical test was performed.
ASDAS, Ankylosing Spondylitis Disease Activity; axPsA, axial psoriatic arthritis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BMI, body mass index; DAPSA, Disease Activity in Psoriatic Arthritis; n, Number; PASI, Psoriasis Area and Severity Index; pPsA, peripheral psoriatic arthritis; SpA, SpondyloArthritis.

None of the patients diagnosed with pPsA or not diagnosed with PsA had active inflammatory and/or structural (post)inflammatory changes in the sacroiliac joints and/or spine on imaging. Among patients not diagnosed with PsA, four (4.9%) presented with radiographic sacroiliitis ≥2 unilaterally; one of them (1.9%) had radiographic sacroiliitis fulfilling the mNY criteria. After MRI assessment, axPsA in these four patients could be excluded: three cases showed typical imaging patterns of osteitis condensans ilii (OCI) and one did not present any active inflammatory or structural changes in the SIJ.

Table 2 Laboratory and imaging characteristics of patients diagnosed with psoriasis with pPsA, axPsA and patients not diagnosed with PsA

	Patient group				
Patient characteristic	All patients seen at rheumatology (N=100)	pPsA (N=5)	axPsA (N=14)	No PsA (N=81)	P value*
HLA-B27 positive—n (%)	16 (16.0)	0	4 (28.6)	12 (14.8)	0.204
CRP (mg/L)—mean (SD)	3.5 (6.1)	8.0 (15.4)	8.0 (10.8)	2.5 (3.1)	0.039
Elevated CRP (>5 mg/L)—n (%)	17 (17.0)	1 (20.0)	5 (35.7)	11 (13.6)	0.041
Peripheral arthritis, current (last 7 days)—n (%)	11 (11.0)	5 (100.0)	3 (21.4)	3 (3.7)	0.012
Radiographic sacroiliitis as per mNY criteria—n (%)	5 (5.0)	0	4 (28.6)	1 (1.2)†	<0.001
Radiographic sacroiliitis ≥grade 2 unilaterally—n (%)	9 (9.0)	0	5 (35.7)	4 (4.9)†	<0.001
Active inflammation, sacroiliac joint (MRI)—n (%)	8 (8.0)	0	8 (57.1)	0	<0.001
Structural (post) inflammatory changes, sacroiliac joint (MRI)—n (%)	8 (8.0)	0	8 (57.1)	0	<0.001
Active inflammation, spine (MRI)—n (%)	13 (13.0)	0	13 (92.9)	0	<0.001
Structural (post) inflammatory changes, spine (MRI)—n (%)	8 (8.0)	0	8 (57.1)	0	<0.001

*Statistically significant differences between the axPsA and noPsA groups of patients were determined by using Mann–Whitney U test for continuous data and χ^2 test for categorical data
†In those four patients not diagnosed with axPsA suspicious findings by conventional radiography were observed (one of the even fulfilling the mNYc), but those were then judged as not compatible with axPsA after MRI evaluation.
axPsA, axial psoriatic arthritis; CRP, C reactive protein; HLA-B27, human leucocyte antigen B27; mNY, modified New York; N, number; pPsA, peripheral psoriatic arthritis.

Significant differences were noted in the proportion of patients that had radiographic sacroiliitis in this group compared with the axPsA group (table 2).

Previous and current treatments

A substantial proportion of patients with psoriasis seen at rheumatology were using non-steroidal anti-inflammatory drugs (NSAIDs) at screening (42%), although no significant differences were noted in NSAIDs use between patients diagnosed with axPsA and patients not diagnosed with PsA (57.1% vs 38.3%; p=0.185). Among all patients seen, a minority reported previous use of non-opioid and opioid analgesics (10% and 5%, respectively) (table 3).

The most common systemic psoriasis therapy was methotrexate, used by 11% of patients in total. Common topical psoriasis therapies included steroids and vitamin D analogues, used by 78% and 52% of the patients, respectively (table 3).

DISCUSSION

This prospective, multicentre study is, to our knowledge, one of the first studies that applied a dermatologist-centred screening/referral tool focusing on detecting axial involvement in patients with psoriasis. Furthermore, the current algorithm was useful for the detection of PsA in patients with psoriasis by applying a straightforward and simple criterion such as age (18 years of age or older), confirmed diagnosis of psoriasis, chronic back pain, defined as back pain lasting ≥3 months, having back pain onset prior to 45 years of age and not treated with biologics or targeted synthetic DMARD within the last 12 weeks.

In addition, in order to capture inflammatory/structural postinflammatory changes in the axial skeleton objectively, our study included MRI of sacroiliac joints and spine as a part of the rheumatological diagnostic approach for all patients. Our data provide further support for previous reports on the prevalence of PsA with and without axial involvement among patients with psoriasis and highlights the demographic and clinical characteristics of these patients with a special focus on imaging data.

We have found that 19% of patients seen by a rheumatologist in our study were diagnosed with PsA (5/100 with pPsA and 14/100 with axPsA), whereas 73.7% (14/19) of patients with PsA had axial involvement that is clearly related to the screening methodology focusing on axial symptoms. A study published in 2019 reported an overall prevalence of PsA among patients with psoriasis of 19.7%,[13] whereas previous studies suggest that 25%–70% of patients diagnosed with PsA have axial involvement[14]

One study investigated presence of axial involvement in patients with PsA as defined by radiographic sacroiliitis ≥grade 2 unilaterally.[15] In this study, 45% of patients presented with radiographic sacroiliitis ≥grade 2 unilaterally and 35% of patients fulfilled the mNY criteria for radiographic sacroiliitis.[15] In our study, we have found that 28.6% and 35.7% of patients with axPsA presented with sacroiliitis ≥grade 2 unilaterally and as per the mNY criteria, respectively. However, we also investigated the overlap between radiographic and MRI findings and found that, while all four patients who fulfilled the mNY criteria for radiographic sacroiliitis also presented with active and/or structural (post)inflammatory changes in the sacroiliac joints on MRI, in five other patients, evidences of involvement of sacroiliac joints were only detected on MRI (figure 2). These findings highlight the importance of MRI in detecting axial involvement in patients with PsA in the absence of definite radiographic changes in the sacroiliac joints. Furthermore, even MRI of sacroiliac joints would have resulted in missing of patients with isolated spinal involvement, which represent a substantial proportion of patients with axial involvement in PsA. Additionally, also in the group not diagnosed with axPsA, suspicious findings by conventional radiography were observed in four patients, which were then judged as not compatible with axPsA but rather due to other causes such as OCI after MRI evaluation. This stresses again the rather low specificity of borderline abnormalities seen in conventional radiographs of the SIJs and highlights the importance of MRI assessments in patients with suspected inflammatory axial involvement.

Previous data reported suggest that males and females are, in general, equally affected by PsA.[16] Among patients with axPsA, whereas Carvalho *et al* reported that males more commonly present with axial involvement,[17] Nas *et al* have found a larger

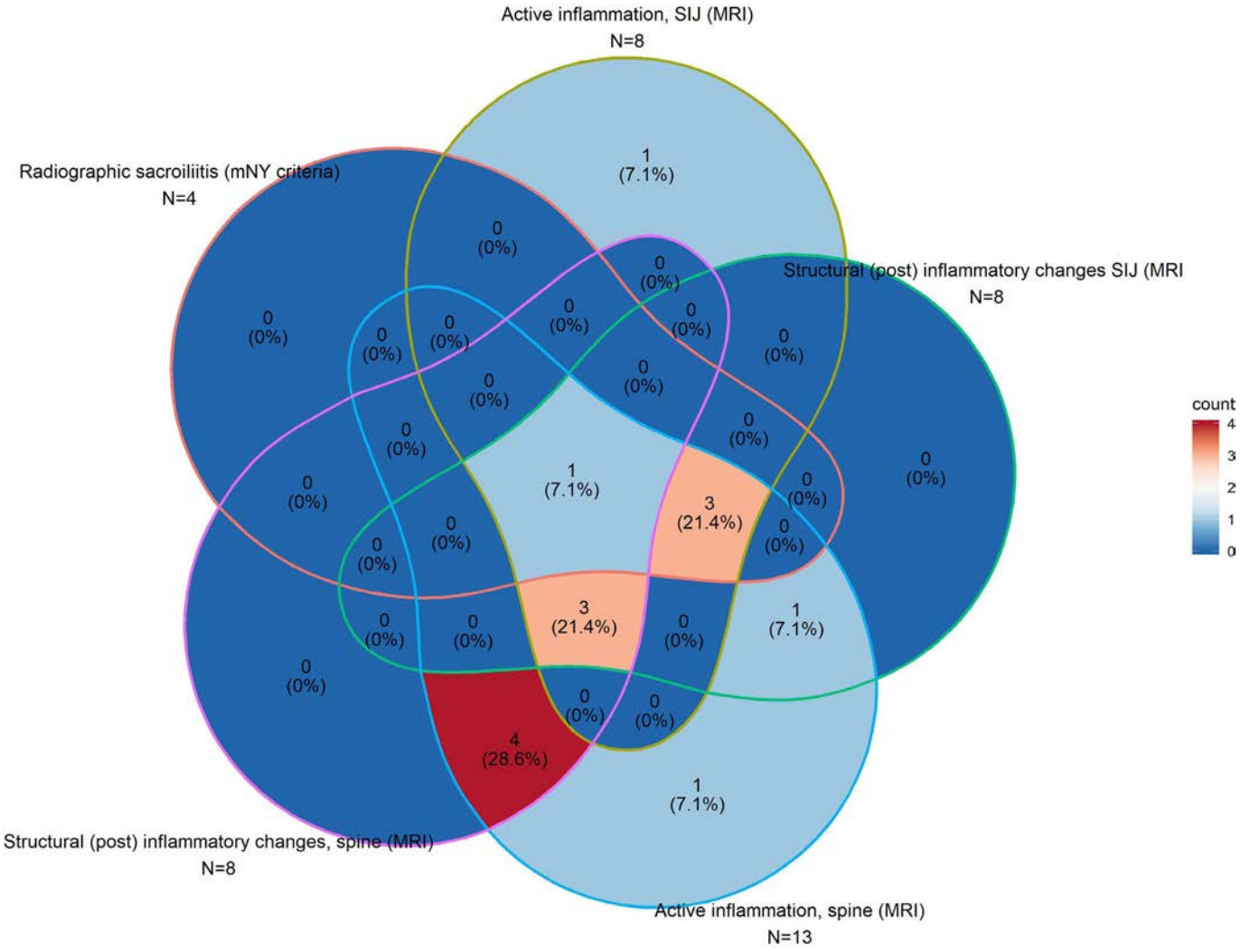

Figure 2 Imaging features of axial involvement in patients with psoriasis diagnosed with axPsA. This Venn diagram represents imaging overlapping and non-overlapping imaging features in patients diagnosed with axPsA. There are five features spread across the image: radiographic sacroiliitis as per mNY criteria at the upper left corner, active inflammation on MRI of SIJ at the top, structural (post)inflammatory changes on MRI of SIJ at the upper right corner, active inflammation on MRI of spine at the bottom and structural (post)inflammatory changes on MRI of spine at the bottom left. For each, we see the number of patients who presented with a feature defined by a coloured lining and the patients that have overlapping features. The number of overlapping features in patients is also represented in colour. For example, we see that out of 13 patients with active inflammation in spine (MRI), 4 also had structural post inflammatory changes in spine (MRI) as coloured in red. Following, out of eight patients with structural changes SJI (MRI), three had structural post inflammatory changes in spine (MRI) and three radiographic sacroiliitis (mNY criteria) as represented in light red. mNY, modified New York; SIJ, sacroiliac joint.

proportion of females with axial involvement compared with males (59.8% vs 40.2%).[18]

With regard to laboratory findings, a larger proportion of patients with axPsA in our study were HLA-B27 positive compared with patients not diagnosed with PsA (28.6% vs 14.8%) although the difference was not statistically significant.[16 17 19] Interestingly, none of the patients diagnosed with PsA without axial involvement in our study were HLA-B27 positive. In addition, elevated C reactive protein (CRP) level has been considered strongly associated with incidence of PsA according to a recently published systematic literature review.[20] While only one patient with pPsA in our study presented with elevated CRP (>5 mg/L), we have found that 35.7% of patients with axPsA had elevated CRP, and a significant difference was noted when compared with the group of patients not diagnosed with PsA. This finding is consistent with data reported by one study that demonstrated an association between elevated CRP and axial involvement in patients with PsA.[21]

A major strength of this study is its prospective design that allowed collection of high quality data since there were no missing data from records of patients who underwent a complete clinical and imaging investigation and included in this analysis. Furthermore, we collected data from patients attending 14 different dermatology sites in the Berlin area, which increased the representativeness of this population.

Our study has limitations. First, patients with PsO not fulfilling the referral strategy have not been evaluated; thus, the specificity of the strategy and the negative predictive value could not be evaluated. Furthermore, no validated and established PsA screening tool was part of this project and therefore no comparisons of the performances between our screening tool and already existing screening tools for PsA in general could be applied. In

Table 3 Previous and current treatments of patients diagnosed with psoriasis with pPsA, axPsA and patients not diagnosed with PsA

	Patient group				
Patient characteristic	All patients seen at rheumatology (N=100)	pPsA (N=5)	axPsA (N=14)	No PsA (N=81)	P value*
NSAIDs use, current—n (%)	42 (42.0)	3 (60.0)	8 (57.1)	31 (38.3)	0.185
Analgesics (non-opioid)	10 (10.0)	0	2 (14.3)	8 (9.9)	0.620
Analgesics (opioid)	5 (5.0)	0	2 (14.3)	3 (3.7)	0.102
Systemic psoriasis therapy—n (%)					
Methotrexate	11 (11.0)	0	2 (14.3)	9 (11.1)	0.732
Systemic retinoids	2 (2.0)	0	1 (7.1)	1 (1.2)	0.155
Phosphodiesterase inhibitor	1 (1.0)	0	1 (7.1)	0	0.016
Systemic glucocorticoids	1 (1.0)	0	1 (7.1)	0	0.016
Other therapies	3 (3.0)	1 (20.0)	0	2 (2.5)	0.552
Topical psoriasis therapy—n (%)					
Topical steroids	78 (78.0)	5 (100.0)	12 (85.7)	61 (75.3)	0.394
Vitamin D analogues	52 (52.0)	1 (20.0)	4 (28.6)	47 (58.0)	0.041
Topical retinoids	1 (1.0)	0	0	1 (1.2)	0.676
Topical calcineurin inhibitors	1 (1.0)	0	0	1 (1.2)	0.676
UVB therapy	1 (1.0)	0	0	1 (1.2)	0.676

*Statistically significant differences between the axPsA and noPsA groups of patients were determined by using Mann–Whitney U test for continuous data and χ^2 test for categorical data
axPsA, axial psoriatic arthritis; N, number; NSAIDs, non-steroidal anti-Inflammatory drugs; pPsA, peripheral psoriatic arthritis; UVB, ultraviolet B.

addition to that, our screening approached specifically focused on patients with chronic back pain that started before the age of 45 years and therefore patients with a later onset of their axial disease or those with isolated peripheral involvement of their PsA would have been missed. Further, imaging—representing at the same time one the major strength of the study—had a relatively high impact on the final judgement on the presence or absence of axial involvement. Finally, a relatively small number of patients diagnosed with axPsA introduces some uncertainty in the estimation of the effects in the given populations.

To conclude, our study revealed that application of a dermatologist-centred screening tool may be useful for the detection of PsA (and specifically axPsA) in patients with psoriasis. The tool is easy to apply and not time-consuming, which makes its application feasible in daily practice ideally in combination with a screening for peripheral disease. In addition, the study provided evidence for the important role of imaging (and specifically MRI) in diagnosing axPsA. These results provide valuable real-world insights into the possibility of diagnosing axPsA early with the ultimate goal of improving the care and quality of life of patients living with the disease.

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

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval This study involves human participants and was approved by Local Ethics Committee of Charité—Universitätsmedizin Berlin, protocol number 2018–7929 and was conducted according to the principles of the Declaration of Helsinki. Participants gave informed consent to participate in the study before taking part.

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CLINICAL SCIENCE

Remission and low disease activity (LDA) prevent damage accrual in patients with systemic lupus erythematosus: results from the Systemic Lupus International Collaborating Clinics (SLICC) inception cohort

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ABSTRACT

Objective To determine the independent impact of different definitions of remission and low disease activity (LDA) on damage accrual.

Methods Patients with ≥2 annual assessments from a longitudinal multinational inception lupus cohort were studied. Five mutually exclusive disease activity states were defined: remission off-treatment: clinical Systemic Lupus Erythematosus Disease Activity Index (cSLEDAI)-2K=0, without prednisone or immunosuppressants; remission on-treatment: cSLEDAI-2K score=0, prednisone ≤5 mg/day and/or maintenance immunosuppressants; low disease activity Toronto cohort (LDA-TC): cSLEDAI-2K score of ≤2, without prednisone or immunosuppressants; modified lupus low disease activity (mLLDAS): Systemic Lupus Erythematosus Disease Activity Index-2K score of 4 with no activity in major organ/systems, no new disease activity, prednisone ≤7.5 mg/day and/or maintenance immunosuppressants; active: all remaining visits. Only the most stringent definition was used per visit. Antimalarials were allowed in all. The proportion of time that patients were in a specific state at each visit since cohort entry was determined. Damage accrual was ascertained with the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI). Univariable and multivariable generalised estimated equation negative binomial regression models were used. Time-dependent covariates were determined at the same annual visit as the disease activity state but the SDI at the subsequent visit.

Results There were 1652 patients, 1464 (88.6%) female, mean age at diagnosis 34.2 (SD 13.4) years and mean follow-up time of 7.7 (SD 4.8) years. Being in remission off-treatment, remission on-treatment, LDA-TC and mLLDAS (per 25% increase) were each associated with a lower probability of damage accrual (remission off-treatment: incidence rate ratio (IRR)=0.75, 95% CI 0.70 to 0.81; remission on-treatment: IRR=0.68, 95% CI 0.62 to 0.75; LDA:

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Remission off-treatment and on-treatment, low disease activity Toronto cohort (LDA-TC) and lupus low disease activity (LLDAS) have been proposed as targets in systemic lupus erythematosus (SLE) treatment.

WHAT THIS STUDY ADDS

⇒ This is the first study examining the independent impact of remission off-treatment and on-treatment, LDA-TC and LLDAS on damage accrual.

⇒ Remission off-treatment and on-treatment, LDA-TC and LLDAS are associated with lower probability of damage in a multinational multiethnic inception cohort.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study reinforces the relevance of remission off-treatment and on-treatment, LDA-TC and LLDAS as potential targets in the management of patients with SLE.

IRR=0.79, 95% CI 0.68 to 0.92; and mLLDAS: IRR=0.76, 95% CI 0.65 to 0.89)).

Conclusions Remission on-treatment and off-treatment, LDA-TC and mLLDAS were associated with less damage accrual, even adjusting for possible confounders and effect modifiers.

INTRODUCTION

Remission and low disease activity (LDA) have been proposed as targets for the management of systemic lupus erythematosus (SLE).[1] These states have been associated with a lower probability of mortality, damage, flares, hospitalisation, costs and cardiovascular events and with a better health-related quality of life.[2] However, there are various definitions of these states.

The Definition of Remission in Systemic Lupus Erythematosus (DORIS) group is an international task force whose aim was to provide a validated definition of remission. Its 2021 version includes a clinical Systemic Lupus Erythematosus Disease Activity Index (cSLEDAI)=0, Physician Global Assessment (PGA) score of <0.5 (0–3), prednisone ≤5 mg/day, and/or immunosuppressive drugs and biologics at maintenance dose. The group acknowledged that remission off-treatment is the ultimate goal but infrequently achieved; thus, remission on-treatment was recommended.[3]

LDA has several definitions. The Toronto Cohort definition of LDA (low disease activity Toronto cohort (LDA-TC)) includes a cSLEDAI ≤2, without prednisone or immunosuppressive drugs,[4] while the Asia-Pacific Lupus Collaboration (APLC) definition of lupus low disease activity state (LLDAS) includes a Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) of ≤4, with no activity in major organ systems (renal, neurological, cardiopulmonary, vasculitis and fever), with no new features of disease activity compared with previous assessment, PGA score ≤1.0, prednisone of ≤7.5 mg/day and/or immunosuppressive drugs at maintenance dose.[5] All states allow antimalarials.

DORIS remission off-treatment and on-treatment, LDA-TC and LLDAS have been associated with lower probability of damage accrual in several cohorts[4 6–21]; however, the independent impact of each state has rarely been evaluated. Therefore, it is possible that at least part of the protective effect of a less stringent definition resulted from the inclusion of patients fulfilling a more stringent definition of a disease activity state.

Thus, we aimed to determine the independent impact of these states on damage accrual, as well as their impact on specific organ damage. We conducted these analyses in a large multinational, multiethnic disease inception cohort.

METHODS

The Systemic Lupus International Collaborating Clinics (SLICC) cohort is a multinational, multiethnic inception cohort which includes patients recently diagnosed with SLE recruited from 33 centres in Asia, Europe and North America from 1999 to 2011. These patients met the American College of Rheumatology revised classification criteria and were enrolled within 15 months of diagnosis. Data were collected per protocol at enrolment and annually and entered in a centralised database. At each annual visit, disease activity (Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)-2K[22]), damage accrual (Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI)[23]) and the average medications doses were recorded. Laboratory tests necessary for assessing disease activity and damage variables were performed locally.[24]

Study population

We selected all patients with at least two visits.

Disease activity states

Disease activity states were categorised based on DORIS,[3] the Toronto Cohort (TC)[4] and APLC[18] definitions; however, remission and LLDAS were defined without the inclusion of PGA because this measure was not collected in the SLICC cohort, hence modified lupus low disease activity (mLLDAS). Definitions of remission not including the PGA have previously been proposed by the Padova group.[16] Five mutually independent disease activity states are thus included:

1. Remission off-treatment: cSLEDAI-2K (excluding serology)=0, without prednisone and immunosuppressive drugs at the visit date.
2. Remission on-treatment: cSLEDAI-2K=0, prednisone of ≤5 mg/day and/or immunosuppressive drugs at maintenance dose at the visit date.
3. LDA-TC, defined as a cSLEDAI-2K≤2, without prednisone or immunosuppressive drugs at the visit date.
4. mLLDAS: SLEDAI-2K score of ≤4, with no activity in major organ systems, with no new features of disease activity compared with the previous assessment, prednisone of ≤7.5 mg/day and/or immunosuppressive drugs at maintenance dose at the visit date.
5. Active: all other visits.

If more than one definition was met, the most stringent definition fulfilled per visit was used.

Antimalarials were allowed in all groups.

The outcome was an increase in the total SDI score between two consecutive visits and an increase in the score per organ system included in the SDI.

Covariates

As achieving a disease activity state could be driven by patient or clinical characteristics that are also associated with the outcome, the following potential confounder or effect modifiers were included: sociodemographic variables including age at diagnosis, sex, race/ethnicity (classified as white from the USA, white (other), black, Asian, Hispanic and other), years of formal education, disease and treatment related variables including disease duration at baseline, the highest dose of prednisone before baseline and antimalarial use (antimalarial use was recorded at every visit).

Statistical analyses

We described the mean (SD) for continuous variables and the number (percentage) for categorical variables at baseline.

To determine the impact on the increase of the SDI, univariable and multivariable generalised estimated equation (GEE) negative binomial regression models were used. To create mutually exclusive groups, disease activity was categorised into five states, as noted, with the most stringent definition fulfilled per visit selected. The proportion of the time that patients were in the specific state at each visit since cohort entry was determined by dividing the number of years in a given state by the total follow-up at each visit for each patient. Possible effect modifiers and confounders adjusted for included the aforementioned covariates. Time-dependent covariates were determined at the same annual visit as the disease activity state; the outcome SDI was assessed at the subsequent visit. The interval between visits were included as an offset variable. The association with damage accrual is reported as incidence rate ratio (IRR) compared to

those with active disease. Sensitivity analysis including only those patients with at least 5 and 10 years of follow-up was performed. Additionally, two alternative models were considered: the first one included remission off-treatment, remission on-treatment, LDA (LDA-TC and mLLDAS together as one state) and active; the second one included remission (on-treatmentand off-treatment as one state), LDA (LDA-TC and mLLDAS as one state) and active.

To determine the impact on the increase of damage within each organ, univariable and multivariable GEE logistic regression models were used. In these cases, the outcome was the increase (or not) per organ damage, and visits were included until the maximum score per organ was achieved. Additionally, for premature gonadal failure, only women aged younger than 40 at diagnosis were included. Possible effect modifiers and confounders adjusted for included sex, age at diagnosis, race/ethnicity, education, baseline disease duration, follow-up time, the highest-ever glucocorticoid dose prior to cohort entry, antimalarials and the score of the same organ damage.

For these analyses, we have chosen 25% of the follow-up time as the unit; that is, a significant IRR should be interpreted as a patient staying in a given state 25% longer time has a probability (IRR) of preventing damage (25% vs 0% or 30% vs 5%, etc) compared with those with active disease.

All analyses were performed using SPSS V.28.0.

RESULTS

There were 1652 patients; 1464 (88.6%) were female; median age at diagnosis was 34.2 (SD 13.4) years; and mean baseline disease duration was 5.6 (SD 4.2) months. Patients had a mean follow-up of 7.7 (SD 4.8) years, 7.5 (4.8) visits per patient, and a total of 12 236 follow-up visits were included. Seven hundred and sixty-two patients (46.1%) had an increase in SDI score of ≥1 during follow-up. The SDI increased in 1267 visits, in 992 by 1 point, in 194 by 2 points, in 61 by 3 points, in 16 by 4 points and in 4 by 5 points. Two thousand five hundred and fifty-five (20.9%) of the visits were classified as remission off-treatment, 2419 (19.8%) as remission on-treatment, 556 (4.5%) as LDA-TC, 680 (5.6%) as mLLDAS and 6026 (49.2%) as active. These data are depicted in table 1.

In the multivariable model, being in remission off-treatment, remission on-treatment, LDA-TC and mLLDAS (per 25% increase in time spent in a specified state vs the active state) were predictive of a lower probability of damage accrual: remission off-treatment, IRR=0.75 (95% CI 0.70 to 0.81); remission on-treatment, IRR=0.68 (95% CI 0.62 to 0.75); LDA-TC, IRR=0.79 (95% CI 0.68 to 0.92); and mLLDAS, IRR=0.76 (95% CI 0.65 to 0.89). Univariable and multivariable models are depicted in table 2. Similar results were found in the sensitivity analysis including those patients with at least 5 or 10 years of follow-up (data not shown). The alternative models are depicted in online supplemental table 1.

Neuropsychiatric damage was accrued in 196 (11.9%) patients, musculoskeletal damage in 195 (11.8%), ophthalmological damage in 186 (11.3%) and renal damage in 159 (9.6%) patients (table 3). In the multivariable models, remission off-treatment and on-treatment and LDA-TC were associated with a lower probability of ophthalmological and renal damage; remission off-treatment and on-treatment were associated with lower probability of neuropsychiatric, cardiovascular, musculoskeletal and skin damage; remission off-treatment was associated with a lower probability of lung and gonadal damage; LDA-TC was associated with a lower probability of peripheral vascular damage; and mLLDAS was associated with a lower probability of diabetes. Univariable and multivariable models of the impact of disease activity states on organ damage accrual are depicted in table 4.

Table 1 Characteristics of SLICC patients included in this study

Characteristic	Number (%) or mean (SD)
At baseline	
Female sex	1464 (88.6)
Age at diagnosis (years)	34.2 (13.4)
Ethnicity	
White, USA	512 (31.0)
White, other	304 (18.4)
Black	277 (17.7)
Asian	251 (15.2)
Hispanic	259 (15.7%)
Other	49 (3.0)
Education level (years)	11.5 (2.0)
Disease duration at baseline (months)	5.6 (4.2)
Highest prednisone dose before baseline (mg/day)	27.4 (25.7)
SDI baseline	0.2 (0.6)
Follow-up (visits=12 236)	
Disease activity state	
Remission off-treatment	2555 (20.9)
Remission on-treatment	2419 (19.8)
LDA-TC	556 (4.5%)
mLLDAS	680 (5.6)
Active	6026 (49.2)
Antimalarials use	8771 (71.7)

LDA-TC, low disease activity Toronto cohort; mLLDAS, modified lupus low disease activity state; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; SLICC, Systemic Lupus International Collaborating Clinics.

DISCUSSION

In this large multinational, multiethnic cohort, we have examined, for the first time, the independent impact of remission off-treatment and on-treatment, LDA-TC and mLLDAS on damage accrual after adjustment for possible confounders. Achieving any of these possible targets was associated with a lower probability of damage accrual. The more annual visits the patient remained in a state, the lower the probability of damage accrual. In the alternative models, when visits were classified into four states (remission off-treatment, remission on-treatment, LDA (including LDA-TC and mLLDAS) and active) and in three states (remission (on-treatment and off-treatment), LDA (including LDA-TC and mLLDAS) and active), similar results were found.

Rates of remission and LDA vary around the world, with remission being most frequent in European populations (almost 90% for at least 1 year in the Padova cohort)[25] but less frequent in Latin American (20% achieved remission at least once during the follow-up).[6] As the SLICC cohort is a multinational, multiethnic cohort, the proportion of patients in remission on and off-treatment is consistent with the literature.[2] However, the relatively low proportion of visits in LDA-TC and mLLDAS but not in remission suggests that a better gradation of response state between remission and active is needed.

Our results are consistent with those from other cohorts; for example, in the GLADEL, Almenara and the Cagliari cohorts, LLDAS (excluding those in remission off-treatment and on-treatment) was associated with lower damage,[6 13 26] while in the Padova cohort,[21] those in remission accrued less damage than those in LLDAS; however, in the Toronto cohort,[4] those in

Table 2 Univariable and multivariable models of the impact of disease activity states on overall damage accrual

	Univariable model	Multivariable model
	IRR (95% CI)	IRR (95% CI)
Disease activity state		
Remission off-treatment	**0.74 (0.69 to 0.80)**	**0.75 (0.70 to 0.81)**
Remission on-treatment	**0.69 (0.63 to 0.76)**	**0.68 (0.62 to 0.75)**
LDA-TC	**0.76 (0.66 to 0.89)**	**0.79 (0.68 to 0.92)**
mLLDAS	**0.75 (0.64 to 0.89)**	**0.76 (0.65 to 0.89)**
Male sex	**1.62 (1.35 to 1.95)**	**1.29 (1.09 to 1.52)**
Age at diagnosis	**1.02 (1.02 to 1.02)**	**1.03 (1.02 to 1.03)**
Ethnicity		
White, USA	Ref.	Ref.
White, other	1.08 (0.87 to 1.34)	1.05 (0.87 to 1.27)
Black	**1.68 (1.36 to 2.08)**	**1.50 (1.23 to 1.83)**
Asian	0.81 (0.64 to 1.04)	0.83 (0.66 to 1.05)
Hispanic	1.33 (1.09 to 1.62)	1.27 (1.04 to 1.55)
Other	1.06 (0.69 to 1.61)	1.10 (0.72 to 1.68)
Educational level (years)	**0.95 (0.92 to 0.98)**	0.98 (0.95 to 1.01)
Disease duration at baseline	0.86 (0.71 to 1.04)	0.97 (0.80 to 1.16)
Antimalarial use	**0.65 (0.56 to 0.74)**	**0.76 (0.65 to 0.87)**
Highest prednisone dose before baseline	**1.01 (1.01 to 1.01)**	**1.00 (1.00 to 1.01)**
SDI before	**1.12 (1.08 to 1.16)**	1.03 (0.99 to 1.07)

Bold indicates values which are statistically significant.
IRR, incidence rate ratio; LDA-TC, low disease activity Toronto cohort; mLLDAS, modified lupus low disease activity state; Ref., reference; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

LDA-TC (and not in remission) and those in remission accrued damage similarly.

While different definitions of remission were evaluated in the Padova cohort, the more stringent the definition, the lower the probability of damage accrual.[11] However, in the APLC cohort, several definitions of remission were evaluated (with or without prednisone, with or without immunosuppressive drugs, with or without serological activity) and the HRs were similar for all definitions.[10] Additionally, LLDAS was significantly associated with reduction of damage accrual, independent of the definition of remission used, except for the least stringent definition. It probably reflects the small number of patients in LLDAS but not in remission according to the least stringent definition.[18] Similarly, in the Hopkins cohort, remission with or without prednisone presented similar risk ratios for damage accrual.[9]

Table 3 Proportion of patients with an increase in organ damage

Organ	Number (%)
Ophthalmological	186 (11.3)
Neuropsychiatric	196 (11.9)
Renal	159 (9.6)
Lung	91 (5.5)
Cardiovascular	101 (6.1)
Peripheral vascular	68 (4.1)
Gastrointestinal	49 (3.0)
Musculoskeletal	195 (11.8)
Skin	103 (6.2)
Gonadal	31/1032 (3.0)
Diabetes	45 (2.7)
Cancer	68 (4.1)

Table 4 Univariable and multivariable models of the impact of disease activity states on specific organ damage accrual

	Univariable	Multivariable*
	OR (95% CI)	OR (95% CI)
Ophthalmological		
Remission off-treatment	0.88 (0.77 to 1.01)	**0.84 (0.72 to 0.97)**
Remission on-treatment	**0.79 (0.64 to 0.96)**	**0.72 (0.59 to 0.88)**
LDA-TC	**0.71 (0.52 to 0.96)**	**0.69 (0.50 to 0.94)**
mLLDAS	0.91 (0.71 to 1.17)	0.88 (0.69 to 1.13)
Neuropsychiatric		
Remission off-treatment	**0.80 (0.68 to 0.99)**	**0.85 (0.73 to 0.99)**
Remission on-treatment	**0.55 (0.42 to 0.72)**	**0.66 (0.53 to 0.82)**
LDA-TC	0.75 (0.51 to 1.09)	0.76 (0.54 to 1.05)
mLLDAS	**0.63 (0.40 to 1.00)**	0.75 (0.53 to 1.05)
Renal		
Remission off-treatment	**0.52 (0.39 to 0.67)**	**0.71 (0.54 to 0.92)**
Remission on-treatment	**0.43 (0.31 to 0.61)**	**0.54 (0.38 to 0.78)**
LDA-TC	**0.12 (0.03 to 0.51)**	**0.27 (0.10 to 0.77)**
mLLDAS	**0.43 (0.22 to 0.87)**	0.65 (0.36 to 1.17)
Lung		
Remission off-treatment	**0.59 (0.44 to 0.80)**	**0.71 (0.53 to 0.95)**
Remission on-treatment	**0.77 (0.59 to 0.99)**	0.85 (0.68 to 1.07)
LDA-TC	**0.52 (0.29 to 0.92)**	0.63 (0.40 to 1.01)
mLLDAS	0.58 (0.34 to 1.00)	0.68 (0.43 to 1.07)
Cardiovascular		
Remission off-treatment	**0.79 (0.64 to 0.99)**	**0.73 (0.58 to 0.92)**
Remission on-treatment	**0.70 (0.53 to 0.93)**	**0.66 (0.51 to 0.92)**
LDA-TC	0.97 (0.73 to 1.30)	0.89 (0.68 to 1.17)
mLLDAS	0.64 (0.36 to 1.10)	0.62 (0.36 to 1.05)
Peripheral vascular		
Remission off-treatment	0.89 (0.69 to 1.15)	0.97 (0.75 to 1.25)
Remission on-treatment	**0.66 (0.45 to 0.98)**	0.75 (0.52 to 1.08)
LDA-TC	**0.03 (0.00 to 0.83)**	**0.06 (0.00 to 0.87)**
mLLDAS	1.07 (0.68 to 1.67)	1.16 (0.78 to 1.72)
Gastrointestinal		
Remission off-treatment	1.02 (0.79 to 1.33)	1.05 (0.81 to 1.37)
Remission on-treatment	1.12 (0.81 to 1.56)	1.17 (0.86 to 1.59)
LDA-TC	0.99 (0.58 to 1.70)	1.01 (0.60 to 1.69)
mLLDAS	1.14 (0.66 to 1.96)	1.27 (0.77 to 2.09)
Musculoskeletal		
Remission off-treatment	**0.89 (0.83 to 0.96)**	**0.70 (0.58 to 0.84)**
Remission on-treatment	0.93 (0.84 to 1.02)	**0.77 (0.62 to 0.94)**
LDA-TC	0.96 (0.85 to 1.08)	0.82 (0.62 to 1.09)
mLLDAS	1.04 (0.92 to 1.17)	0.92 (0.69 to 1.22)
Skin		
Remission off-treatment	**0.66 (0.52 to 0.85)**	**0.69 (0.53 to 0.90)**
Remission on-treatment	**0.47 (0.32 to 0.70)**	**0.52 (0.36 to 0.75)**
LDA-TC	1.07 (0.85 to 1.36)	1.06 (0.82 to 1.37)
mLLDAS	0.71 (0.44 to 1.13)	0.72 (0.46 to 1.12)
Gonadal		
Remission off-treatment	**0.43 (0.22 to 0.84)**	**0.48 (0.25 to 0.94)**
Remission on-treatment	0.68 (0.39 to 1.19)	0.77 (0.45 to 1.32)
LDA-TC	1.07 (0.63 to 1.83)	1.12 (0.66 to 1.89)
mLLDAS	0.48 (0.11 to 2.09)	0.65 (0.18 to 2.30)
Diabetes		
Remission off-treatment	0.73 (0.50 to 1.05)	0.73 (0.51 to 1.05)
Remission on-treatment	0.60 (0.35 to 1.02)	0.61 (0.37 to 1.02)
LDA-TC	0.67 (0.24 to 1.83)	0.66 (0.25 to 1.74)
mLLDAS	**0.28 (0.11 to 0.69)**	**0.32 (0.16 to 0.64)**
Cancer		

Continued

Table 4 Continued

	Univariable	Multivariable*
	OR (95% CI)	OR (95% CI)
Remission off-treatment	1.24 (1.00 to 1.53)	1.10 (0.87 to 1.40)
Remission on-treatment	1.36 (1.05 to 1.76)	1.19 (0.90 to 1.56)
LDA-TC	1.10 (0.71 to 1.70)	1.03 (0.65 to 1.63)
mLLDAS	1.28 (0.86 to 1.89)	1.17 (0.79 to 1.73)

*Adjusted for included sex, age at diagnosis, race/ethnicity, education, baseline disease duration, follow-up time the highest-ever glucocorticoid dose prior to cohort entry, antimalarials and the score of the same organ damage.
LDA-TC, low disease activity Toronto cohort; mLLDAS, modified lupus low disease activity state.

Remission off-treatment and on-treatment and LDA-TC but not mLLDAS were associated with a lower probability of renal and ophthalmological damage. In the case of renal damage, this may be related to better control of disease activity, as it has been associated with renal damage in other cohorts[27 28] and/or to the self-selection of a greater number of non-renal lupus in the remissions and LDA groups. Similar to our results, a longer percentage of the follow-up on remission on-treatment and LLDAS (including remission) were associated with a lower rate of some items of renal damage (end-stage renal disease and glomerular filtration rate <50%) in the Hopkins cohort.[9] Regarding ophthalmological damage, our results are consistent with previous reports that found an association between disease activity and glucocorticoid dose and cataracts.[29 30]

Remissions off-treatment and on-treatment were associated with lower probability of neuropsychiatric, cardiovascular, musculoskeletal and skin damage. In the Hopkins cohort, remission on-treatment and LLDAS (including remission) were associated with a lower probability of neuropsychiatric damage (remission with cranial or peripheral neuropathy and LLDAS with seizures). Nevertheless, in the Hopkins cohort, remission was not associated with a lower risk of cardiovascular damage, but LLDAS (including remission) was associated with a lower probability of myocardial infarction.[9] In the Hopkins cohort, a longer duration of remission was associated with a lower probability of several items of musculoskeletal damage (avascular necrosis and osteoporosis with fracture), and the LLDAS (including remission) was associated with lower probability of musculoskeletal damage (deforming or erosive arthritis, avascular necrosis, osteomyelitis and osteoporosis with fracture).[9] In a recent metaregression, glucocorticoid dose was associated with a higher risk of cardiovascular events, osteonecrosis and osteoporosis with fracture.[31] In the LUpus in MInorities: NAture versus nurture (LUMINA) cohort, disease activity was associated with skin damage.[32]

Remission off-treatment was associated with a lower probability of lung and gonadal damage, and this is consistent with a report from the Hopkins cohort in which a longer duration of remission on-treatment and LLDAS (including remission) was associated with a lower probability of gonadal failure.[9] In the LUMINA cohort, disease activity and glucocorticoids were associated with lung damage in the univariable models but not in the multivariable model.[33]

LDA-TC was associated with a lower probability of peripheral vascular damage; however, in the LUMINA cohort, disease activity and glucocorticoid dose were not statistically significantly associated with peripheral vascular damage.[34]

mLLDAS was associated with a lower probability of diabetes; similarly, in the Hopkins cohort, LLDAS (including remission) was associated with lower probability of diabetes.[9]

Remission off-treatment and on-treatment, LDA-TC and mLLDAS are associated with a lower probability of damage accrual. It would be expected that remission, in particular remission off-treatment, was associated with a lower probability of damage accrual; nevertheless, according to these data, LLDAS and LDA could be good targets in SLE management. These data are relevant to propose treat-to-target strategies and to define outcomes for clinical trials.[1] However, there are some domains that seem to require a more stringent definition of LDA, probably due to the deleterious effect of glucocorticoids. These data could reinforce the partial safety of low dose of prednisone,[35] which is important as glucocorticoid withdrawal is not always possible, and, in some patients, a prednisone dose of ≤5 mg/day could be acceptable.[36–38] Based on the results of remission on-treatment and LDA-TC, it seems that allowing a relatively safe dose of glucocorticoids and/or immunosuppressive drugs is better than allowing LDA but without treatment. These results are consistent with the notion that prednisone should be tapered as quickly as possible but withdrawn only when disease activity is under control and slowly.[38–40] However, these results should be interpreted carefully as they have overlapping CIs. Additionally, these results suggest that the longer the patient remains in remission or an LDA state, the better the outcome, in line with observations from several other cohorts.[9 11 17 21 26] According to these data, remission could be an achievable state in many patients, and it should remain as the ideal target in SLE treatment. However, as more stringent definitions (remission off-treatment and on-treatment) are less frequently achieved in patients with a higher risk of poorer outcomes (like non-white populations or with more severe manifestations), less stringent definitions could be more realistic outcomes for the treatment of SLE patients.[2 41–43] For example, European Alliance of Associations for Rheumatology (EULAR) and Pan American League of Associations of Rheumatology (PANLAR) guidelines recommended remission or LDA as the therapeutic goal.[44 45]

This study has some limitations. First, as the PGA was not included in the SLICC cohort, we could not use the original definition of remission and LLDAS. We believe the PGA is relevant for the definition of remission and LLDAS; however, the PGA has not been consistently reported by different investigators, as reported in a recent systematic review,[46] leading to some problems in its interpretation. However, the recent effort to standardise it (the Physician Global Assessment International Standardisation COnsensus in Systemic Lupus Erythematosus (PISCOS) study) should solve this problem.[47] Nevertheless, based on our results, definitions of remission and LDA without the PGA could be useful, particularly by physicians not properly trained in scoring it. Additionally, as recommended by the group for the PISCOS study, it is important to point out that the PGA should be scored by the same physician at all visits. Second, as visits were performed annually, it is possible that we have missed some fluctuations in disease activity occurring between the scheduled visits, however, as we have recorded the treatment between two visits, it is likely that an increase in disease activity would have been captured as it would have led to an increase in the treatment. Third, we do not know if achievement of remission or LLDAS is related to the underlying disease or more aggressive therapy. Also, we do not know how achievement of remission

or LLDAS mediates decreased damage accrual - is it related to more mild underlying disease, more aggressive therapy, or other factors. Fourth the average duration of follow-up (7.7 years), may have resulted in an overrepresentation of damage occurring earlier versus later in in the disease course. Fifth, as we have examined several outcomes and alternative models, it is possible that some associations have been influenced by multiple comparisons. However, it is important to point out that the lack of a gold standard approach for multiple test adjustment could lead to different results using the same information; based on this, some researchers have suggested to not overcorrect the data but rather to make use of the effect size in these cases.[48]

However, the main strength of this study is the inclusion of a large multinational, multi-ethnic inception cohort, with a relatively long follow-up which allowed us to evaluate the independent impact of each disease activity state on global damage accrual as well as on specific organ damage accrual.

In conclusion, remission on- and off-treatment, LDA-TC and mLLDAS were associated with less damage accrual, even after adjusting for possible confounders and effect modifiers. This highlights the importance of treating-to-target in SLE. If we want to use remission and LDA as treatment goals, their definitions should allow adequate differentiation between these states. The high rate of remission should encourage the use of remission on-treatment or off-treatment as our ideal target, with LDA (LDA-TC and LLDAS) being only an alternative target.

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CLINICAL SCIENCE

Leflunomide versus azathioprine for maintenance therapy of lupus nephritis: a prospective, multicentre, randomised trial and long-term follow-up

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ABSTRACT

Objectives Previous studies have compared mycophenolate mofetil and azathioprine as maintenance therapy for lupus nephritis (LN). Leflunomide is an immunosuppressant widely used in the treatment of rheumatoid arthritis. The aim of this investigator-initiated study was to compare the efficacy and safety of leflunomide versus azathioprine as maintenance therapy for LN.

Methods 270 adult patients with biopsy-confirmed active LN from 7 Chinese Rheumatology Centres were enrolled. All patients received induction therapy with 6–9 months of intravenous cyclophosphamide plus glucocorticoids. Patients who achieved complete response (CR) or partial response (PR) were randomised to receive prednisone in combination with leflunomide or azathioprine as maintenance therapy for 36 months. The primary efficacy endpoint was the time to kidney flare. Secondary outcomes included clinical parameters, extrarenal flare and adverse effects.

Results A total of 215 patients were randomly allocated to the leflunomide group (n=108) and azathioprine group (n=107). Kidney flares were observed in 17 (15.7%) leflunomide-treated patients and 19 (17.8%) azathioprine-treated patients. Time to kidney flare did not statistically differ (leflunomide: 16 months vs azathioprine: 14 months, p=0.676). 24-hour proteinuria, serum creatinine, serum albumin, serum C3 and serum C4 improved similarly. Extrarenal flare occurred in two patients from the azathioprine group and one patient from the leflunomide group. The incidence of adverse events was similar in the 2 groups: leflunomide 56.5% and azathioprine 58.9%.

Conclusions The efficacy and safety profile of leflunomide are non-inferior to azathioprine for maintenance therapy of LN. Leflunomide may provide a new candidate for maintenance therapy in patients with LN.

Trial registration number NCT01172002.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT?

⇒ Lupus nephritis (LN) is a common severe complication of systemic lupus erythematosus with significant unmet clinical needs. So far, only two randomised controlled trials (RCTs) have investigated maintenance therapy for LN, confirming that mycophenolate mofetil and azathioprine are effective medications in maintenance phase, which are not available or tolerable in all patients.

WHAT DOES THIS STUDY ADD?

⇒ This is the first study of leflunomide in maintenance therapy of LN. This prospective, randomised, open-label trial shows that the efficacy and safety profile of leflunomide are non-inferior to azathioprine for the maintenance therapy of LN. Besides, the 6-year extended follow-up data provide evidence that leflunomide is not only effective in controlling kidney and extrarenal flares but is also quite safe and well tolerated.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The results support leflunomide as a potential candidate treatment for LN during the maintenance phase. The prolonged, double-blind, placebo-controlled follow-up studies in larger and more diverse patient populations are needed to further verify the long-term effect of leflunomide in the maintenance therapy of LN.

INTRODUCTION

Lupus nephritis (LN) is a common severe complication of systemic lupus erythematosus (SLE) and a major cause of morbidity and mortality. Approximately 50%–60% of adult patients with SLE develop kidney involvement during their illness. In addition, 10%–30% of patients with LN progress to kidney failure requiring kidney replacement therapy. Although the kidney failure risk associated with LN has substantially improved since the 1970s, the rate of kidney replacement therapy has remained consistent and appears to have increased since 2000.[1] Therefore, there are still significant unmet needs in the management of LN.

The guidelines for LN treatment have been updated recently by the European Alliance of Associations for Rheumatology and Kidney Disease Improving Global Outcomes.[2 3] The initial phase of treatment is termed the induction phase, which is followed by a prolonged maintenance phase of treatment to achieve durable remission, and limit the risk of LN flare. Maintenance therapy lasts 2–3 years or longer, depending on the risk of relapse. Mycophenolate mofetil (MMF) and azathioprine (AZA) are commonly used in maintenance therapy.

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The long-term use of these drugs is associated with considerable toxicity and is not effective in all patients.

Leflunomide (LEF) is a prodrug that is rapidly converted to its active metabolite A771726, which inhibits de novo pyrimidine nucleotide biosynthesis mediated especially by dihydroorotate dehydrogenase, thereby preventing DNA synthesis. LEF is a recommended disease-modifying anti-rheumatic drug for the treatment of rheumatoid arthritis. Its use has been reported in other autoimmune diseases, such as psoriatic arthritis, antineutrophil cytoplasmic autoantibody-associated vasculitis, SLE and Takayasu disease.[4] Preclinical studies found that LEF reduced the amount of autoantibodies and immune complex deposits on glomeruli in MRL/lpr mice.[5 6] A couple of clinical trials have evaluated LEF in the treatment of immune-related kidney diseases. The results showed that the efficacy of LEF was non-inferior to cyclophosphamide (CYC) as induction therapy for LN,[7] and it was also effective in immunoglobulin A nephropathy by improving kidney function while decreasing loss of urine protein.[8]

Here, we reported the results of a 36-month study comparing LEF and AZA as maintenance therapy for LN patients who showed a complete response (CR) or partial response (PR) to induction therapy with the NIH-CYC regimen. The results provided the first evidence supporting that LEF may be an effective and safe choice for maintenance therapy in patients with LN.

METHODS

Study design

We conducted a prospective, multicentre, randomised, open-label trial comparing LEF with AZA for the maintenance of remission in patients with LN. The study comprised two phases. In phase 1, active biopsy-proven LN patients were recruited and treated with the standard NIH-CYC regimen for induction therapy. After 6–9 months of the induction phase, those who achieved CR or PR were admitted into the second maintenance phase. Patients were randomised into the LEF group or AZA group. Criteria for CR included the following: 24-hour urine protein quantity <0.5 g/24 hours, inactive urinary sediment (red blood cell (RBC) <5/high-power field (HPF), white blood cell (WBC) <5/HPF), normal serum albumin and improved or stabilised kidney function (serum creatinine (SCr) change was within ±25% of baseline value). PR was defined as significant improvement in 24-hour urine protein (at least a 50% decrease in the 24-hour urine protein to <3 g/24 hours if the baseline urine protein was >3.5 g/24 hours, or to ≤1 g/24 hours if the baseline urine protein did not reach the level of nephrotic syndrome), serum albumin ≥30 g/L and stable or improved kidney function (SCr change was within ±25% of baseline value). The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice principles. Details of the protocol are available in the online supplementary methods.

Study participants

For the first induction phase of the study, patients with active LN were recruited. The inclusion criteria were: age 18–65 years, SLE according to the American College of Rheumatology classification criteria,[9] biopsy-proven class III/IV/V active LN diagnosed by International Society of Nephrology/Renal Pathology Society 2003 (biopsy performed less than 3 months before study entry), 24-hour proteinuria ≥1 g and SLE Disease Activity Index (SLEDAI) score ≥8. The exclusion criteria were treatment with CYC within 3 months, pulse intravenous glucocorticoids (GCs) (methylprednisolone: >200 mg/day) within 6 weeks, severe infection, severely abnormal kidney function with estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2, pregnant, breast feeding, previous malignancy, previously documented allergy to CYC, AZA or LEF (see online supplementary methods, p5–p6). Patients who showed a clinical response (CR or PR) 6–9 months after induction treatment were randomly assigned (in a 1:1 ratio) to AZA or LEF groups in the subsequent maintenance phase of the study.

Randomisation and masking

Patients fulfilling the inclusion/exclusion criteria were allocated to the LEF or AZA group by randomisation. Randomisation was performed using a computerised, interactive voice-response system with stratification according to centre, age, gender and kidney biopsy classification. This is an open label study without masking.

Intervention and assessment schedule

During the induction phase, all patients received intravenous pulse CYC therapy (0.5–1 g/m^2) once a month for 6 months combined with oral GCs (with an initial dose equivalent to 1 mg prednisone/kg/d for 4 weeks that was tapered by 10% every 2 weeks to no more than 10 mg/day at the end of the induction phase). If necessary, induction therapy was extended to 9 months for those who showed inadequate clinical response after 6 months of treatment.

During the maintenance phase, patients were randomised to receive LEF (Airuohua) (20 mg/d) or AZA (initial dose 50 mg/d, target dose 100 mg/d). Patients received prednisone or its equivalent (maximum dose, 10 mg per day) with dose reduction based on the investigator's judgement. The protocol suggested that the GC dose be reduced to 7.5 mg/day at months 9–12 and 5 mg/day at months 12–15. Patients were assessed every 2 months until month 12, followed by every 4 months until month 36, early withdrawal, or termination due to treatment failure.

OUTCOMES

The primary endpoint was the time to kidney flare during 36 months of maintenance-phase follow-up. A kidney flare was defined as (i) the recurrence or development of nephrotic syndrome (24 hours proteinuria ≥3.5 g and serum albumin <30 g/L), (ii) abnormal kidney function (>30% increase in SCr within 1 month directly attributed to lupus and confirmed 2 weeks later, or (iii) 2-fold increase in proteinuria (24 hours proteinuria >1 g in patients with CR or doubling of proteinuria in patients with PR at the end of induction). A kidney flare could occur with or without new or increased haematuria (≥5 RBC / HPF) or the appearance of cellular casts.

Key secondary endpoints included the number of patients achieving CR; kidney-associated variables, including 24 hours proteinuria, SCr and serum albumin over time; frequency of extrarenal flares; immunologic variables (C3, C4, and anti-double-stranded DNA antibodies); and safety profile in each group. Disease activity was measured by the SLEDAI-2000 (SLEDAI-2K) scoring system.[10]

Sample size

This study was designed as a non-inferiority trial. The non-inferiority margin was set at 12% for the primary outcome (flare at 36 months of maintenance-phase follow-up), meaning that the lower bound of the two-sided 95% CI for the difference in flare rates between LEF and AZA (as reference) should exceed −12%.

A previous study in patients with SLE reported flare rates of 15% in the LEF arm and 20% in the AZA arm. Assuming that the flare rates in LEF and AZA groups at 36 months would differ by 5%, a sample size of 158 patients was needed to yield a power of 80% and establish the non-inferiority of LEF to AZA, with a one-sided α level of 0.025. The sample size calculation made the conservative assumption that the dropout rate would be as high as 20%. Therefore, the required sample size was 200.

Patient and public involvement

See online supplementary methods section (page 13–14).

Statistical analysis

IBM-SPSS (version number: 25.0) was used for data statistics and analysis. The difference between groups for all data was considered significant at $p<0.05$. Details of the statistical analysis are available in the online supplementary methods.

RESULTS

Patients and treatments

270 biopsy proven active LN patients were treated with CYC regimen combined with GCs from seven centres in mainland China. After 6–9 months of the induction therapy, 215 patients achieved CR/PR (41 patients received an extended 9 month CYC treatment, and among them, 29 patients achieved clinical response (11 CR patients and 18 PR patients)). Detailed characteristics were listed in online supplementary table 1, and online supplementary figure 1). This intention-to-treat population was randomly assigned to the LEF group (n=108) or AZA group (n=107) for a 36 month maintenance therapy from August 2010 to November 2018. The demographics and baseline disease characteristics did not significantly differ between the two groups, as described in table 1. A total of 137 patients (63.7%) completed the 36 months of maintenance treatment: 72 (66.7%) in the LEF group and 65 (60.1%) in the AZA group (figure 1).

Table 1 Demographic and disease characteristics of patients at baseline of maintenance therapy

Characteristics	LEF group (N=108)	AZA group (N=107)
Age (year)	30.8±9.1	33.2±10.9
Female sex—no. (%)	98 (90.7%)	92 (86.0%)
Race or ethnic group—no. (%)		
Han	100%	100%
Body weight (kg)	56.2±8.3	55.8±7.5
Systolic BP (mm Hg)	123.8±10.4	122.7±10.0
Diastolic BP (mm Hg)	77.6±7.5	76.6±8.4
Duration of LN (months)	12.8±28.0	14.7±31.0
Clinical remission—no. (%)		
CR	69 (63.9%)	77 (72.0%)
PR	39 (36.1%)	30 (28.0%)
Kidney biopsy class—no. of patients (%)		
III or III+V	33 (30.6%)	29 (27.1%)
IV or IV+V	67 (62.0%)	62 (57.9%)
Pure V	8 (7.4%)	16 (15.0%)
Urinary protein (mg/24 hours)	542±502	451±426
Active urine sediment—no. of patients (%)	5 (4.6%)	9 (8.4%)
SCr (μmol/L)	67.2±20.8	66.8±19.0
Estimated GFR (mL/min/1.73 m^2)	132.6±44.0	132.7±38.3
Estimated GFR category—no. (%)		
≥60 mL/min/1.73 m^2	73 (98.6%)	75 (98.7%)
≥90 mL/min/1.73 m^2	63 (85.1%)	65 (86.7%)
Immunologic factors		
Serum C3 (mg/dL)	848±236	891±203
Serum C4 (mg/dL)	180±103	194±70
Patients receiving drugs at baseline		
Prednisone use (mg/day)	9.9±0.8	9.8±0.8
HCQ use—no. (%)	89 (82.4%)	93 (86.9%)
ACEI/ARB use—no. (%)	31 (28.7%)	26 (24.3%)
SLEDAI score	2.3±2.9	2.1±3.0

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blockers; AZA, azathioprine; BP, blood pressure; CR, complete response; GFR, glomerular filtration rate; Han, the Han nationality; HCQ, hydroxychloroquine; LEF, leflunomide; LN, lupus nephritis; PR, partial response; SCr, serum creatinine; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

Treatments

Most patients received 20 mg/day of LEF or 100 mg/day of AZA in the maintenance phase (mean body weight in AZA group was 55.8 kg (±7.5 kg) and mean dose of AZA was 1.5–2 mg/kg/day). For 14 patients in the LEF group, the dosage was temporally reduced to 10 mg/day due to adverse events (AEs) (mild elevation in liver enzymes or decrease in white blood cells) but returned to 20 mg/day within 2 months. For 9 patients in the AZA group, the dosage was temporarily reduced to 50 mg/day due to AEs but increased to 100 mg/day shortly after.

At baseline, the mean dosage of GCs was approximately 10 mg/day (prednisone or equivalent) (table 1). Patients in both groups underwent GC dosage reduction to 7.5 mg/day and 5 mg/day afterward. The proportion of patients treated with 5 mg/day GCs was 86.3% in the LEF group (69/80) and 94.7% in the AZA group (71/75) at 24 months. At 36 months, 24 patients in the LEF group and 18 patients in the AZA group had their GC dosage further decreased to 2.5 mg/day.

Study endpoints

The time to kidney flare, the primary endpoint of the study, was compared between the groups using Kaplan-Meier survival curves. Time to kidney flare was not statistically different in the LEF group (17/108 patients, 15.7%; median time: 16 months) compared with that in the AZA group (19/107 patients, 17.8%; median time 14 months) during the 36 months of follow-up (figure 2). During the first 6 months, 5 in the LEF group and 5 in the AZA group experienced kidney flare. Afterward, there were around four–five cases with kidney flare per year in both groups.

One patient from the LEF group and 3 patients from the AZA group met the criteria for a kidney flare based on the recurrence/development of nephrotic syndrome, and 16 from the LEF group and 16 from the AZA group were diagnosed with kidney flare based on proteinuria increases. Kidney flare combined with new or increased haematuria were found in 6 patients (3 in the LEF group and 3 in the AZA group, respectively). In both groups, no kidney flare event was based on abnormal kidney function.

Key secondary endpoints were also comparable between LEF and AZA groups. The proportion of patients who achieved and maintained CR over 36 months was similar between LEF and AZA groups (61 (56.4%) in the LEF group vs 58 (54.2%) in the AZA group).

For other kidney-associated parameters, there were no significant differences between LEF and AZA groups with respect to 24-hour proteinuria, serum albumin, SCr and eGFR over a 3-year period (figure 3A–D and online supplementary table 2). Sustained doubling of SCr or kidney failure was not observed in both groups. Subgroup analysis revealed that patients who had

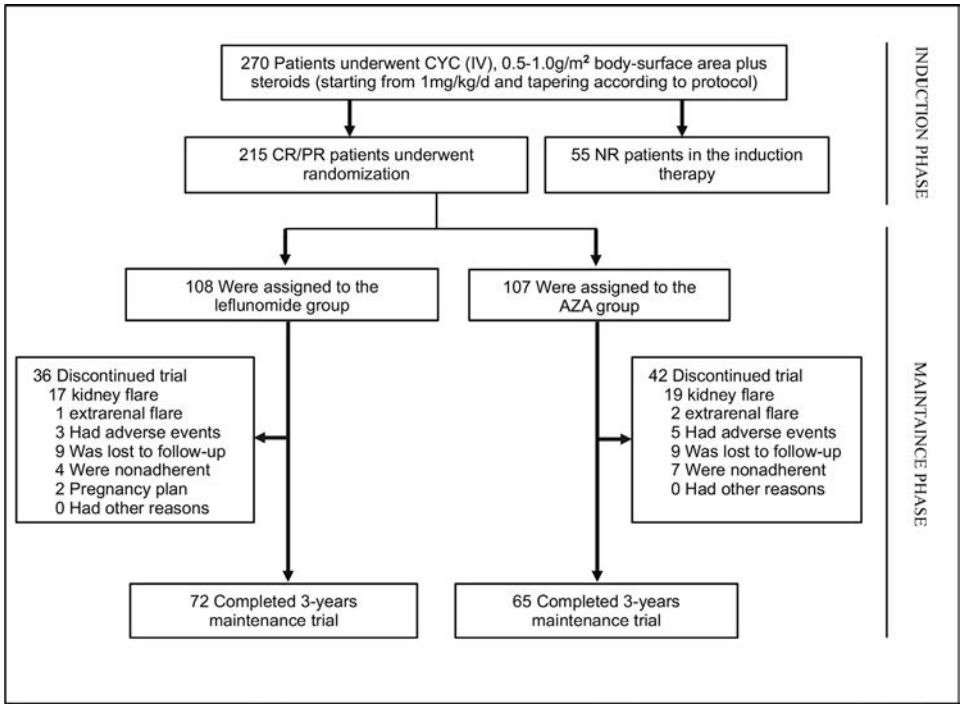

Figure 1 Enrolment and randomisation. AZA, azathioprine; CR, complete response; CYC, cyclophosphamid; NR, no response; PR, partial response.

CR at baseline during the remission phase appeared to have a lower risk of kidney flare if they were allocated to the LEF group (6.7%) compared with the AZA group (14.3%), but the difference was not statistically significant.

Regarding extrarenal flare, there was one case in the LEF group and two cases in the AZA group. For the case in the LEF group, the patient had headache, arthritis and fever, with a SLEDAI score of 13. In the AZA group, one case presented with rash and vasculitis (SLEDAI score=12), and the other case showed rash, arthritis and a low platelet count (SLEDAI score=11). Disease activity represented by SLEDAI scores and C3 and C4 levels did not differ over time between the two groups (figure 3E and F and online supplementary table 2).

Safety and tolerability

There was no difference between the two groups in terms of the incidence of AEs: 56.5% (61 of 108 patients) in the LEF group and 58.9% (63 of 107 patients) in the AZA group (table 2). There were no events of death, severe infection or malignancy in the study. There was no serious AE during the study. Haematological abnormality and liver dysfunction were the most common AEs in both groups. However, most AEs were mild, and patients recovered after routine management. The proportion of patients with AEs leading to permanent treatment discontinuation was similar between the LEF group (2/108 patients: 1 case of leucopenia and 1 case of liver dysfunction) and AZA group (5/107 patients: 3 cases of leucopenia, 1 case of thrombocytopenia and 1 case of liver dysfunction).

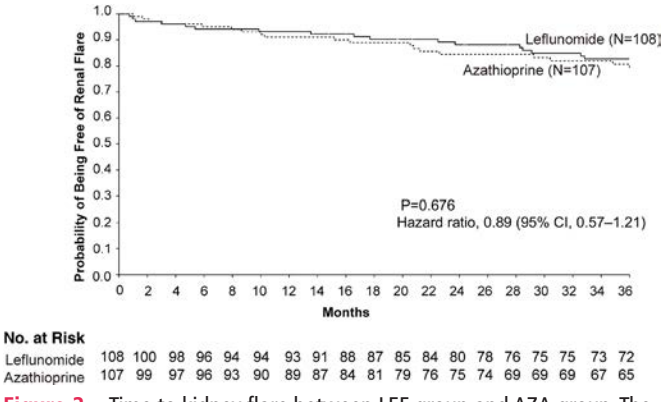

Figure 2 Time to kidney flare between LEF group and AZA group. The primary end point of the study was compared by using Kaplan-Meier survival curves. AZA, azathioprine; LEF, leflunomide.

Long-term extended follow-up

After the 3-year study, many patients maintained in remission and continued to be followed up. For those in sustained remission, immunosuppressive drugs were further tapered or stopped. For LEF, the dosage was gradually reduced from 10 mg/day to 10 mg every other day. Similarly, AZA was reduced from 50 mg/day to 50 mg every other day. The target GC dosage was 2.5 mg/day (prednisone or equivalent). Patients were not encouraged to stop GCs.

90 patients continued using study drugs for more than 4 years, including 48 in the LEF group and 42 in the AZA group. The reasons that patients stopped LEF or AZA treatment included kidney flare (7 in the LEF group from the 4th–6th year and 6 in the AZA group), intention for pregnancy (6 in the LEF group and 2 in the AZA group), sustained remission and lost to follow-up. At the end of 5 years, 37 patients continued LEF or AZA treatment (22 in the LEF group and 15 in the AZA group), and 19 patients had been treated for more than 6 years (10 in the LEF group and 9 in the AZA group). There was no kidney failure event during the study. Only one patient stopped AZA because of intolerance during the extended follow-up, suggesting the long-term safety of both LEF and AZA.

DISCUSSION

Maintenance therapy is important in the treatment of LN and SLE disease. The aim of maintenance therapy is to consolidate

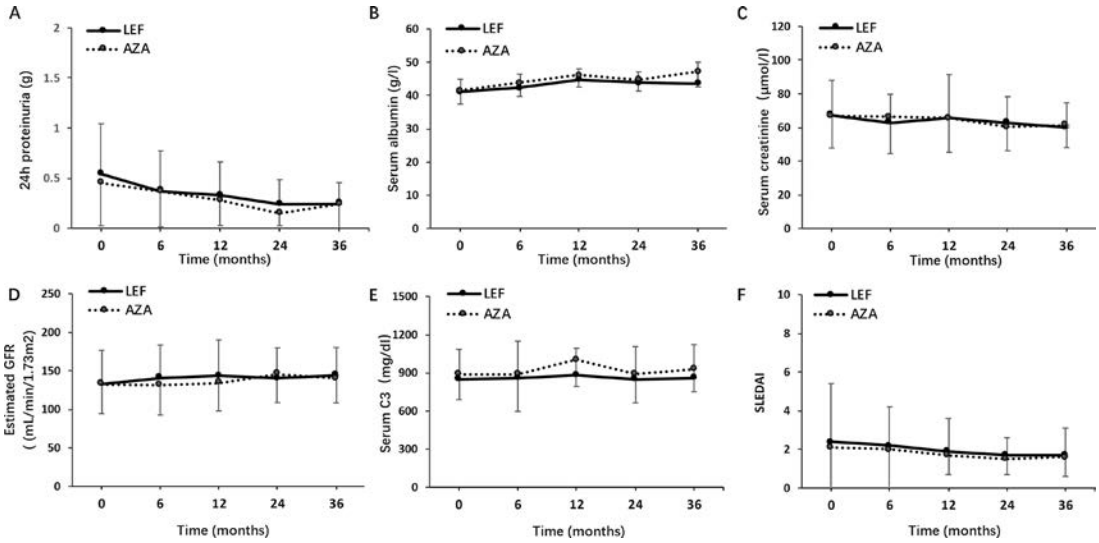

Figure 3 **Change from baseline in laboratory parameters**. The differences in 24-hour proteinuria (A), serum albumin (B), SCr (C), eGFR (D), serum C3 (E) and SLEDAI (F) over a 3-year period between LEF and AZA groups were analysed. AZA, azathioprine; eGFR, estimated glomerular filtration rate; LEF, leflunomide; SCr, serum creatinine; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

responses into durable complete remissions and limit the risk of disease flare-up.[11] It is well recognised that sustained remission effectively reduces cumulative damages and improves the quality of life for patients with SLE. In the current study, we compared the time to and rate of kidney flare between patients in LEF and AZA groups after they achieved CR or PR with initial CYC-based induction therapy. In our study, the rate of kidney flare was 15.7% in the LEF group and 17.8% in the AZA group during the 36 months of follow-up. In the previous 3-year maintenance study in Aspreva Lupus Management Study (ALMS) patients, kidney flares were observed in 15 of 116 patients given MMF (12.9%) compared with 26 of 111 patients given AZA (23.4%). MMF was significantly more effective than AZA in the 3-year maintenance treatment.[12] In contrast, MMF was not superior to AZA in the MAINTAIN Nephritis Trial, in which the two drugs were compared after a short course of the Euro-CYC regimen. Kidney flare occurred in 19% of patients in the MMF group (10/53) compared with 25% in the AZA group (13/52) after a mean follow-up of 4 years.[13] During a 10-year follow-up, the MAINTAIN Trial did not reveal an advantage of MMF over AZA as maintenance therapy for LN.[14] Therefore, compared with the previous two maintenance studies of LN, the rate of kidney flare in our cohort appeared to be lower, particularly in the AZA group, but still comparable. The reason behind this discrepancy might be as follows. (1) All participants in our study were Chinese compared with the 100% Caucasian cohort in the MAINTAIN study and ~70% non-Asian ancestry patient population in the ALMS study. Racial differences may partially account for treatment responses. (2) Patients in our study were given more vigorous induction therapy with higher CYC dosages and thus might have been in a more stable condition when enrolled. At baseline, the mean 24-hour urinary protein was ~500 mg/24 hours in the current study, which was notably lower than that in the ALMS study (906±819.93 mg/24 hours in the MMF group and 820.0±754.33 mg/24 hours in the AZA group). As an early proteinuria response is associated with favourable long-term kidney outcomes, the baseline disease status likely contributes to the future risk of kidney flares.

LN is a disease with significant unmet clinical needs. In addition to the increasing list of new medications introduced into this field, drug repurposing has also attracted substantial interest. LEF has been extensively used in the treatment of rheumatoid arthritis worldwide, with a good safety profile and long-term use experience. In the current study, LEF was non-inferior to AZA in terms of effectiveness and AEs in the long-term treatment of patients with LN. Our findings support LEF as a potential candidate treatment for LN during the maintenance phase. The 6 years of data provide evidence that LEF is not only effective in controlling kidney and extrarenal flares but is also quite safe and well tolerated. Transient liver dysfunction and mild leucopenia were common AEs. Compared with calcineurin inhibitors, kidney injury was rarely reported for LEF, supporting its extended use in patients with kidney diseases.[15] Pregnancy is a concern with LEF treatment. Patient dropouts because of pregnancy or pregnancy planning were more frequently observed in the LEF arm compared with the AZA arm. For patients wanting to conceive, administering cholestyramine could effectively remove the drug from the body.[16]

Table 2 Summary of patients with AEs over the 3 year study.

Safety population, n (%)	LEF	AZA
Any AEs	61 (56.5%)	63 (58.9%)
AEs occurring in ≥5% of patients in either treatment group		
Leucopenia	31 (28.7%)	31 (29.0%)
Anaemia	13 (12.0%)	13 (12.1%)
Thrombocytopenia	7 (6.5%)	6 (5.6%)
Elevated liver enzymes	23 (21.3%)	22 (20.6%)
Irregular menstruation or amenorrhoea	7 (7.1%)	5 (5.4%)
Any grade 3 AEs		
Leucopenia	0	1 (0.9%)
Cerebrovascular accident	0	1 (0.9%)
Elevated liver enzymes	4 (3.7%)	2 (1.9%)
Any AEs leading to permanent treatment discontinuation		
Leucopenia	1 (0.9%)	3 (2.8%)
Elevated liver enzymes	1 (0.9%)	1 (0.9%)
Thrombocytopenia	0	1 (0.9%)

AE, adverse events; AZA, azathioprine; LEF, leflunomide.

Adding LEF to the LN treatment strategy is of clinical significance. First, only a few clinical randomised controlled trials have investigated maintenance therapy for LN, and they required long-term follow-up and were limited by a low frequency of events. The current study provides a relatively high level of evidence supporting LEF in the maintenance treatment of LN with comparable efficacy to the standardised regimen of AZA. We recognise the increasing use of MMF as the first-line treatment for LN, and the ALMS study supported the superiority of MMF over AZA in the maintenance therapy for LN,[12] despite the negative findings from the MAINTAIN study. However, they should not prevent the use of AZA or the potential use of LEF in LN treatment because MMF is not appropriate for all patients. For example, the significantly increased risk of infection remains a concern for MMF use in Asians, therefore, most of our patients could not tolerate the recommended dosage of MMF for induction therapy (up to 3 g/day).[17 18] The dose of MMF used in ALMS study was 2 g/day, while the recommended dosage of MMF for maintenance therapy was 1–2 g/day.[2 3] This might potentially limit the performance of MMF in real-world practice as compared with that in the clinical trial.[19] Second, LEF is a drug with a new mechanism of action in the treatment of LN. Thus, LEF might improve the effectiveness of LN treatment and potentially act as an adjunct therapy or a candidate for combination/multitarget therapy. Although it is beyond the scope of this study, investigating combination therapies in future studies is intriguing. Finally, LEF has several advantages, including easy accessibility, long-term safety profile and cost effectiveness, that may benefit patients, especially those in developing countries with limited access to new drugs or with tolerance and efficacy issues with current drugs.

There are several limitations to the current study. First, the study was an open-label study, not a double-blinded trial. However, the primary outcome (kidney flare) was strictly defined by objective lab examination results and, therefore, unlikely to have been influenced by the open-label design. Second, the current study is a multicentre study based in mainland China. Whether the results can be verified in patients from other ethnic groups requires larger international studies. Third, the trial was designed for 3 years. Therefore, it is still too early to conclude the long-term effect of LEF in terms of hard outcomes, such as death and kidney failure. However, according to our experience, no patients in the study population have developed kidney failure.

In summary, to the best of our knowledge, this multicentre, randomised-controlled, open-label study is the first to report the non-inferiority of LEF to AZA for the maintenance therapy of LN in terms of its efficacy and safety profiles. Therefore, LEF may provide a candidate drug in the treatment of LN.

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Correction notice This article has been corrected since it published Online First. Affiliation number 4 has been corrected.

Contributors LL and CB wrote the first draft of the study protocol. JX, LS, XZ, LH, ZL and LD provided modification comments and final approval. CW, MD and SW were responsible for data collection. QF, CW and MD were responsible for data management and statistical analysis. QF, LL and CB accessed and verified the underlying data. The first draft of the manuscript was completed by QF and CW. All authors reviewed the subsequent manuscripts and approved the final version. LL and CB are the guarantors.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

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CLINICAL SCIENCE

Phase 3, multicentre, randomised, placebo-controlled study evaluating the efficacy and safety of ustekinumab in patients with systemic lupus erythematosus

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ABSTRACT

Objective Evaluate the efficacy and safety of ustekinumab, an anti-interleukin-12/23 p40 antibody, in a phase 3, randomised, placebo-controlled study of patients with active systemic lupus erythematosus (SLE) despite receiving standard-of-care.

Methods Active SLE patients (SLE Disease Activity Index 2000 (SLEDAI-2K) ≥6 during screening and SLEDAI-2K ≥4 for clinical features at week 0) despite receiving oral glucocorticoids, antimalarials, or immunomodulatory drugs were randomised (3:2) to receive ustekinumab (intravenous infusion ~6 mg/kg at week 0, followed by subcutaneous injections of ustekinumab 90 mg at week 8 and every 8 weeks) or placebo through week 48. The primary endpoint was SLE Responder Index (SRI)-4 at week 52, and major secondary endpoints included time to flare through week 52 and SRI-4 at week 24.

Results At baseline, 516 patients were randomised to placebo (n=208) or ustekinumab (n=308). Following the planned interim analysis, the sponsor discontinued the study due to lack of efficacy but no safety concerns. Efficacy analyses included 289 patients (placebo, n=116; ustekinumab, n=173) who completed or would have had a week 52 visit at study discontinuation. At week 52, 44% of ustekinumab patients and 56% of placebo patients had an SRI-4 response; there were no appreciable differences between the treatment groups in the major secondary endpoints. Through week 52, 28% of ustekinumab patients and 32% of placebo patients had a British Isles Lupus Assessment Group flare, with a mean time to first flare of 204.7 and 200.4 days, respectively. Through week 52, 70% of ustekinumab patients and 74% of placebo patients had ≥1 adverse event.

Conclusions Ustekinumab did not demonstrate superiority over placebo in this population of adults with active SLE; adverse events were consistent with the known safety profile of ustekinumab.

Trial registration number NCT03517722.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ An unmet need remains for improved treatment options for patients with systemic lupus erythematosus (SLE), who continue to experience a high disease burden. A phase 2 study of ustekinumab, a monoclonal antibody inhibiting the interleukin-12/23 p40 subunit, demonstrated efficacy in patients with active SLE.

WHAT THIS STUDY ADDS

⇒ In the phase 3 LOTUS study of ustekinumab in patients with active SLE, the primary and major secondary endpoints were not achieved; thus, there was insufficient evidence to support continuation of this study. Safety results were consistent with the known safety profile of ustekinumab.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The LOTUS results add to the body of research in SLE treatments and improve the understanding of the pathogenesis of SLE. Additionally, aspects of the LOTUS study design may be useful in optimising future studies of SLE treatments.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a heterogeneous and biologically complex chronic autoimmune disease that can present with a wide-ranging constellation of symptoms affecting multiple organ systems, with patients commonly experiencing arthralgia/arthritis and skin rashes.[1] Conventional therapies include oral glucocorticoids, antimalarial and/or immunosuppressive therapies to control inflammation. Therapies approved more recently are belimumab, a monoclonal antibody targeting B lymphocyte stimulator,[2] anifrolumab, a type 1 interferon (IFN) receptor antagonist,[3] and voclosporin in lupus nephritis, an immunosuppressant inhibiting calcineurin.[4] Advances in general medical care have resulted in improved outcomes in these patients; however, disease burden with SLE remains high with patients often experiencing significant work disability and an increased risk of mortality compared with the general population.[5–7]

The aetiology of SLE remains unclear, with several molecular pathways implicated in the pathogenesis of this disease. Elevated levels of interleukin (IL)-12 and IL-23 have been found in serum and tissue samples from patients with SLE,[8–10] with expression of the shared p40 subunit being upregulated in untreated SLE patients in comparison with treated patients.[11] Ustekinumab, a monoclonal antibody inhibiting the IL-12/23 p40 subunit,[12] is approved for patients with moderate-to-severe plaque psoriasis and active psoriatic arthritis and was identified in a previous meta-analysis as being a top candidate for repositioning in SLE.[13]

The efficacy and safety of ustekinumab in patients with active SLE was evaluated in a phase 2, randomised, placebo-controlled study.[14 15] Among patients who entered the optional long-term extension, greater proportions of ustekinumab-treated patients achieved an SLE Responder Index (SRI)-4 composite response at week 24 compared with placebo, and response rates were maintained through 2 years.[16] Here, we report the efficacy and safety results of the subsequent phase 3, randomised, placebo-controlled study (LOTUS; ClinicalTrials.gov: NCT03517722) of ustekinumab in patients with active SLE.

METHODS

Patients

Eligible patients were aged 16–75 years (inclusive) with a diagnosis of SLE and a documented history of meeting the Systemic Lupus International Collaborating Clinics classification criteria for SLE ≥3 months prior to first study agent administration. Patients had active SLE (screening SLE Disease Activity Index 2000 (SLEDAI-2K) ≥6 and baseline SLEDAI-2K ≥4 for clinical features) despite receiving stable doses of ≥1 of the following: oral glucocorticoids (≤20 mg/day prednisone or equivalent), antimalarials (≤250 mg/day chloroquine, ≤400 mg/day hydroxychloroquine) or immunomodulatory drugs (mycophenolate mofetil ≤2 g/day, mycophenolic acid ≤1.5 g/day, azathioprine/6 mercaptopurine ≤2 mg/kg/day, oral methotrexate (MTX) ≤25 mg/week, or subcutaneous or intramuscular MTX ≤20 mg/week). All patients had to have ≥1 previous well-documented unequivocally positive test for ≥1 of the following: antinuclear, anti-dsDNA, or anti-Smith antibodies as well as ≥1 positive test result during screening. Other inclusion criteria included: ≥1 British Isles Lupus Assessment Group (BILAG)[17] A and/or ≥2 BILAG B domain scores at screening and Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI)[18] activity score ≥4 or≥4 joints with pain and signs of inflammation (active joints) at screening and/or week 0.

Concomitant use of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, nonsteroidal anti-inflammatory drugs, or other analgesics, or select topical medications for cutaneous disease was permitted at stable doses. Patients were excluded if they had any unstable or progressive manifestation of SLE (eg, active class III or IV glomerulonephritis, systemic vasculitis, or active central nervous system involvement) or other inflammatory diseases that might confound efficacy assessments. Patients could not have received previous treatment with systemic immunomodulatory drugs; adrenocorticotropic hormone; oral or intravenous cyclophosphamide or intravenous cyclophosphamide; B-cell targeted therapies or B-cell depleting therapy (or have evidence of continued B-cell depletion following such therapy); immunomodulatory biological therapy within prespecified timeframes prior to screening or study agent administration. All patients were naïve to ustekinumab.

Study design

Patients were randomised (3:2) to ustekinumab (intravenous infusion of ~6 mg/kg at week 0, then subcutaneous injections

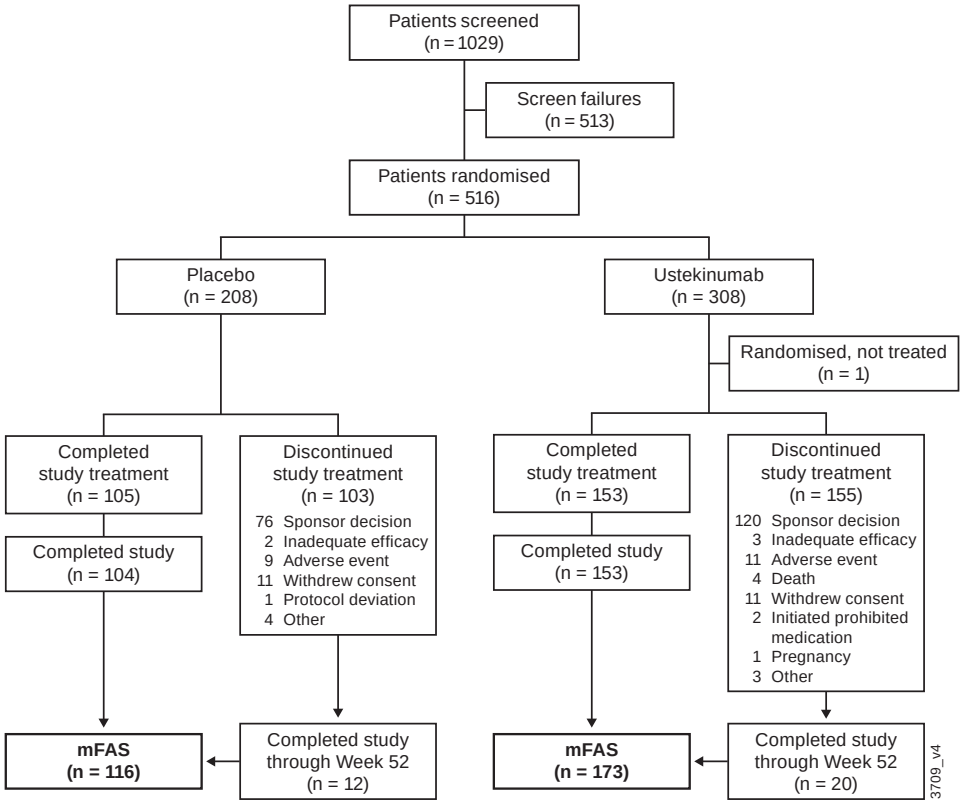

Figure 1 Patient disposition of LOTUS participants. mFAS, (including patients who either completed their week 52 visit or would have had a week 52 visit at the time of study discontinuation by the sponsor). mFAS, modified full analysis set.

of ustekinumab 90 mg at week 8 and every 8 weeks thereafter) or placebo (infusion at week 0, then subcutaneous injections at week 8 and every 8 weeks) with crossover to ustekinumab at week 52. The planned study duration included study agent administration through week 160, with safety follow-up through week 176. However, following a prespecified interim efficacy analysis, the study was discontinued early due to lack of efficacy.

Randomisation included the following stratification factors: race (white, black or other), presence of lupus nephritis (ever; yes/no), composite of baseline SLE medications and SLEDAI-2K score (high medications and SLEDAI-2K≥10, high medications and SLEDAI-2K<10, medium medications and SLEDAI-2K≥10, medium medications and SLEDAI-2K<10). High medication use was defined as receiving any of the following: ≥15 mg/week MTX, or ≥1.5 mg/kg/day azathioprine/6 mercaptopurine, or ≥1.5 g/day mycophenolate mofetil/≥1.125 g/day mycophenolic acid, and/or ≥15 mg/day prednisone or equivalent; all other medication use was classified as medium.

Assessments

Global clinical efficacy was assessed using the SRI-4 composite response: ≥4 points reduction in SLEDAI-2K score, no new BILAG A or no more than 1 BILAG B domain score, and no worsening (<10% worsening from baseline) of physician global assessment, without meeting the treatment failure criteria. Other assessments included active joint assessment (tender and swollen joints and signs of inflammation), CLASI activity score for mucocutaneous disease, the Physical and Mental Component Summary scores of the Medical Outcomes Study 36-item Short-Form Health Survey (SF-36 PCS/MCS; minimal clinically important difference (MCID): change ≥2.5)[19] and the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue; MCID: change ≥4) score for fatigue. SLE flares were assessed using the BILAG with a severe flare defined as ≥1 new BILAG A domain score and a moderate flare defined as ≥2 new BILAG B domain scores.

Safety was monitored throughout the study through adverse event (AE) reporting and routine blood chemistry and haematology tests. Blood samples were collected at regular intervals for assessing the pharmacokinetics and pharmacodynamics of ustekinumab and the presence of antibodies to ustekinumab. Serum levels of pharmacodynamic markers were assessed using the Meso Scale Discovery platform (IFNγ and p40), Quanterix's single molecule array (Simoa) technology (IFNα), and the high-sensitivity Single Molecule Counting Erenna Immunoassay (IL-17F and IL-22) in a representative biomarker subgroup. Samples from demographically matched healthy subjects (n=30) were procured independently (BioIVT, Westbury, NY) as a control group for biomarker analyses. Antibodies to ustekinumab were assessed using a validated drug-tolerant electrochemiluminescent immunoassay.

Statistical methods

The primary endpoint was the proportion of patients achieving an SRI-4 composite response at week 52. Secondary endpoints were to be tested in a hierarchical manner as follows: time to first flare (≥1 new BILAG A or ≥2 new BILAG B scores) through week 52, proportion of patients with an SRI-4 composite response at week 24, proportion of patients with joint response (≥50% improvement in active joints) at week 52 in patients with ≥4 affected joints at baseline, proportion of patients who achieved a reduction in glucocorticoid dose at week 40 and sustained that reduction through week 52 in patients receiving glucocorticoids at baseline, proportion of patients with CLASI response (≥50% improvement in CLASI activity score) at week 52 in patients with a baseline CLASI ≥4, and proportion of patients who achieved reduction in glucocorticoid dose at week 40 and sustained that reduction through week 52 and achieved SRI-4 composite response at week 52.

For the primary and binary major secondary endpoints, patients with missing data or those meeting ≥1 treatment failure criteria were classified as non-responders. Treatment failure criteria were as follows: increase in baseline dose or initiation of permitted SLE medications between weeks 12 and 52, initiation of a protocol-prohibited medication, or discontinuation of study agent for any reason before week 52. Continuous endpoints were analysed using a mixed model for repeated measures to test differences between treatment groups and adjust for missing data. The models included baseline SLEDAI score as a covariate and treatment, baseline medication use for SLE (high, medium), race, visit, and an interaction of treatment and visit as fixed effects.

The planned sample size of 500 patients (ustekinumab, 300; placebo, 200) would yield ~98% power to detect a significant difference in SRI-4 response rates at week 52 in the two treatment groups assuming response rates of 35% in the placebo group[15 20] and 53% in the ustekinumab group. This assumption in response rates would yield an absolute difference of 18% over placebo or an OR of 2.09 with an alpha level of 0.05.

A preplanned interim analysis was performed by an independent data monitoring committee 24 weeks after ~50% of the planned enrollment had been randomised. If the proportion of patients achieving an SRI-4 composite response in the ustekinumab group was ≥2% greater than that in the placebo group, then the study would continue without modification.

The prespecified efficacy analyses were intended to include the full analysis set (FAS; all randomised patients who received ≥1 dose of study agent); however, on study discontinuation by the sponsor, efficacy analyses were performed using the modified FAS (mFAS) and included only patients who either completed their week 52 visit or would have had a week 52 visit at the time of study discontinuation by the sponsor. Sensitivity analyses assessed the primary endpoint in subpopulations defined by baseline characteristics: sex, age, weight, body mass index, geographical region, race, ethnicity, SLE medication use, presence of lupus nephritis, SLEDAI-2K score, PGA score, urine protein/creatinine ratio, C3 and C4 levels, and anti-dsDNA status.

Safety analyses included all patients who received ≥1 administration of study agent. The incidence of antibodies to ustekinumab was summarised for all patients who received ustekinumab and had ≥1 available serum sample (post-ustekinumab administration). Pharmacokinetic and pharmacodynamic analyses included patients who received ≥1 dose of ustekinumab (partial or complete; IV or SC) and had ≥1 available blood sample (post-ustekinumab administration).

Patient and public involvement

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

RESULTS

Patient disposition and baseline characteristics

LOTUS was conducted at 140 sites in 20 countries. Of 1029 patients screened, 516 were randomised to placebo (n=208) or ustekinumab (n=308) (figure 1). Following the preplanned interim analysis, the futility criteria were met, and the sponsor

Table 1 Baseline demographic and disease characteristics

	All randomised patients		mFAS*	
	Ustekinumab	Placebo	Ustekinumab	Placebo
Patients, n†	308	208	173	116
Female	291 (94.5)	191 (91.8)	165 (95.4)	108 (93.1)
Age	42.9±11.4	44.5±12.3	43.4±11.4	45.8±11.3
Race				
White	208 (67.5)	136 (65.4)	130 (75.1)	86 (74.1)
Black	24 (7.8)	18 (8.7)	14 (8.1)	10 (8.6)
Asian	57 (18.5)	46 (22.1)	22 (12.7)	20 (17.2)
Disease duration (years)	8.8±8.0	9.1±7.6	8.8±8.6	9.4±7.7
SLEDAI-2K (0–105)	10.4±3.4	10.5±3.7	10.5±3.8	10.5±3.7
Physician's global assessment (VAS, 0–3)	1.8±0.4	1.8±0.4	1.8±0.5	1.8±0.4
BILAG				
≥1 BILAG A	144 (46.8)	79 (38.0)	71 (41.0)	43 (37.1)
≥2 BILAG B	170 (55.2)	124 (59.6)	103 (59.5)	69 (59.5)
Tender joint count	15.0±11.4	13.9±10.4	16.6±12.1	14.8±11.0
Swollen joint count	9.1±6.8	8.4±6.4	9.8±6.9	8.7±6.3
Joints with both tenderness and inflammation	8.7±6.5	7.8±6.0	9.5±6.8	8.2±6.0
CLASI activity score (0–70)				
Patients, n	307	208	172	116
Mean±SD	8.4±6.8	7.9±6.4	7.6±6.0	8.0±5.4
ANA‡	282/302 (93.4)	189/204 (92.6)	155/167 (92.8)	105/113 (92.9)
Anti-dsDNA (>75 kIU/L)‡	113 (36.7)	77 (37.0)	59 (34.1)	36 (31.0)
Low complement‡				
C3	129 (41.9)	90 (43.3)	66 (38.2)	49 (42.2)
C4	79 (25.6)	57 (27.4)	35 (20.2)	26 (22.4)
Patients with lupus nephritis	52 (16.9)	48 (23.1)	31 (17.9)	23 (19.8)
Concomitant medications				
Oral glucocorticoids	249 (80.8)	163 (78.4)	140 (80.9)	92 (79.3)
Dose (mg/day)	9.7±4.8	9.6±5.5	9.3±4.5	8.8±4.6
Antimalarials	223 (72.4)	155 (74.5)	122 (70.5)	86 (74.1)

Data reported as n (%), n/N (%), or mean ± standard deviation unless otherwise noted.
*The mFAS included patients who either completed their week 52 visit or would have had a week 52 visit at the time of study discontinuation by the sponsor.
†Patients were enrolled at sites located in Argentina (7 sites), Bulgaria (3 sites), Canada (1 site), China (3 sites), Colombia (6 sites), Germany (4 sites), Hungary (3 sites), Japan (18 sites), Lithuania (4 sites), Poland (8 sites), Portugal (1 site), Republic of Korea (3 sites), Russian Federation (7 sites), Serbia (7 sites), Spain (5 sites), South Africa (4 sites), Taiwan (5 sites), Thailand (5 sites), Ukraine (6 sites), USA (40 sites).
‡Analyses of ANA, anti-ds-DNA, C3, and C4 were performed by a central laboratory. The presence of ANA (determined as either positive or negative) was assessed using the Kallestad HEp-2 indirect fluorescent antibody method (Bio-Rad Laboratories). Anti-dsDNA was measured using the QUANTA Lite dsDNA SC ELISA (INOVA diagnostics) with the following reference values: negative defined as <30 IU/mL, borderline defined as 30–75 IU/mL, positive defined as >75 IU/mL. C3 levels were measured using Tina-quant complement C3c V.2 kit (Roche Diagnostics) with a reference range of 0.90–1.80 g/L. C4 levels were measured using the Tina-quant complement C4 V.2 kit (Roche Diagnostic) with a reference range of 0.1–0.4 g/L.
ANA, anti-nuclear antibodies; BILAG, British Isles Lupus Assessment Group; CLASI, Cutaneous Lupus Erythematosus Disease Area and Severity Index; dsDNA, double-strand DNA; mFAS, modified full analysis set; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; VAS, Visual Analogue Scale.

discontinued the study on 26 June 2020. Data for this report were collected from 3 May 2018 to 5 November 2020.

At the time of study discontinuation, 104 patients in the placebo group and 153 in the ustekinumab group had completed study participation through week 52 (figure 1). The mFAS comprised 116 placebo patients and 173 ustekinumab patients who had completed their week 52 visit or would have had a week 52 visit (based on their last scheduled visit) at the time the study was discontinued.

Baseline demographic and disease characteristics are shown in table 1. Among all randomised patients, the placebo group had a lower proportion of female patients and the mean age was higher when compared with the ustekinumab group. Patients in the placebo group had, on average, fewer active joints as well as greater proportions of patients with ≥2 BILAG B domain scores and lupus nephritis. In addition, the proportion of patients with ≥1 BILAG A domain score was higher in the ustekinumab group. Overall, the baseline demographic and disease characteristics of patients included in the mFAS were similar to those for the total study population (table 1).

Efficacy

The primary and major secondary endpoints were not achieved (figure 2). In the mFAS population, 44% of ustekinumab patients and 56% of placebo patients had an SRI-4 composite response at week 52 (figure 2). Sensitivity analyses of the primary endpoint in subpopulations defined by various demographic and disease characteristics were consistent with the mFAS (data not shown). Through week 52, 28% of patients in the ustekinumab group and 32% of patients in the placebo group had a BILAG flare, with a mean time to first flare of 204.7 and 200.4 days, respectively (figure 3). There were no appreciable differences between treatment groups in the response rates for SRI-4 at week 24 or

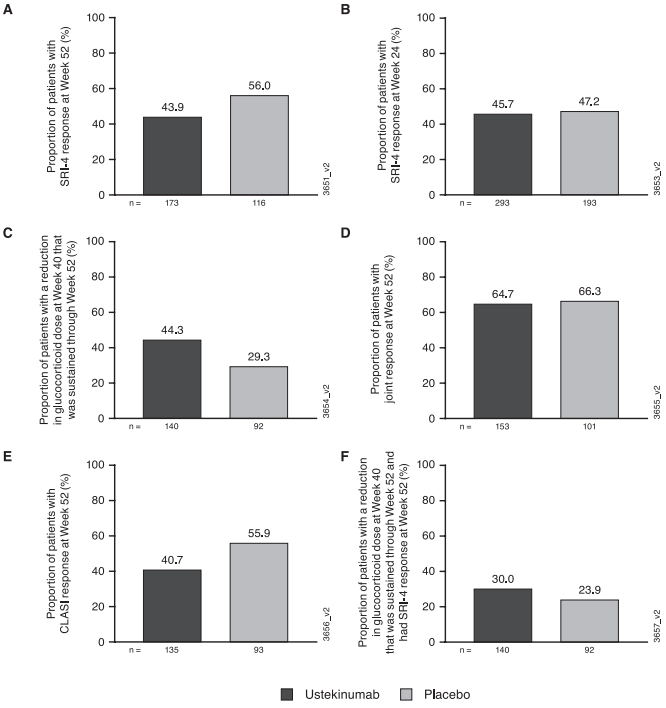

Figure 2 The proportion of patients achieving an SRI-4 composite response at week 52 (A) and week 24 (B), a reduction in glucocorticoid dose at week 40 that was sustained through week 52 (C), joint response at week 52 (D), CLASI response at week 52 (E), and a reduction in glucocorticoid dose at week 40 that was sustained through week 52 together with an SRI-4 composite response at week 52 (F). Analyses were performed using the modified Full Analysis Set population, excluding patients whose week 52 visit was projected to occur after the early study discontinuation by the sponsor. CLASI, Cutaneous Lupus Erythematosus Disease Area and Severity Index; SRI-4, Systemic Lupus Erythematosus Responder Index-4.

joint or CLASI activity improvement at week 52 (figure 2). In a post hoc analysis of the 197 patients who were not included in the mFAS population, 46% (55/120) of patients in the ustekinumab group and 34% (26/77) in the placebo group had an SRI-4 composite response at week 24 (nominal p=0.125).

Among patients receiving concomitant glucocorticoids at baseline, 44% of those in the ustekinumab group had a reduction in glucocorticoid dose at week 40 that was sustained through week 52 vs 29% of placebo patients (nominal p=0.040). There was a trend favouring ustekinumab in the proportion of patients with both a reduction in glucocorticoid dose at week 40 that was sustained through week 52 and an SRI-4 composite response at week 52 (30% vs 24%, nominal p=0.380). No treatment effect with ustekinumab was observed in an exploratory analysis of SRI-4 response rates at week 52 with patients stratified by week 40 glucocorticoid dose (≥7.5 mg or <7.5 mg) (data not shown).

Among patients in the mFAS, 11% in the placebo group and 9% in the ustekinumab group had a clinically meaningful improvement in FACIT-Fatigue score at week 52; 58% and 48%, respectively, had an improvement ≥MCID in SF-36 PCS score, and 43% and 38%, respectively, had an improvement ≥MCID in SF-36 MCS score.

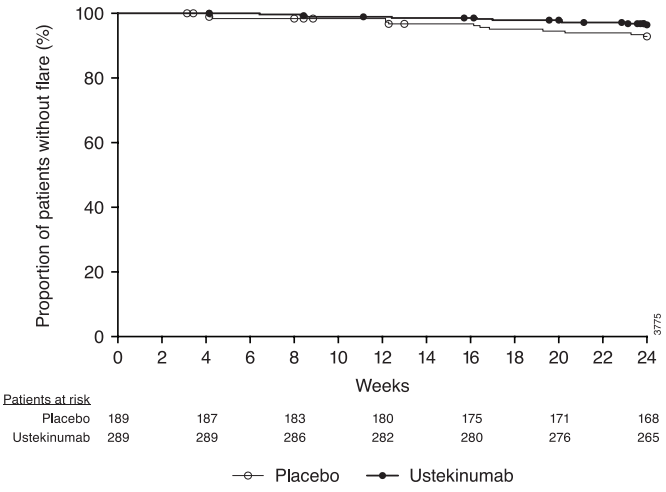

Figure 3 Time to first BILAG flare. BILAG, British Isles Lupus Assessment Group

Safety

Through week 52, 74% of placebo patients and 70% of ustekinumab patients reported ≥1 AE (table 2), with infections being the most common type (44% and 43%, respectively). Serious AEs occurred in 28 (13%) patients in the placebo group and 37 (12%) in the ustekinumab group; serious infections occurred in 8 (4%) and 15 (5%) patients, respectively (table 2). Serious infections reported in both treatment groups through week 52 were pneumonia (placebo, n=1; ustekinumab, n=4) and urinary tract infection (placebo, n=2; ustekinumab, n=1). Other serious infections in the placebo group were herpes zoster, sepsis, urosepsis, bronchitis, and diverticulitis (all singular events). In the ustekinumab group, serious infections through week 52 included gastroenteritis, staphylococcal endocarditis, tonsillitis and vulval cellulitis. During the extension, four patients (ustekinumab group) reported a serious infection: COVID-19 (n=2), gastritis (n=1) and pulmonary tuberculosis (n=1; negative chest radiograph and Quantiferon TB gold test at screening). No opportunistic infections occurred. AEs reported during the extension were similar in type and frequency to those reported through week 52 (table 2).

Five patients reported a major adverse cardiovascular event: acute myocardial infarction in the placebo group (n=2), cerebral infarction (n=1) and embolic stroke (n=1) in the ustekinumab group and acute myocardial infarction in a placebo→ustekinumab patient. Two malignancies occurred: diffuse large B-cell lymphoma (placebo, n=1) and gastric cancer (ustekinumab, n=1) through week 52; both patients discontinued study agent. No additional malignancies occurred through week 176.

One death was reported in the placebo group (splenic rupture). Five deaths occurred in the ustekinumab group: hypovolaemic shock, cardiac failure due to lupus myocarditis, haemorrhagic stroke (history of arterial hypertension), staphylococcal endocarditis, and COVID-19 (history of asthma).

Five patients, all in the ustekinumab group, had an infusion reaction; of these, two discontinued as a result. Injection-site reactions occurred in five ustekinumab patients and no placebo patient through week 52. After week 52, one patient (placebo→ustekinumab group) had an injection-site reaction. All injection-site reactions were considered mild.

Immunogenicity

Through week 48, 300 patients received ≥1 partial or complete dose of ustekinumab and had ≥1 post-administration serum sample. Of these patients, 24 (8%) tested positive for antibodies to ustekinumab, with 16 testing positive for neutralising antibodies. Through week 52, 1/24 (4%) patient who was positive

Table 2 Adverse events through end of study in LOTUS

	Placebo (weeks 0–52)	Ustekinumab (weeks 0–52)	Placebo→ ustekinumab (weeks 52–176)	Ustekinumab (weeks 52–176)	All ustekinumab*
Patients, n	208	307	88	134	395
Mean duration of follow-up (weeks)	50.4	50.1	29.7	29.7	55.6
Patients with ≥1 AE	155 (74.5)	214 (69.7)	26 (29.5)	37 (27.6)	246 (62.3)
Patients with ≥1 SAE	28 (13.5)	37 (12.1)	5 (5.7)	7 (5.2)	49 (12.4)
Patients with ≥1 infection	92 (44.2)	132 (43.0)	9 (10.2)	23 (17.2)	149 (37.7)
Patients with ≥1 serious infection	8 (3.8)	15 (4.9)	0	4 (3.0)	19 (4.8)
COVID-19-related AEs	0	2 (0.7)	0	4 (3.0)	6 (1.5)
COVID-19-related SAEs	0	2 (0.7)	0	2 (1.5)	4 (1.0)
Patients with ≥1 infusion reaction	0	5 (1.6)	--	--	--
Patients with ≥1 injection-site reaction	0	5 (1.6)	1 (1.1)	0	6 (1.5)
AEs leading to discontinuation	9 (4.3)	11 (3.6)	0	1 (0.7)	12 (3.0)
Deaths†	1 (0.5)	4 (1.3)	0	1 (0.7)	5 (1.3)

*All patients who received ≥1 dose of ustekinumab, including patients who crossed over from placebo.
†One death occurred in the placebo group (splenic rupture). In the ustekinumab group, 4 deaths occurred prior to week 52 (hypovolaemic shock, cardiac failure due to systemic lupus erythematosus myocarditis (patient was discharged against medical advice), haemorrhagic stroke (patient had a history of arterial hypertension) and staphylococcal endocarditis), and one death (COVID-19; history of asthma) occurred after week 52.
AE, adverse event; SAE, serious adverse event.

for antibodies to ustekinumab and 4/276 (1%) patients who were negative for antibodies to ustekinumab experienced an injection-site reaction.

Pharmacokinetics

Among patients randomised to ustekinumab, 303 were included in the pharmacokinetic analyses. Median trough serum ustekinumab concentrations reached steady state by week 24 (2.31 µg/mL) and were maintained through week 80 (2.09 µg/mL). Median serum ustekinumab concentrations at week 24 were similar for patients with and without renal disease (2.15 and 2.31 µg/mL, respectively); however, these results should be interpreted with caution due to the small number of patients with lupus nephritis (n=51 with available data, mean glomerular filtration rate (GFR): 0.93 mL/s/m^2; other patients, n=252, mean GFR: 0.98 mL/s/m^2).

Pharmacodynamics

Serum samples from 201 patients (ustekinumab, n=115; placebo, n=86) were used for pharmacodynamic analyses. Baseline characteristics for this population were similar to those of the FAS (online supplemental table 1). Baseline serum concentrations of IFNα, IFNγ and p40 in LOTUS patients were higher than those from healthy controls; serum levels of IL-17F and IL-22 were similar in LOTUS patients and healthy controls. There were no apparent differences in baseline levels of any of the assessed biomarkers between the treatment groups (figure 4). At week 24, serum p40 levels were increased and IFNγ levels were decreased in the ustekinumab group compared with baseline, with no apparent changes in the placebo group (figure 4). No treatment effect was seen in serum concentrations of IFNα, IL-17F and IL-22 (data not shown). Among the biomarkers analysed, changes in serum levels did not appear to be associated with SRI-4 response at week 24 (online supplemental figure 1).

DISCUSSION

In the earlier phase 2 study of ustekinumab in patients with SLE, a significantly greater proportion of ustekinumab-treated patients achieved an SRI-4 composite response at week 24 compared with placebo (primary endpoint).[15] However, these results were not confirmed in the larger phase 3 LOTUS study. The high response rate seen in the placebo/standard-of-care (SOC) group in LOTUS, with nearly 60% of these patients achieving an SRI-4 response at week 52, may have blunted the ability to assess the

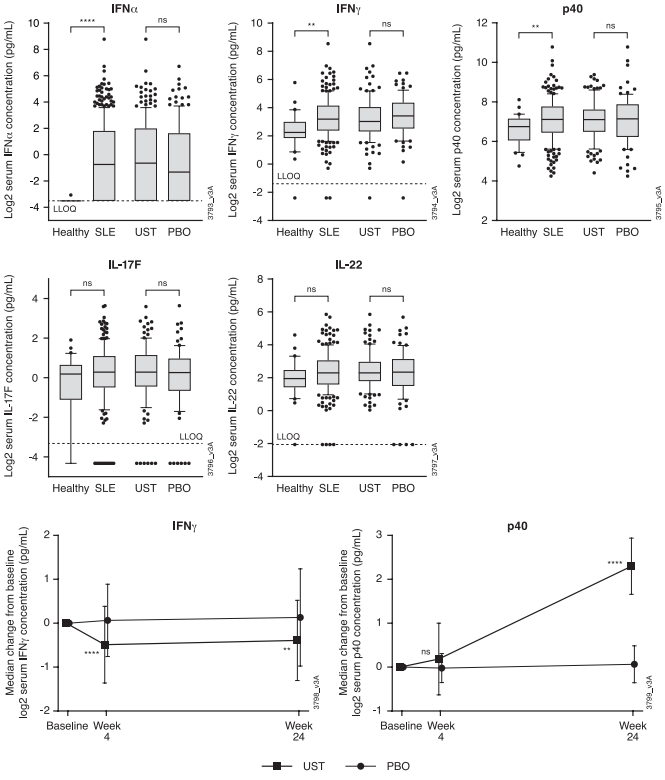

Figure 4 Baseline serum concentrations of IFNα, IFNγ, p40, IL-17F and IL-22 in LOTUS patients and healthy controls and median change from baseline in serum concentrations of IFNγ and p40 through week 24. Each box represents the 25th to 75th percentiles. Lines inside the boxes represent the median. The upper line extends to the largest value ≤1.5 × IQR; the lower line extends to the smallest value ≤1.5 × IQR. Data beyond the end of the lines represents outlier points. **P<0.01, ****p<0.0001. IFN, interferon; IL, interleukin; LLOQ, lower limit of quantification; PBO, placebo; SLE, systemic lupus erythematosus; UST, ustekinumab.

efficacy of ustekinumab. In recent trials of other compounds in patients with SLE, response rates in placebo/SOC groups were lower than that observed in LOTUS (week 24 SRI-4 response rates: placebo/SOC group, 48% vs baricitinib, 64%[21] and week 52 SRI-4 response rates: placebo/SOC group, 48% vs belimumab, 61%).[22] The week 52 SRI-4 response rate of 56% in the LOTUS placebo/SOC group limits the ability to see a signal for ustekinumab.

Race and ethnicity have been shown to influence both organ damage accrual and response to treatment in SLE patients.[23 24] The majority of patients in both the phase 2 and LOTUS studies were white, which may have biased the populations towards having less severe disease. However, sensitivity analyses by demographic and disease characteristics were similar to results in the overall mFAS. There were important differences between these studies that should be noted. The phase 2 study was smaller (102 patients), and placebo patients crossed over to ustekinumab at week 24 (the time point for primary endpoint analysis). Patients in ustekinumab and placebo groups, respectively, in the phase 2 study had slightly higher mean baseline SLEDAI-2K scores of 10.6 and 11.4 compared with LOTUS patients (All patients: 10.4 and 10.5; mFAS: 10.5 and 10.5). However, on average, patients in the phase 2 study[15] had a lower prevalence of lupus nephritis, fewer swollen, tender and active joints, and less severe skin disease at baseline than did LOTUS patients. Additionally, in the phase 2 study, BILAG A domain manifestations were more common in the placebo group (52%) vs ustekinumab (45%), while in the LOTUS population, BILAG A domain manifestations were more common in the ustekinumab group (All patients: 47% vs 38%; mFAS: 41% vs 37%).

Concomitant use of glucocorticoids was permitted in the phase 2 study at stable doses through week 28 with limited exemptions for dose adjustment, thus tapering was not generally permitted in the phase 2 study. In contrast, glucocorticoid tapering was strongly encouraged when clinically appropriate between weeks 24 and 40 in LOTUS, but a mandatory tapering regimen was not included in the study design. In addition, no dose adjustment was permitted between weeks 40 and 52, during which the primary endpoint was assessed. Because steroid tapering was not mandatory in LOTUS, investigators could discontinue tapering if disease activity increased without meeting the treatment failure criteria, thus favouring the placebo group in achieving the primary endpoint at week 52. Including such a directive regimen may provide more information on the glucocorticoid-sparing properties of a medication, but can result in a lower response to a study medication as measured by standard outcomes such as the SRI-4.

In both the phase 2 and LOTUS studies, a modified version of the SLEDAI-2K was used. All descriptors had to be present at the time of the screening visit, excluding seizure, fever, pericarditis/pleuritis, mucosal ulcers, diffuse alopecia and lupus headache. However, during postbaseline efficacy assessment visits, the presence of some variables was assessed based on the preceding 30 days while the presence of other variables (including visual disturbance, cranial nerve disorder (motor power and sensory deficit), cerebrovascular accident (motor and sensory deficit), vasculitis, arthritis, myositis (motor power), rash and alopecia (patchy)) was only assessed on the day of the study visit. Post hoc sensitivity analyses completed in both studies using the BILAG to reconstruct the SLEDAI-2K taking into consideration the preceding 30 days for all variables resulted in inconsistencies in response rates in both the phase 2 and LOTUS studies. One can speculate that this was due to activities that occurred during the preceding 30 days not being included in the SLEDAI score.

No new safety signals were identified in the LOTUS study, and the overall safety results were consistent with the known safety profile of ustekinumab. Infections were the most commonly reported type of AE.

The pharmacodynamic effects observed following ustekinumab treatment in LOTUS were generally consistent with those observed in the phase 2 study.[25] In both studies, comparable post-treatment increases in p40 levels and decreases in IFNγ levels were observed. Changes in p40 levels were not associated with an SRI-4 response in either study; however, while SRI-4 responders in the phase 2 study had greater decreases in IFNγ than did non-responders, no association was observed between decreases in IFNγ levels and SRI-4 response in the LOTUS patient population. Treatment with ustekinumab did not result in reductions in IL-17F or IL-22 in either study. In contrast, decreases in serum levels of IL-17F and/or IL-22 have been consistently observed following ustekinumab treatment in patients with psoriasis[26] and psoriatic arthritis,[27] in which ustekinumab has demonstrated significant clinical efficacy. Thus, taken together with the clinical efficacy assessments, these results suggest that although IL-23 may be involved in the pathogenesis of SLE, it is not an overarching target for these patients.

In summary, although the phase 2 results appeared robust, the phase 3 LOTUS study met futility criteria and was discontinued early. The primary and key secondary endpoints were not achieved in the overall study population or in the subpopulations evaluated in these analyses; despite a numerical trend suggesting that steroid tapering was possible to a greater extent in the ustekinumab group compared with the placebo group, there was insufficient evidence to support continuation of development of ustekinumab in patients with SLE.

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Ethics approval This study involves human participants and was approved by In the US, the protocol was approved by Central IRB/IEC - Chesapeake IRB, 6940 Columbia Gateway Drive, Suite 110, Columbia, MD, 21046. Participants gave informed consent to participate in the study before taking part.

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TRANSLATIONAL SCIENCE

Pathogenic neuropsychiatric effect of stress-induced microglial interleukin 12/23 axis in systemic lupus erythematosus

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ABSTRACT

Objectives The central nervous system disorder in systemic lupus erythematosus (SLE), called neuropsychiatric lupus (NPSLE), is one of the most severe phenotypes with various clinical symptoms, including mood disorder, psychosis and delirium as diffuse neuropsychological manifestations (dNPSLE). Although stress is one of the aggravating factors for neuropsychiatric symptoms, its role in the pathogenesis of dNPSLE remains to be elucidated. We aimed to investigate stress effects on the neuropsychiatric pathophysiology in SLE using lupus-prone mice and patients' data.

Methods Sleep disturbance stress (SDS) for 2 weeks was placed on 6–8-week-old female MRL/*lpr* and control mice. Behavioural phenotyping, histopathological analyses and gene and protein expression analyses were performed to assess SDS-induced neuroimmunological alterations. We also evaluated cytokines of the cerebrospinal fluid and brain regional volumes in patients with dNPSLE and patients with non-dNPSLE.

Results SDS-subjected MRL/*lpr* mice exhibited less anxiety-like behaviour, whereas stressed control mice showed increased anxiety. Furthermore, stress strongly activated the medial prefrontal cortex (mPFC) in SDS-subjected MRL/*lpr*. A transcriptome analysis of the PFC revealed the upregulation of microglial activation-related genes, including *Il12b*. We confirmed that stress-induced microglial activation and the upregulation of interleukin (IL) 12/23p40 proteins and increased dendritic spines in the mPFC of stressed MRL/*lpr* mice. IL-12/23p40 neutralisation and tyrosine kinase 2 inhibition mitigated the stress-induced neuropsychiatric phenotypes of MRL/*lpr* mice. We also found a higher level of cerebrospinal fluid IL-12/23p40 and more atrophy in the mPFC of patients with dNPSLE than those with non-dNPSLE.

Conclusions The microglial IL-12/23 axis in the mPFC might be associated with the pathogenesis and a promising therapeutic target for dNPSLE.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Neuropsychiatric lupus with diffuse neuropsychological manifestations (dNPSLE) is attributed to a variety of factors, including vascular occlusions, blood–brain barrier impairment, cytokines, autoantibodies and direct neuronal cell damage.
⇒ Stress affects the activation status of central neurons and glial cells, probably leading to neuroinflammation.
⇒ Stress, particularly chronic stress, is involved in the development of autoimmune diseases, including lupus, and has adverse effects on the disease activity with neuropsychiatric symptoms.

WHAT THIS STUDY ADDS

⇒ Stress-subjected MRL/*lpr* mice showed disinhibited behaviour, microglial activation with IL-12/23p40 upregulation and neuronal activation in the medial prefrontal cortex (mPFC).
⇒ Anti-IL-12/23p40 neutralising antibody or tyrosine kinase 2 inhibitor ameliorated these stress-elicited neuropsychiatric phenotypes in MRL/*lpr*.
⇒ A higher level of IL-12/23p40 in the cerebrospinal fluid and more atrophic changes in the mPFC were observed in patients with dNPSLE than those with non-dNPSLE.

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

⇒ An association of the stress-elicited IL-12/23 axis in the mPFC with the pathogenesis of disinhibited agitative behaviour in patients with dNPSLE was suggested.
⇒ Blockade of IL-12/23 signalling in the mPFC may be a novel therapeutic target for dNPSLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease with a predilection for young women of childbearing age, leading to a profound impact on their lives. Patients with SLE manifest multiple organ disorders such as skin rash, arthritis, nephritis, haematologic abnormality and inflammation in the central nervous system (CNS).[1] Among them, CNS diseases occur in up to 50% of patients

and demonstrate a wide range of symptoms, including headache, stroke, anxiety, depression, cognitive dysfunction, seizures, psychosis and acute confusional state (ACS). The CNS disorder, neuropsychiatric SLE (NPSLE), is one of the most severe manifestations and is further classified into diffuse neuropsychological syndrome (dNPSLE) and focal neurological syndrome (fNPSLE). Although a variety of factors, including vascular occlusions, blood–brain barrier impairment, cytokines, autoantibodies and direct neuronal cell damage, have been suggested for the development of dNPSLE,[2 3] its pathogenesis remains poorly understood. In addition, neuropsychiatric symptoms often occur independently of the systemic disease activity among patients with SLE, making it difficult to predict the development of dNPSLE.[4] Rheumatologists have been using glucocorticoids and pancytotoxic immunosuppressants for treatment, but there is no available therapeutic strategy that targets the disease-specific pathogenesis.[1 5]

Stress as emotional and physiological challenges can affect the neural activation status in the CNS. In the short term, it promotes allostasis for adaptation to the surrounding environment, but in the long term, it exhausts the body physically and mentally. It has been known that stress affects the functions of several physiological systems via the neuroendocrine pathway.[6] Stress also targets and remodels the CNS itself structurally and functionally, contributing to alterations in behavioural and physiological responses.[7] In animal models, stress induced by sleep deprivation atrophies hippocampal and cortical neurons, resulting in impaired retention and memory.[7–9] Also, sleep deprivation increases cytokines and oxidative stress markers in neurons.[7] We previously reported sleep disturbance stress (SDS) induced brain microinflammation in the presence of CNS-specific autoreactive CD4-positive T cells, exacerbating clinical symptoms of experimental autoimmune encephalomyelitis (EAE).[10] Thus, stress together with immune cells would be involved in the exacerbation of autoimmune diseases. Indeed, SDS is reported to impair metabolism and upregulate proinflammatory cytokine in humans.[11 12] Stress exposure also increases the risk of developing autoimmune diseases, including lupus, and has adverse effects on the disease activity with neuropsychiatric manifestations in SLE.[13 14] However, the molecular mechanisms of stress on the dNPSLE pathogenesis are still unknown. Therefore, we here investigated whether SDS promotes neuropsychiatric symptoms and the molecular pathogenesis using lupus-prone mice and its association with patients with dNPSLE.

PATIENTS, MATERIALS AND METHODS

Detailed information about each experiment and statistical analysis is described in online supplemental patients, materials and methods.

Study design

Briefly, we used lupus-prone mouse models with SDS load. In animal experiments, 6–8-week-old female mice were used for each experiment. Behavioural phenotyping, histopathological analyses, RNA sequencing (RNA-seq), flow cytometry and ELISA were performed to assess SDS-induced behavioural changes and neuroimmunological alterations. We retrospectively reviewed medical records of the patients with SLE whose serum and cerebrospinal fluid (CSF) were preserved at Hokkaido University and Kitasato University between 2006 and 2020. We also collected the clinical data of 71 consecutive patients who underwent brain MRI at Hokkaido University Hospital between 2019 and 2020. We evaluated serum and CSF cytokine levels using ELISA and atrophic brain regions using voxel-based morphometry (VBM) in the patients.

Statistical analysis

Experimental data using mice were analysed with an unpaired Student's t-test and two-way analysis of variance with Tukey-Kramer post-hoc multiple comparisons test or paired t-test for the unpaired or paired values of continuous variables, respectively. For the analyses of human sample data, we used the Mann-Whitney U test and Kruskal-Wallis test with post-hoc Steel-Dwass multiple comparison method for the values of continuous variables and χ^2 test for the proportions of categorical variables. To explore differentially expressed genes in the RNA-seq analysis, we used adjusted p values with false discovery rate correction by the Storey method for genes with an absolute fold change (FC) over 1.5 compared with control strains. A receiver operating characteristics analysis was performed to evaluate the diagnostic ability of the data for dNPSLE with the area under the curve (AUC). Neuroimaging data were analysed by analysis of covariance with age, sex, disease duration, total intracranial volume and white matter (WM) volume as confounding factors. In the linear correlation analysis, Pearson's product moment correlation coefficients were calculated. P values lower than 0.05 were considered statistically significant. We used JMP Pro V.14 (SAS Institute, USA) for all analyses. All statistical tests were two-sided, and all experiments were performed at least two times.

RESULTS

Sleep disturbance stress-induced abnormal behaviour in lupus-prone mice

We employed MRL/MpJJmsSlc-*lpr*/*lpr* (MRL/*lpr*) female mice, which show SLE-like manifestations, including nephritis and CNS symptoms.[15] For the stress load, we used SDS loading cages, in which continuous stress to inhibit regular sleep is imposed on mice on a free rotation wheel for 2 weeks (figure 1A, online supplemental figure 1A). Mice housed in SDS cages showed more activity throughout the night and higher levels of serum corticosterone than those housed in a normal cage (online supplemental figure 1B-F). We then performed behavioural phenotyping of SDS-subjected MRL/*lpr* mice and of control MRL/MpJJmsSlc-+/+ (MRL/MpJ) mice. We employed an elevated plus maze test (EPM) and open field test (OF) to assess risk-taking agitation-like behaviours, which are often observed in patients with dNPSLE with ACS as vigilance and psychomotor overactivity.[16] In the EPM, SDS-subjected control mice tended to show more anxiety-like behaviour than mice without stress, which is commonly seen in chronic stress-exposed mice. In contrast, SDS-subjected MRL/*lpr* showed significantly less anxiety-like behaviour than control (figure 1B,C). There was a slight but significant difference in total travelling distance in the EPM between the SDS-subjected MRL/*lpr* and control mice (figure 1D). Consistently, SDS-subjected MRL/*lpr* mice showed less anxiety-like behaviour than SDS-free MRL/*lpr* mice without any decrease in general locomotor activity in the OF (figure 1E–G). To exclude the possibility that systemic inflammation makes SDS-subjected mice disinhibited on the behaviour, we evaluated the SDS effect in NOD/ShiJcl, which is a mouse model of chronic inflammatory autoimmune disease like Sjögren's syndrome (SS). In contrast to SDS-subjected MRL/*lpr*, SDS-receiving NOD/ShiJcl demonstrated similar behaviour to SDS-free mice in the EPM (online supplemental figure 1G,H). Thus, stress had disinhibitory effects on the behaviours of MRL/*lpr* mice.

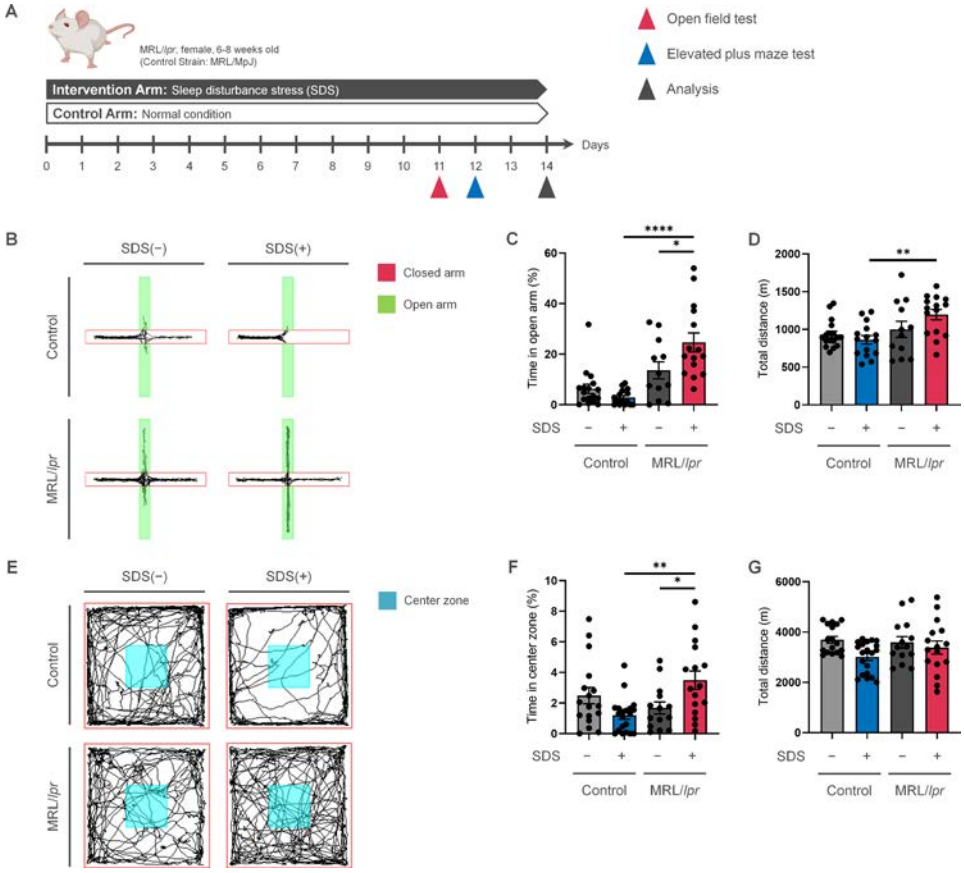

Figure 1 Stress-elicited abnormal behaviour and mPFC activation in lupus-prone mice. (A) The study protocol for chronic SDS. (B–D) EPM in SDS-subjected or SDS-free MRL/*lpr* and control mice (MRL/MpJ). (B) representative trajectories. (C) Percentage of time spent in the open arms and (D) total travelled distance to assess anxiety-like behaviour and general locomotor activity, respectively (n=12–17 per group). (E–G) OF results. (E) representative tracking images. (F) Percentage of time spent in the centre zone and (G) total distance travelled to assess anxiety-like behaviour and general locomotor activity, respectively (n=14–20 per group). Data are means±SEM. *p<0.05, **p<0.01 and ****p<0.0001 using a two-way ANOVA with post-hoc Tukey-Kramer multiple comparison test. ANOVA, analysis of variance; EPM, elevated plus maze test; mPFC, medial prefrontal cortex; OF, open field test; SDS, sleep disturbance stress.

Persistent neuronal activation in medial prefrontal cortex of stress-subjected MRL/*lpr* mice

One of the representative vrain regions receiving stress stimulus is the paraventricular nucleus (PVN).[17] PVN neurons send axonal projections to the ventral tegmental area (VTA) as the origin of dopaminergic neurons that project to the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc).[18 19] Previous reports demonstrated that acute single stress activates the mPFC and represses anxiety-like behaviour, while chronic stress causes anxiety by inhibiting the mPFC and activating NAc neurons.[20 21] Therefore, we investigated neuronal activity in these regions in the presence or absence of SDS (figure 2A,B). In the PVN, both SDS-subjected strains showed a higher number of phosphorylated cFos (p-cFos)-positive cells than SDS-free mice (online supplemental figure 2A-C). In the VTA, more p-cFos positive cells were observed in SDS-subjected mice than SDS-free mice regardless of the mouse strain (figure 2C–E). In contrast, only SDS-subjected MRL/*lpr* mice showed elevated neuronal activation in the mPFC (figure 2F–H). Meanwhile, both stressed strains showed more p-cFos positive cells in the NAc compared with SDS-free strains (figure 2I–K). In contrast, among NOD/ShiJcl mouse strain, SDS-subjected mice showed similar number of p-cFos-positive cells to SDS-free mice (online supplemental figure 2D,E). These findings suggest that the mPFC is an important region for anxiolytic effect for disinhibited behaviour over NAc activation induced-anxious effect in SDS-subjected MRL/*lpr* mice.

Enhanced microglia activation signatures in the pFc of stressed MRL/*lpr* mice

We hypothesised that the stress load alters gene expression profiles in the mPFC of MRL/*lpr* mice. An RNA-seq transcriptome analysis of the PFC among the strains detected 7952 differentially expressed genes with an absolute FC over 1.5. A principal component analysis revealed a stress-induced effect on the gene expressions corresponding to the mouse strains (figure 3A). Among these genes, 509 genes were significantly upregulated or downregulated in SDS-subjected MRL/*lpr* mice compared with the other mouse groups (figure 3B). Using these genes, an ingenuity pathway analysis identified pathways associated with inflammatory and neuronal signalling, such as phagosome formation, neuroinflammation, Th1 pathway, G-protein-coupled receptor and cAMP response element binding protein (CREB) signalling (figure 3C, online supplemental figure 3A). Consistently, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses detected pathways related to the positive regulation of cytokine production, G protein-coupled receptor activity and neuroactive ligand receptor interaction (figure 3D, online supplemental figure 3B). Considering the inflammatory nature of lupus, we focused on 635 genes associated with the inflammatory pathways analysed above. SDS-subjected MRL/*lpr* mice demonstrated relatively high expressions for microglia-activating genes, such as *H2-Eb1*, *Nos2*, *Il12b* and *Fcgr4*, and low expressions for microglial-inactivating genes, including *Arg1*, *Nr4a3*

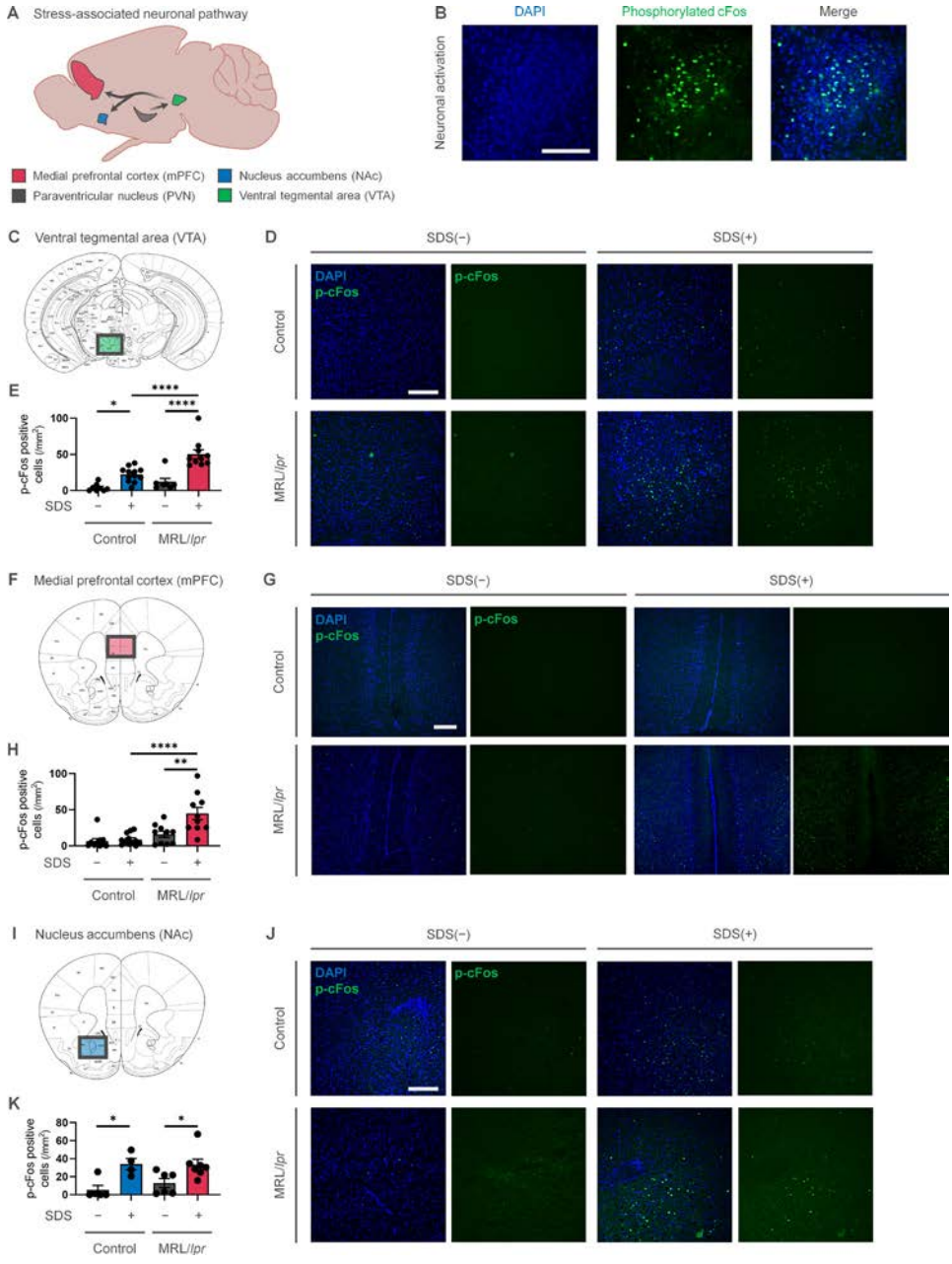

Figure 2 Neuronal activation of stress-responsive brain regions. (A) The representative stress-responsive neuronal pathway. The PVN sends axonal connections to the VTA, which projects to the mPFC and NAc. (B) Representative images of phosphorylated cFos (p-cFos)-immunostained cells with DAPI. Scale bar: 20 µm. (C–K) Evaluation of neuronal activation using immunohistochemistry of p-cFos-positive cells in the (C–E) VTA, (F–H) mPFC and (I) to K) NAc between SDS-subjected or SDS-free MRL/*lpr*, and control mice. (C, F and I) Schematic drawings of the VTA, mPFC and NAc were taken from Franklin and Paxinos (1997).[64] (D, G, J) representative immunohistochemical images of activating p-cFos-positive neuronal cells with DAPI in the VTA, mPFC and NAc. Scale bars: (D) and (J) 50 µm and (G) 100 µm. (H) Quantification of p-cFos-positive cells in the VTA (n=7–12 per group), mPFC (n=10–12 per group), and NAc (n=4–8 per group). Data are means±SEM. *p<0.05, **p<0.01 and ****p<0.0001 using a two-way ANOVA with the post-hoc Tukey-Kramer multiple comparison test. ANOVA, analysis of variance; DAPI, 4′,6-diamidino-2-phenylindole; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; p-cFos, phosphorylated cFos; PVN, paraventricular nucleus; VTA, ventral tegmental area.

and *Treml4* (figure 3E). The *Il12b* gene encodes interleukin 12 (IL-12) p40 subunit, which is shared by IL-12 and IL-23, both of which are critical for inflammation development. We confirmed that *Il12b* gene had the highest expression in the PFC of SDS-subjected MRL/*lpr* mice by quantitative PCR (figure 3F). Although the serum IL-12/23p40 level of SDS-subjected MRL/*lpr* mice was lower than that of SDS-free MRL/*lpr* mice, the highest CSF IL-12/23p40 protein level was observed in SDS-subjected MRL/*lpr* mice (figure 3G, online supplemental figure 3C). We also examined the CSF levels of interferon-γ and IL-17A, which are IL-12/23p40 signaling-inducible cytokines. The CSF interferon-γ and IL-17A tended to be higher levels in SDS-subjected MRL/*lpr* mice (online supplemental figure 3D,E). Thus, the upregulation of microglial proinflammatory genes, including *Il12b*, in the PFC of SDS-subjected MRL/*lpr* mice was observed.

Microglial activation and neuronal alterations in the mPFC of SDS-subjected MRL/*lpr* mice

We next investigated which cell types produce IL-12/23p40 in the PFC. We first analysed the CNS invasion of CD45^{high} immune

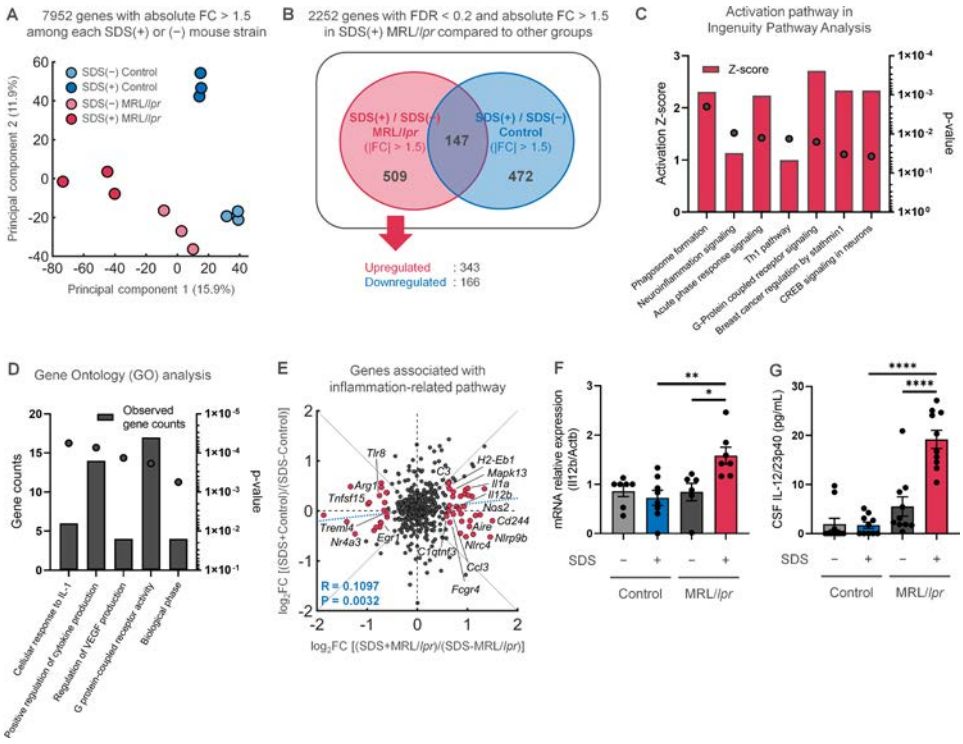

Figure 3 Upregulation of inflammatory gene expression and IL-12/23p40 level in the PFC of stressed MRL/*lpr* mice. (A) Principal component analysis of genes with an absolute FC over 1.5 in SDS-subjected or -free strains (n=3 per group). (B) Venn diagram showing the number of stress-affected differentially expressed genes with an absolute FC >1.5 and false discovery rate-corrected p value <0.2 among the mouse groups. SDS-subjected MRL/*lpr* demonstrated unique expressions of 343 upregulated and 166 downregulated genes compared with SDS-free MRL/*lpr* mice and control strains. (C) Ingenuity pathway analysis and (D) GO analysis of the 343 differentially upregulated genes from SDS-subjected MRL/*lpr* mice. Dots represent p values. (E) SDS-induced changes in inflammation-related gene expressions in the PFC of MRL/*lpr* and controls compared with the respective SDS-free strains. red, SDS-induced MRL/lpr uniquely expressed genes. (R=0.1097, linear regression, Pearson's correlation). (F) *Il12b* mRNA expression relative to SDS-free control mice by quantitative PCR (n=6–8 per group). (G) IL-12/23p40 level in the CSF measured by ELISA (n=10–11 per group). (F, G) data are means±SEM. *p<0.05, **p<0.01 and ****p<0.0001 using a two-way ANOVA with the post-hoc Tukey-Kramer multiple comparison test. ANOVA, analysis of variance; CSF, cerebrospinal fluid; FC, fold change; GO, Gene Ontology; IL, interleukin; PFC, prefrontal cortex; SDS, sleep disturbance stress.

cells, especially T cells. Although peripheral CD45^{high} immune cells were present in the cortex of 14-week-old MRL/*lpr* mice, as previously described,[22] few CD45^{high} cells were detected in the cortex of 8-week-old MRL/*lpr* mice (online supplemental figure 4A,B). Therefore, we focused on CNS cells responsible for IL-12/23p40 production. Public databases of gene expressions in CNS cells demonstrated that microglia and macrophages express *Il12b* gene (online supplemental figure 4C).[23] Consistently, SDS-subjected MRL/*lpr* mice had the highest number of Iba-1^{+}-activated microglia in the mPFC based on the aggregation of a lysosomal activation marker, CD68, without any microglial density changes (figure 4A-C, online supplemental figures 5A,B). A flow cytometric analysis revealed that IL-12/23p40-positive cortical microglia increased in SDS-subjected MRL/*lpr* mice (figure 4D,E, online supplemental figure 5C,D). Additionally, mPFC microglia in NOD/ShiJcl mouse strain did not be affected by SDS (online supplemental figure 5E-G). We then investigated which cells receive IL-12/23p40 in the mPFC. Previous reports showed that neurons express IL-12 receptors and IL-12 enhances neurite outgrowth.[24 25] Indeed, mPFC neurons expressed IL12Rβ1, a subunit of IL-12/23p40 receptor (online supplemental figure 6A). We further found that Stat4, a downstream molecule of IL-12/23 signalling, was highly phosphorylated in the PFC neurons of SDS-subjected MRL/*lpr* mice (figure 4F,G, online supplemental figure 6B,C). Phosphorylation of Stat3, another downstream molecule of IL-12/23 signalling, was not significantly affected by SDS in the PFC neurons of SDS-subjected MRL/*lpr* mice (online supplemental figure 6D-F), indicating mainly activated IL-12 signalling in the PFC neurons. Furthermore, SDS-subjected MRL/*lpr* mice had the most abnormal spines in proximal dendrites to soma of mPFC neurons (figure 4HI), where few dendritic spines are generally expressed.[26] We also observed that MRL/MpJ control mice stereotaxically injected with recombinant IL-12/23p40 demonstrated more abnormal dendritic spines in mPFC neurons (online supplemental figure 6G,H). Therefore, we detected IL-12/23p40 upregulation in activated microglia and Stat4-mediated neuronal alterations in the mPFC of SDS-subjected MRL/*lpr* mice.

Cancelling neuropsychiatric phenotypes in stressed MRL/*lpr* mice by IL-12/23p40 neutralisation and tyrosine kinase 2 inhibition

To investigate whether the blockade of IL-12/23p40 signalling ameliorates the stress-elicited phenotypes in MRL/*lpr* mice, we intracerebroventricularly infused IL-12/23p40 neutralising antibody in SDS-subjected MRL/*lpr* mice (figure 5A). The infused immunoglobulin G (IgG) successfully reached the mPFC, and IL-12/23p40 antibody reduced the CSF IL-12/23p40 level without any change in the serum level of IL-12/23p40 or spleen weight (figure 5B, online supplemental figure 7A–C). Indeed, IL-12/23p40 blockade in the CNS ameliorated stress-induced

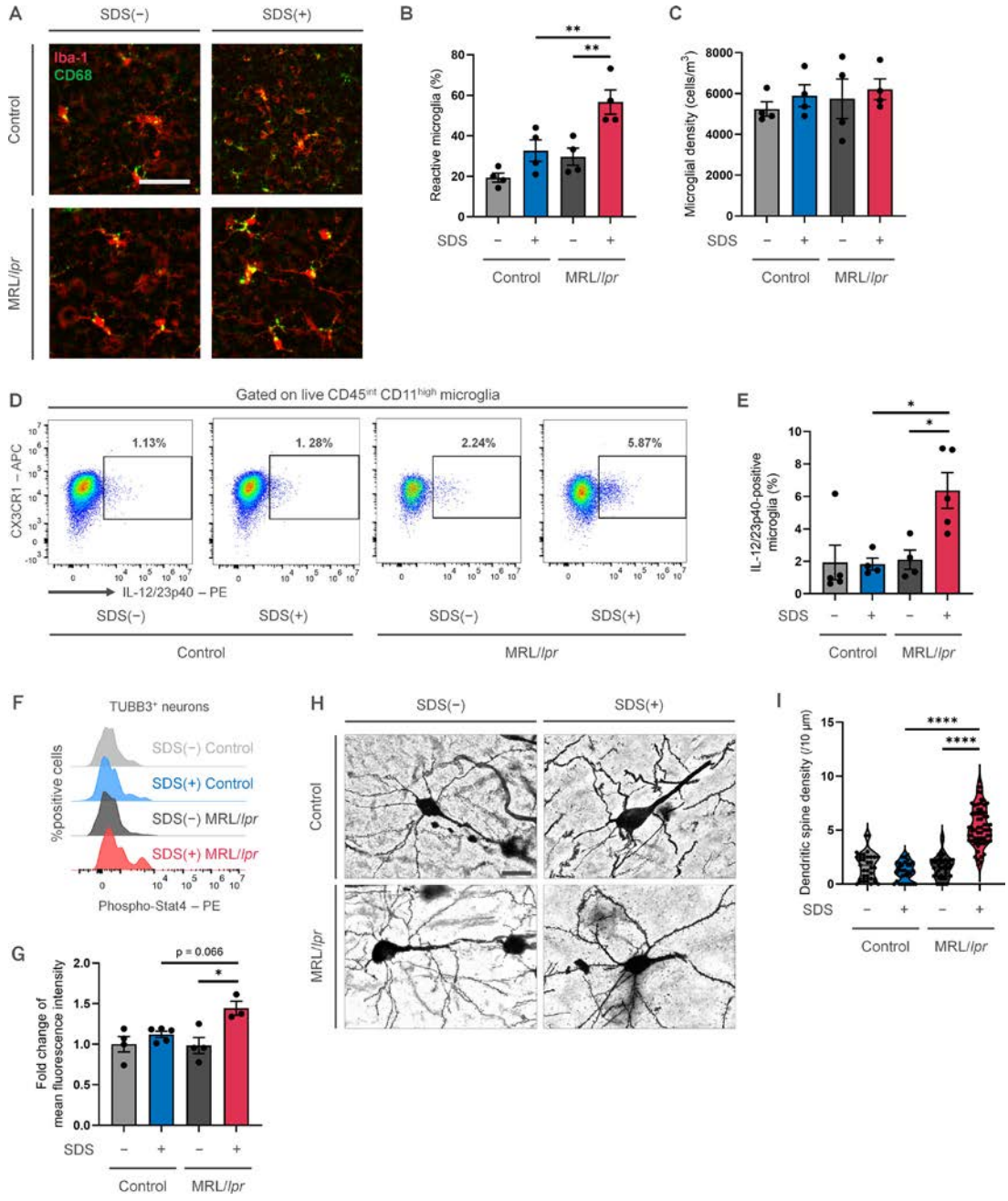

Figure 4 Microglial activation with IL-12/23p40 upregulation and dendritic alterations in stressed MRL/*lpr* mice. (A–C) Microglial activation state analysis in the mPFC. (A) Representative immunohistochemical images of Iba-1-positive microglia with a CD68 lysosomal marker. (B) Percentage of reactive microglia and (C) microglial density (n=4 per group). (D–E) Flow cytometric analysis for IL-12/23p40 production from live $CD45^{int}CD11b^{high}CX3CR1^{+}$ cortical microglia. (D) Representative gating for IL-12/23p40-positive microglia. (E) Percentage of IL-12/23p40-producing microglia (n=4–5 per group). (F and G) Phosphorylated-Stat4 expression in $TUBB3^{+}$ neurons measured by flow cytometry. (F) Representative histogram for phosphorylated-Stat4 and (G) FC of the mean fluorescence intensity for the phosphorylated-Stat4 expression relative to SDS-free control mice (n=4–5 per group). (H–I) Golgi-Cox staining of neurons in the mPFC. (H) Representative images of layer 2/3 pyramidal neurons. (I) Quantification of the dendritic spinal density proximal to the soma of layer 2/3 pyramidal neurons (n=31–62 dendrites per group). Scale bars: (A) 50 µm and (K) 20 µm. (B), (C), (E) and (G) Data are means±SEM. or (I) medians (IQR). *p<0.05, **p<0.01 and ****p<0.0001 using a two-way ANOVA with the Tukey-Kramer test. ANOVA, analysis of variance; FC, fold change; IL, interleukin; mPFC, medial prefrontal cortex; SDS, sleep disturbance stress.

anxiolytic behaviour (figure 5C–E). Moreover, microglial activation and phosphorylated Stat4 levels in the mPFC were reduced (online supplemental figure 7D-F). The abnormal increase of proximal dendritic spines was also remedied (figure 5F,G).

We next employed deucravacitinib, a clinically applied selective inhibitor of tyrosine kinase 2 (Tyk2), which is an IL-12/23-downstream intracellular signalling kinase.[27] We found that Tyk2 inhibitor treatment reduced the spleen weight (figure 6A–C) and suppressed cortical microglial activation with IL-12/23p40 production, phosphorylation of Stat4 in cortical neurons, and anxiolytic behaviour without any effects on general locomotor activity, and increased the number of spines in proximal dendrites (figure 6D-N, online supplemental figure 8). Thus, blockade of the IL-12/23p40-Tyk2 signalling pathway in the CNS successfully inhibited the stress-induced neuropsychiatric phenotypes in SDS-subjected MRL/*lpr* mice.

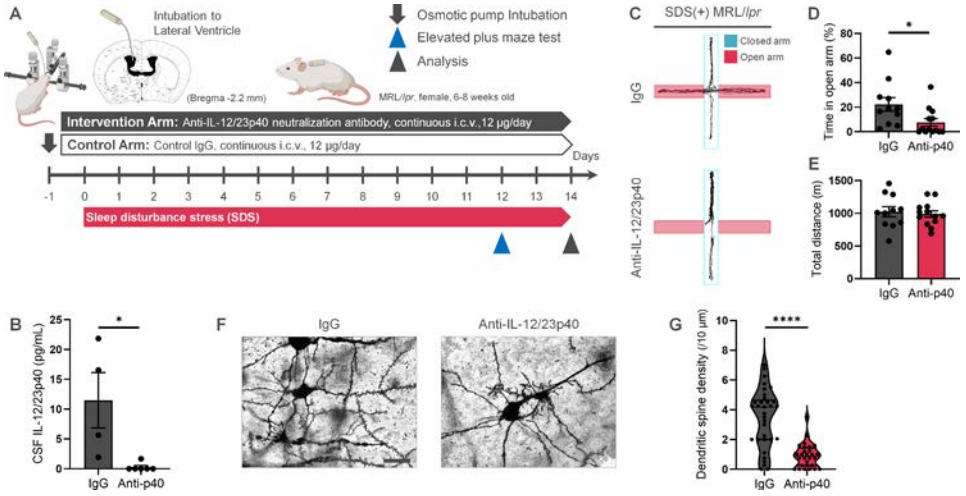

Figure 5 Cancellation of stress-elicited phenotypes in MRL/*lpr* by neutralising antibody-mediated IL-12/23 signalling blockade. (A) Schematic representation of the CNS-targeted IL-12/23p40 blockade in SDS-subjected MRL/*lpr* mice. (B–G) Successful inhibition of SDS-induced neuropsychiatric phenotypes by IL-12/23p40 depletion. (B) ELISA for IL-12/23p40 concentration in the CSF (n=4–6 per group). (C) Representative tracks, (D) percentage of time spent in the open arm and (E) total travelled distance in the EPM (n=11 to 13 per group). (F) Representative Golgi-Cox staining of layer 2/3 pyramidal neurons in the mPFC. (G) Quantification of the spinal density of proximal dendrites to soma (n=28–33 dendrites per group). (F) scale bar, 20 μm. (B), (D) and (E) Data are means±SEM or (G) medians (IQR). *p<0.05 and ****p<0.0001 using an unpaired Student's t-test. CSF, cerebrospinal fluid; CNS, central nervous system; EPM, elevated plus maze test; IgG, immunoglobulin G; IL, interleukin; mPFC, medial prefrontal cortex; SDS, sleep disturbance stress.

High IL-12/23p40 levels in the CSF and mPFC atrophy in patients with dNPSLE

To investigate whether IL-12/23p40 in the CNS affects the pathogenesis of dNPSLE, we measured IL-12/23p40 levels in the CSF and serum of healthy controls and lupus patients with active disease (figure 7A). In the Hokkaido University Hospital cohort, including patients with dNPSLE and patients with SLE without diffuse neuropsychiatric symptoms (non-dNPSLE), which include fNPSLE (online supplemental table 1), CSF IL-12/23p40 levels were highest in patients with dNPSLE, whereas serum levels were similar among all groups (figure 7B, online supplemental figure 9A). Although statistical difference was not observed due to the small number of patients with dNPSLE, the patients without treatment tended to show higher levels of CSF IL-12/23p40 than those treated with glucocorticoid combined with immunosuppressants, suggesting that high variance of CSF IL-12/23p40 levels was likely caused by therapeutic effects of immunosuppressants (online supplemental figure 9B). The CSF IL-12/23p40 level had high diagnostic utility for dNPSLE, with an AUC of 0.8438 (95% CI 0.7377 to 0.9498, sensitivity 78% and specificity 88%) (figure 7C). Using the Kitasato University Hospital cohort (online supplemental table 2), we validated these results, as the higher CSF levels of IL-12/23p40 in patients with dNPSLE than fNPSLE and good diagnostic efficacy for dNPSLE with an AUC of 0.7346 (95% CI 0.5472 to 0.9220, sensitivity 61% and specificity 78%) (figure 7D-E, online supplemental figure 9C). Notably, the patients with ACS or psychosis occupied 70%–80% of the dNPSLE population, and there were few patients with anxiety in both cohorts (online supplemental tables S1 and S2). We investigated the CSF IL-12/23p40 level of the patients with primary SS as a disease control. The IL-12/23p40 levels were significantly higher in the CSF of patients with dNPSLE than that of patients with primary SS (online supplemental figure 9D).

We next investigated morphological changes of the brain in patients with dNPSLE and patients with non-dNPSLE by VBM using another Hokkaido University cohort (figure 7A and online supplemental table 3). The patients with dNPSLE showed less volumes of total grey matter and WM, and more WM lesions than patients with non-dNPSLE (figure 7F, online supplemental figure 9E–H). Based on the results of the SDS mouse model, we calculated the mPFC volume and atrophic Z-score using an age-matched healthy control dataset as a reference, finding a larger mPFC volume reduction with higher Z-score in patients with dNPSLE than patients with non-dNPSLE despite a similar frontal cortex volume (figure 7H1, online supplemental figure 9I). In the Hokkaido University Hospital cohort (online supplemental table 1), patients with dNPSLE received more sleep medications than patients with non-dNPSLE (20/28, 71% vs 6/19, 32%, p=0.0064) at the perioperative period of the CSF collection without any statistical difference in the glucocorticoid dose (online supplemental table 1 and 4). These might indicate a high prevalence of sleep disorder on dNPSLE onset. Together, these results as patients with dNPSLE have increased IL-12/23p40 levels in the CSF and mPFC atrophy, possibly being linked to the results of our mouse model.

DISCUSSION

Previous studies have reported that chronic stress inhibits PFC function with a decreasing density of dendritic spines, impairing behavioural flexibility, including more anxiety-like behaviours.[20 21 28–31] Contrary to these reports, our results demonstrated that stressed lupus-prone mice showed PFC impairment with increasing dendritic spinal density and disinhibited risk-taking and less anxiety-like behaviours. We further showed that the stress-induced activation of microglia in the mPFC plays a key role.

It is well known that activated microglia induce neuroinflammation, predisposing susceptible individuals to neuropsychiatric diseases,[32–34] even though microglia engage with neurons to maintain homeostasis in the CNS.[32–34] In other words, in a pathological state, activated microglia potentially exacerbate neurological diseases by dysregulating neural circuits.[35 36] For

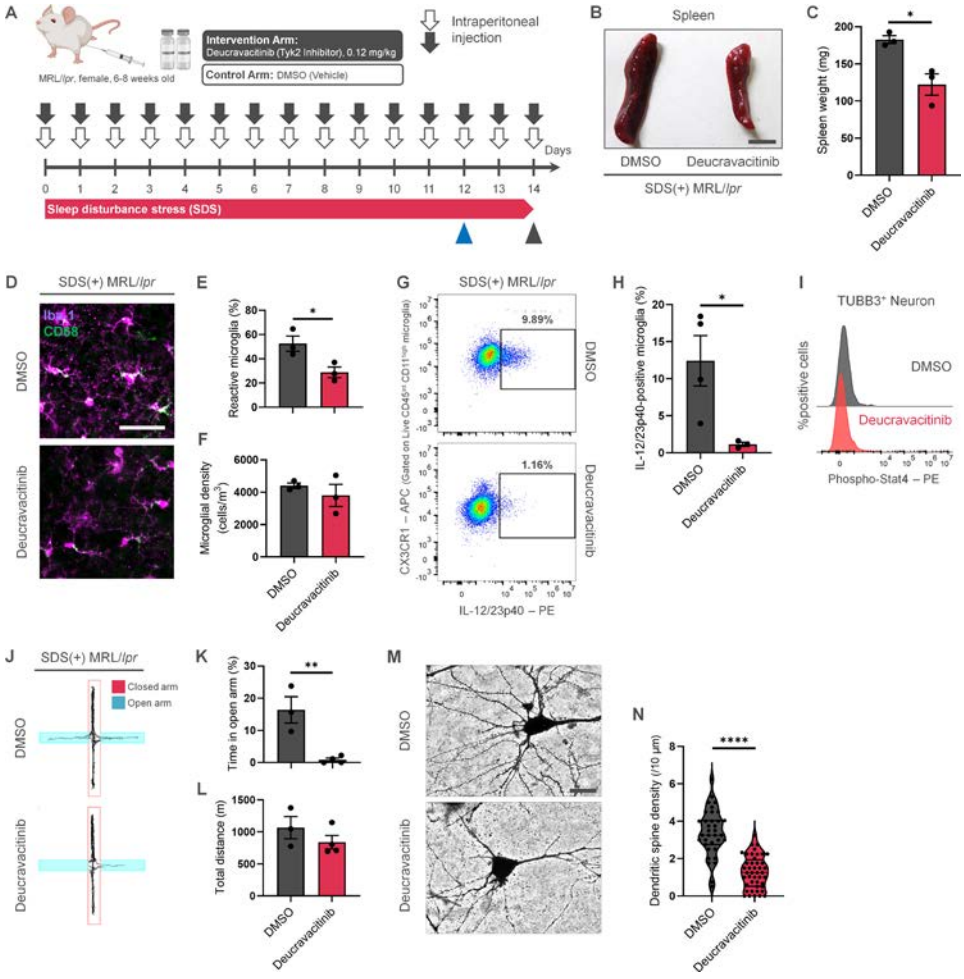

Figure 6 Improvement of stress-elicited neuropsychiatric phenotypes in lupus-prone mice with Tyk2 inhibitor. (A) The experimental protocol for the systemic administration of deucravacitinib in SDS-subjected MRL/*lpr* mice. (B–N) SDS-elicited phenotypes were cancelled by deucravacitinib. (B) Macroscopic findings of the spleen. (C) Spleen weight comparison (n=3 per group). (D) Representative images of activated microglia. (E) Percentage of reactive microglia and (F) microglial density (n=3 per group). (G) Representative gating for IL-12/23p40^{+} microglia and (H) its percentage (n=3 per group). (I) Histogram of phosphorylated-Stat4 expression in TUBB3^{+} neurons. (J) Representative trajectories, (K) percentage of time spent in the open arm and (L) total distance in the EPM (n=3–4 per group). (M) Representative images of layer 2/3 pyramidal neurons in the mPFC. (N) Quantification of the proximal dendritic spinal density (n=35–44 dendrites per group). Scale bars: (B) 10 mm, (D) 50 µm and (M) 20 µm. Data are means±SEM except (N) which shows medians (IQR). *p<0.05, **p<0.01 and ****p<0.0001 using an unpaired Student's t-test. DMSO, dimethyl sulfoxide; EPM, elevated plus maze test; IL, interleukin; mPFC, medial prefrontal cortex; SDS, sleep disturbance stress; Tyk2, tyrosine kinase 2.

instance, homeostatic microglia drive negative feedback mechanisms to inhibit neuronal activation via extracellular ATP to protect the brain from excessive activation, while activated proinflammatory microglia do not trigger these negative feedback mechanisms.[37] In addition, activated microglia alter neuronal functions and eliminate dendritic spines in pyramidal neurons of the mPFC.[38 39] Moreover, microglia from lupus-prone mice exhibit an activated phenotype with upregulated inflammation-related genes, including *Il12b*, and cause neuronal dysfunction leading to neuroinflammation and microglial phagocytosis.[40 41] Although some microglia populations protect lupus model mice from neurodegeneration,[42 43] activated microglia also induce impairment of the blood–brain barrier to cause neuroinflammation development.[44] Thus, it is reasonable that activated microglia can induce the abnormal activation of neuronal circuits in the mPFC to cause neuropsychiatric phenotypes in stressed MRL/*lpr* mice. Although stress is known to induce microglial activation via the norepinephrine-β adrenergic receptor signalling pathway,[45 46] the molecular mechanisms causing the microglial activation in SDS-subjected MRL/*lpr* mice are unknown at present. In addition, the behavioural testing results had relatively high variance in stressed-MRL/*lpr* mice, possibly being derived from the variance of disease penetrance. Further research for understanding the molecular mechanisms of stress-induced microglial activation and neuronal alteration in lupus-prone condition is required.

IL-12/23p40 is a subunit that dimerises with IL-12p35 or IL-23p19 to form IL-12 and IL-23.[47] In autoimmune disorders, IL-12 and IL-23 are associated with the pathogenesis predominantly through T cell-mediated immunity. While some studies reported that IL-12 can relieve neuroinflammation in an EAE model,[48 49] previous research also demonstrated that IL-12 and IL-23 can exacerbate neuroinflammation in EAE.[50 51] They also exacerbate the pathology of Alzheimer's disease.[52] We showed that IL-12/23p40 mainly expressed from activated microglia alter mPFC neurons, modifying the neuronal structure as previously demonstrated.[24] A phase 3 clinical trial for ustekinumab, an IL-12/23p40 neutralisation antibody, in patients with SLE was discontinued due to poor efficacy (Ustekinumab Press Release).[53] However, considering that patients with NPSLE were excluded

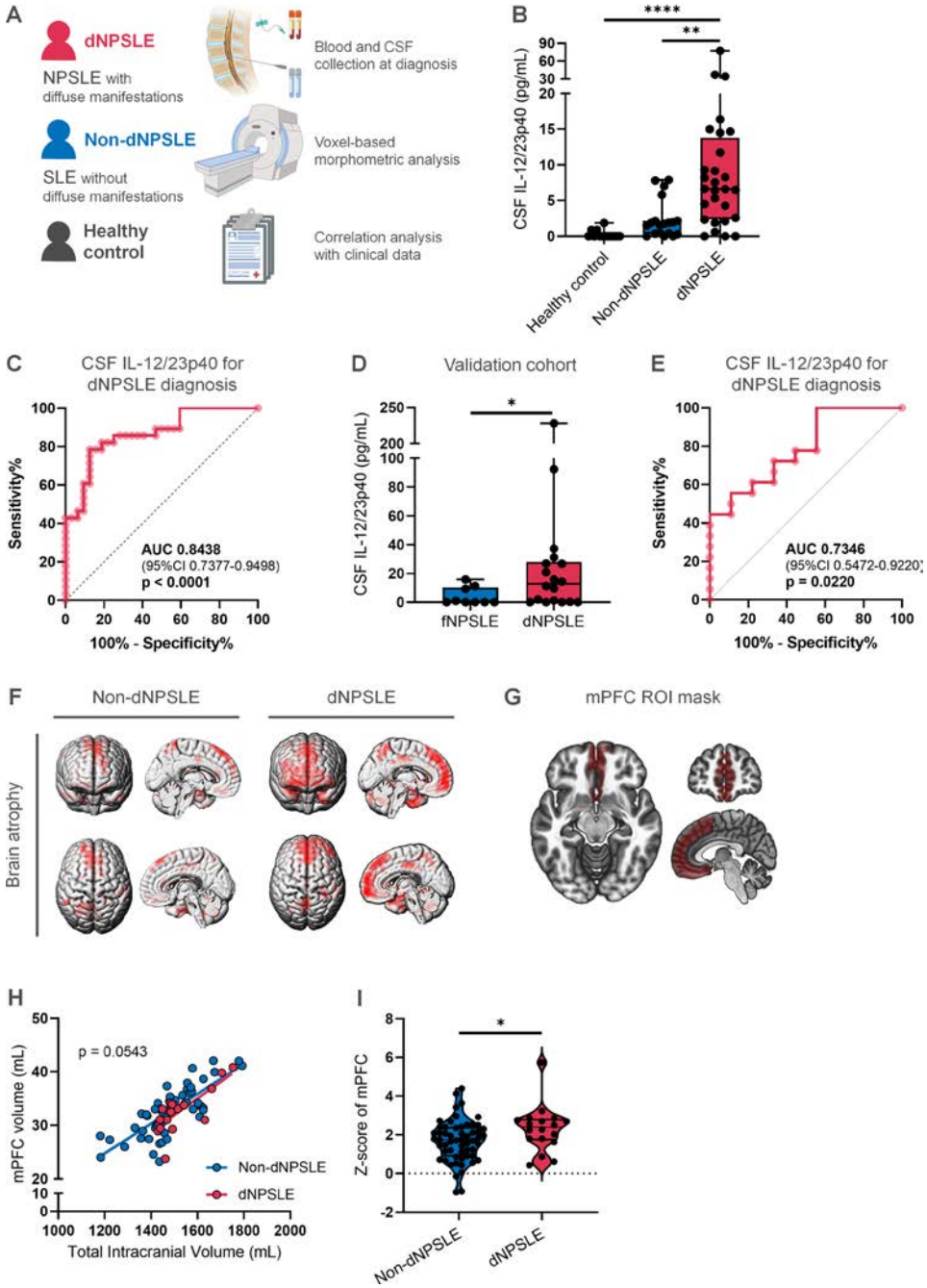

Figure 7 IL-12/23p40 upregulation and mPFC atrophy in patients with NPSLE with diffuse manifestations (dNPSLE). (A) Schema for the evaluation of human clinical samples and data. (B) and (C) The derivation cohort of patients with SLE and HCs at Hokkaido University Hospital. (B) CSF IL-12/23p40 level measured by ELISA (n=13 HCs, n=19 non-dNPSLE and n=28 dNPSLE). (C) An ROC analysis of CSF IL-12/23p40 levels for dNPSLE diagnosis. (D) and (E) The validation cohort of patients with NPSLE at Kitasato University hospital. (D) CSF IL-12/23p40 levels (n=9 fNPSLE and n=18 dNPSLE). (E) An ROC analysis of the CSF IL-12/23p40 level for dNPSLE classification. (F) Representative brains with atrophy rendered on the reference anatomical brain view against the IXI reference dataset. (G) Used brain ROI mask of the mPFC, rendered as red. (H) Correlation between the mPFC volume and TIV (blue, n=57 non-dNPSLE; red, n=18 dNPSLE). (I) Z-scores of mPFC atrophy against the IXI reference dataset. (B), (D) and (I) Data are medians (IQR). *p<0.05, **p<0.01 and ****p<0.0001 using (B) the Kruskal-Wallis test with the post-hoc Steel-Dwass method, (D) and (I) Mann-Whitney U test and (H) ANCOVA adjusting for TIV, age, sex and disease duration of SLE. ANCOVA, analysis of covariance; CSF, cerebrospinal fluid; CNS, central nervous system; dNPSLE, diffuse neuropsychiatric systemic lupus erythematosus; EPM, elevated plus maze test; HCs, healthy controls; IgG, immunoglobulin G; IL, interleukin; mPFC, medial prefrontal cortex; NPSLE, neuropsychiatric systemic lupus erythematosus; ROC, receiver operating characteristic; ROI, region of interest; SDS, sleep disturbance stress; SLE, systemic lupus erythematosus; TIV, total intracranial volume.

from the trial, whether this antibody or the Tyk2 inhibitor has efficacy in patients with dNPSLE should be explored.

As a possible molecular mechanism of dNPSLE, we hypothesised that stress-induced microglial activation, which led to an overactivation of neurons in the mPFC; this overactivation is critical for disinhibited symptoms like agitation, psychosis and ACS. Our study demonstrated that chronic stress induced a risk-taking behaviour with mPFC overactivation in SDS-subjected MRL/*lpr* mice, which would be similar behaviours to the hyperactivity and psychomotor agitation in patients with

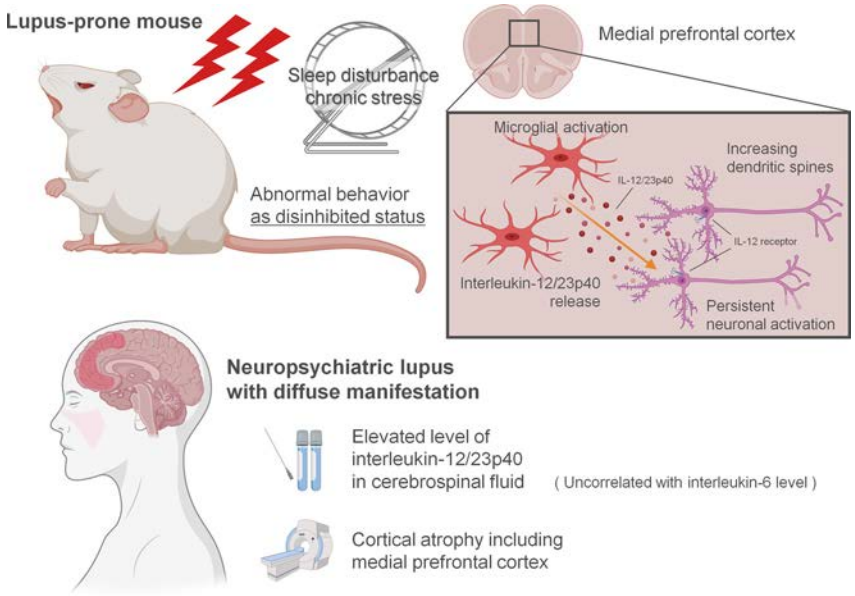

Figure 8 Graphical abstract. SDS-subjected lupus-prone MRL/*lpr* mice demonstrated disinhibited anxiolytic behaviour. Mechanistically, microglial activation, IL-12/23p40 upregulation and neuronal activation with increasing dendritic spines in the medial prefrontal cortex were observed. These stress-induced neuropsychiatric phenotypes were reversed by blockade of the IL-12/23 axis using IL-12/23p40 neutralising antibody or Tyk2 inhibitor. Patients with neuropsychiatric lupus showing diffuse neuropsychological manifestations demonstrated elevated levels of IL-12/23p40 in the cerebrospinal fluid and medial prefrontal cortical atrophy. These results suggest a pathological link between the stress-induced microglial IL-12/23p40 axis with neuronal activation and the development of neuropsychiatric lupus with diffuse manifestations. IL, interleukin; SDS, sleep disturbance stress; Tyk2, tyrosine kinase 2.

NPSLE. Indeed, a specific type of delirium with increased vigilance manifesting agitation, overactivity and hallucinations is observed in patients with NPSLE with ACS. Our mouse model could at least in part explain some of these ACS manifestations as stress-induced and inflammation-induced mPFC overactivation in patients with dNPSLE. Consistently, patients with SLE show an altered mPFC status in decision-making tasks,[54] reduced metabolism and atrophy,[55] and decreased cerebral blood flow.[56] An increase of dendritic spines in PFC neurons is also observed in autism-spectrum disorder and in patients taking antidepressants or N-methyl-D-aspartic acid, which manifest anxiolytic/depressive hyperactive behaviour.[57–60]

Patients with SLE often show more exasperated neuropsychiatric symptoms just after receiving glucocorticoid therapy compared with patients with other autoimmune disorders. These post-glucocorticoid neuropsychiatric symptoms are similar to the diffuse manifestations in NPSLE.[61] Because glucocorticoids sometimes induce mPFC impairment via neuronal alterations,[62 63] IL-12/23 signalling in the altered microglia-neuronal axis in the mPFC demonstrated here may contribute to the development of neuropsychiatric symptoms in patients with SLE undergoing glucocorticoid treatment. Compared with glucocorticoid monotherapy, we thus hypothesise that immunosuppressants such as cyclophosphamide, which would induce microglial apoptosis, combined with glucocorticoid possibly works better for dNPSLE symptoms through inhibiting the interaction between activated microglia and neurons described here. Moreover, JAK/Tyk2-Stat inhibitors would be beneficial for dNPSLE through inhibiting activated microglia-inducing neuronal alteration.

Taken together, we demonstrate that stress has pathogenic neuropsychiatric effects on a lupus rodent model by activating microglia and altering neurons in the mPFC via the IL-12/23 signalling pathway. We also found similar phenomena as an elevated level of CSF IL-12/23p40 and mPFC atrophy in patients with dNPSLE (figure 8). Therefore, our findings suggest that IL-12/23p40 in the mPFC is a therapeutic target for dNPSLE.

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Competing interests None declared.

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

Patient consent for publication Not applicable.

Ethics approval This study involves human participants. Ethical approval for the clinical studies was granted by institutional review board of Hokkaido University Hospital (approval numbers: 019-0055 and 020-0110) and that of Kitasato University School of Medicine (approval number: B20-231) and for the animal experiments and clinical studies was granted by the Institutional Animal Care and Use Committees of Hokkaido University (approval number: 14–0083). This study complied with the Declaration of Helsinki. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository. Data are available upon reasonable request. In detail, RNA-seq datasets that support the observations of this study have been deposited in the Gene Expression Omnibus with the series accession number GSE176429. MRI data have been stored locally following national and Japanese laws on the protection of individuals with regard to the processing of personal data. Other data are available in the main text or the supplementary materials and are available upon reasonable request to the corresponding author.

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CLINICAL SCIENCE

Peroxisome proliferator activated receptor-γ agonist pioglitazone improves vascular and metabolic dysfunction in systemic lupus erythematosus

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ABSTRACT

Objectives Premature cardiovascular events in systemic lupus erythematosus (SLE) contribute to morbidity and mortality, with no effective preventive strategies described to date. Immune dysregulation and metabolic disturbances appear to play prominent roles in the induction of vascular disease in SLE. The peroxisome proliferator activated receptor-gamma agonist pioglitazone (PGZ suppresses vascular damage and immune dysregulation in murine lupus and improves endothelial dysfunction in other inflammatory diseases. We hypothesised that PGZ could improve vascular dysfunction and cardiometabolic parameters in SLE.

Methods Eighty SLE subjects with mild to severe disease activity were randomised to a sequence of PGZ followed by placebo for 3 months, or vice versa, in a double-blind, cross-over design with a 2-month wash-out period. Primary endpoints were parameters of endothelial function and arterial inflammation, measured by multimodal assessments. Additional outcome measures of disease activity, neutrophil dysregulation, metabolic disturbances and gene expression studies were performed.

Results Seventy-two subjects completed the study. PGZ was associated with a significant reduction in Cardio-Ankle Vascular Index (a measure of arterial stiffness) compared with placebo. Various metabolic parameters improved with PGZ, including insulin resistance and lipoprotein profiles. Circulating neutrophil extracellular trap levels also significantly decreased with PGZ compared with placebo. Most adverse events experienced while on PGZ were mild and resolved with reduction in PGZ dose.

Conclusion PGZ was well tolerated and induced significant improvement in vascular stiffness and cardiometabolic parameters in SLE. The results suggest that PGZ should be further explored as a modulator of cardiovascular disease risk in SLE.

Trial registration number NCT02338999.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Premature cardiovascular disease (CVD) in patients with systemic lupus erythematosus (SLE) is associated with significant morbidity and mortality. The underlying mechanisms of premature CVD in SLE are not well defined and no therapeutic agents have shown to significantly reduce CVD risk in SLE.

WHAT THIS STUDY ADDS

⇒ The peroxisome proliferator-activated receptor-γ agonist pioglitazone is associated with improvement in vascular stiffness and various cardiometabolic parameters in SLE.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These results have implications in using non-immunosuppressive therapy that could decrease CVD risk in patients with SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune syndrome with heterogeneous clinical manifestations. While there has been substantial progress in treatment of SLE, this condition is still associated with significant morbidity and mortality, driven in part by premature cardiovascular disease (CVD).[1] Depending on the study and outcome measure, the risk of CVD, especially in young women with SLE, can be as high as 50-fold when compared with matched controls.[2] CVD driven by atherosclerosis develops or progresses in ∼10% of SLE patients/year during short-term follow-up and is one of the most common causes of death.[3 4] The traditional Framingham risk score cannot explain the CVD risk in SLE. Indeed, lupus is now recognised as an independent CVD risk factor.[5]

While the underlying mechanisms of premature CVD in SLE are not well defined, immune

dysregulation coupled with cardiometabolic dysfunction are considered key drivers. This is exemplified by the characterisation of a pathophysiological alliance between type I Interferons (IFNs) and neutrophil dysregulation as inducers of vascular damage in SLE.[6–8] In turn, aberrant formation of neutrophil extracellular traps (NETs) by SLE low-density granulocytes (LDGs) can oxidise lipoproteins and blunt the anti-atherogenic function of high-density lipoprotein (HDL). Furthermore, insulin resistance (IR) is highly prevalent in SLE, may be triggered in part by type I IFNs and other proinflammatory mediators and contribute to cardiometabolic dysfunction and atherosclerosis progression.[9 10] Metabolic syndrome has also been associated with enhanced organ damage, vascular events and mortality in SLE.[11 12] Recent evidence indicates that regulating innate immune pathways and inflammation in SLE can modulate various cardiometabolic parameters, including enhancing HDL's cholesterol efflux capacity.[11 13]

In contrast, several attempts to modulate CV damage in SLE through the use of statins has given inconclusive or negative results.[14 15] The use of some immunomodulators and immunosuppressives has been associated with a modest protective effect, but to date there are no therapeutic agents that have demonstrated to significantly reduce CVD risk in SLE.[16 17]

The thiazolidinediones (TZDs), including pioglitazone (PGZ), are a class of drugs approved for the treatment of patients with type 2 diabetes mellitus (DM). They belong to the family of drugs that activate the peroxisome proliferator-activated receptor-γ (PPAR-γ) and have been found to confer antiatherogenic and anti-inflammatory effects in diabetics and non-diabetic patient groups.[18] In animal models of lupus, TZDs improved vascular damage, endothelial dysfunction and disease activity.[19–21] Furthermore, PGZ improved vascular function and disease activity in rheumatoid arthritis.[22 23]

We hypothesised that PPAR-γ agonists may benefit SLE patients by suppressing inflammatory and immunologic pathways that promote CVD and internal organ damage. To test this hypothesis, we performed a double-blind, placebo-controlled, crossover study to test whether short term use of PGZ improves vascular function, vascular inflammation and various cardiometabolic parameters in SLE.

MATERIALS AND METHODS

Study design and subjects

The study design and conduct complied with relevant regulations regarding the use of human study participants and was conducted in accordance to the criteria set by the Declaration of Helsinki, as authorised by the NIH Office of Human Subject Research. After written informed consent and determination of eligibility, subjects were randomised to a sequence of PGZ followed by placebo (sequence AB), or placebo followed by PGZ (sequence BA) in a 1:1 allocation ratio, in a double-blind cross-over design. The starting dose of PGZ was 30 mg/day, which was titrated up to 45 mg after 1 week if tolerated. There was a 2-month wash-out period between the cross-over (online supplemental figure 1). Eighty SLE subjects that met the American College of Rheumatology Revised Criteria for the Classification of SLE and had mild to severe disease activity (Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI 2K) score between 4 and 20 or SLEDAI 2K ≥2 not considering anti-dsDNA or complement levels), and lack of A flares on the British Isles Lupus Activity Group (BILAG 2004) were enrolled in an outpatient clinical research setting.[24] Eligible subjects were on stable doses of antimalarials and immunosuppressants (for 12 weeks prior to the screening visit) and/or oral glucocorticoids (for 2 weeks prior to the screening visit; prednisone or equivalent <20 mg/day). The primary outcome was change in the vascular function as measured by non-invasive vascular tests and the secondary outcome was decrease in SLE disease activity. The outcome variables were measured at baseline(day 1), and months 3, 5 and 8. The wash-out period was between months 3 and 5. SLE disease activity was determined using SLEDAI 2K, BILAG 2004, Physician Global Assessment (Likert scale 0–3) and patient-reported outcomes 36-item Short Form Survey (SF-36).[25–28] Rate of adverse events (AEs, defined by the National Cancer Institute, Common Terminology Criteria for Adverse Events, V.4.0) was recorded at each visit.

See online supplemental methods for assessments of vascular function, metabolic parameters, LDGs, NETs, transcriptional analysis, flow cytometry and statistical analysis.

RESULTS

Characteristics of the cohort

Eighty subjects were randomised and took at least one dose of the drug and 72 completed all phases of the study. Four subjects withdrew due to AEs (pruritus, weight gain, polyuria), two due to SLE flare, one each due to travel constraints and lost to follow-up (figure 1). Baseline demographics were similar in both sequences (PGZ-Placebo (AB) and Placebo-PGZ (BA); table 1). Consistent with SLE demographics, 87.5% were females, with mean±SD age 45.7±12.1 years and mild-to-moderate disease activity (SLEDAI 2K : 5.1±2.88).

Pioglitazone improves arterial stiffness

PGZ use was associated with a significant decrease in arterial stiffness, as determined by Cardio-Ankle Vascular Index (CAVI) (0.37±0.9 in period 1 and −0.27±0.56 period 2 when PGZ was given vs 0.11±0.65 in period 1 and −0.07±0.66 in period 2 when placebo was given(figure 2). CAVI values decreased by 0.32 points more (95% CI -o.54,-0.10; p=0.005, table 2) in the PGZ group compared with placebo. CAVI values reverted to baseline during the wash-out period and while subjects were on placebo. Other measures of vascular stiffness (PWV and RHI) did not display significant improvement with PGZ (p=0.37 and 0.91, respectively, table 2).

18fluoro-D-glucose positron emission tomography integrated with CT scans were performed on 30 subjects who consented to the procedure and analysis did not reveal significant changes in vascular inflammation after 3 months of PGZ (Aortic arch TBR p=0.84, Global TBR p=0.17). Overall, PGZ use for 3 months in mild-to-moderate SLE resulted in significant improvements in arterial stiffness, as assessed by CAVI.

Pioglitazone improves cardiometabolic parameters

There were improvements in serum lipoproteins and IR with the PGZ use. Serum HDL levels increased with PGZ (4.14±12.29 in period 1; 5.42±11.53 in period 2) compared with placebo (1.28±10.42 in period 1; −1.36±7.91 in period 2). Overall, the increase was 4.72 mg/dL more (95% CI 1.27 to 8.18), p=0.008) in PGZ than in placebo (figure 3A). Similarly, HDL particle size increased by 0.28 (95% CI 0.18 to 0.39, p<0.0001) and the particle number decreased by −1.84 (95% CI −2.90 to −0.78, p=0.0009). Conversely, there was an increase in low-density lipoprotein (LDL) particle size by 0.51 (95% CI 0.38 to 0.71, p<0.0001) and decrease in LDL particle number by −117.1 (95% CI −183.3 to −51.0, p=0.0006) with PGZ(table 2). The concentration of small LDL particles

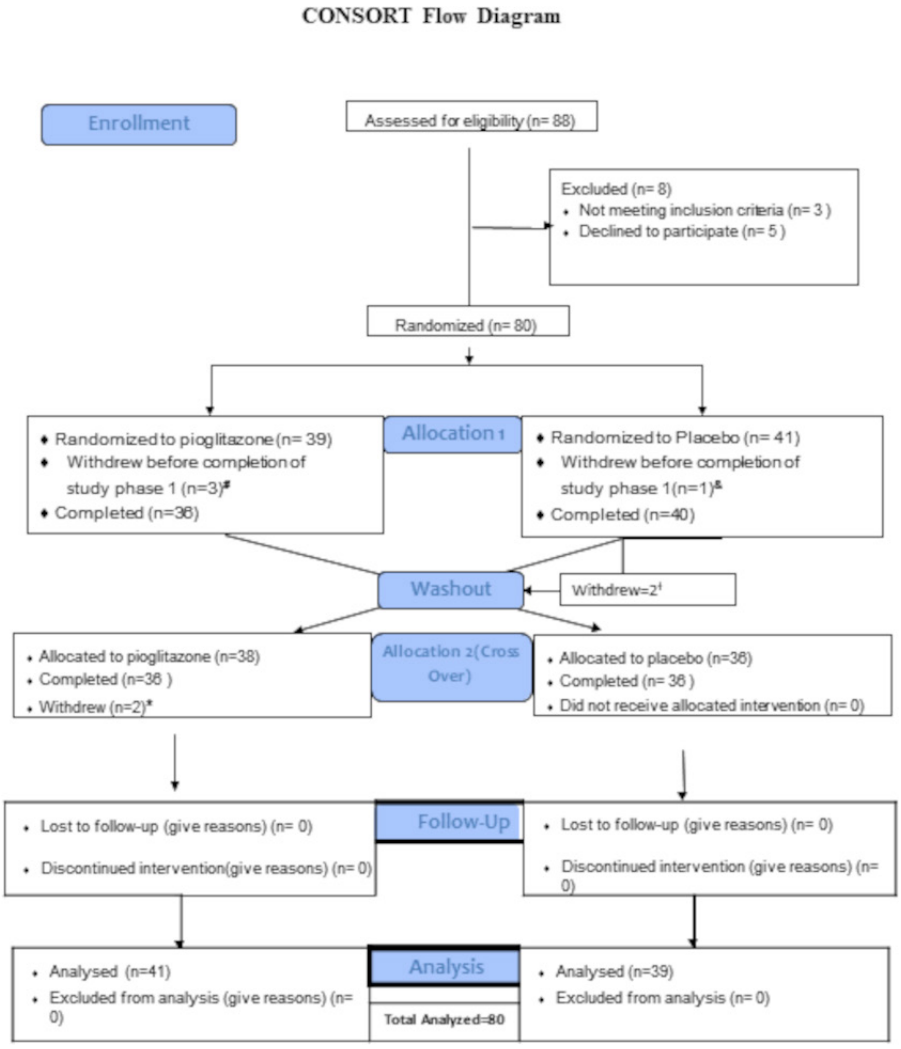

Figure 1 Consolidated Standards of Reporting Trials Flow Diagram. A total of 88 subjects were screened for the trial, with 80 subjects randomised to sequence AB (PGZ-wash-out-placebo N=39) or sequence BA (Placebo-wash-out-PGZ N=41). A total of 72 subjects completed all phases of the clinical trial. #Withdrew due to travel n=1; withdrew voluntarily due to AE (pruritus and increased urinary frequency) n=2; and lost to follow-up n=1. †Withdrawn due to SLE flare n=2. *Subject withdrew voluntarily due to weight gain n=2. AEs, adverse events; SLE, systemic lupus erythematosus.

(s-LDLP) decreased by −254.76 (95% CI −354.13 to −155.39, p<0.0001) whereas the concentration of large LDL particles increased by 236.68 (95% CI 182.47 to 290.89, p<0.0001) on PGZ treatment (figure 3B,C). Serum triglyceride levels decreased with PGZ by −20.94±39.57 mg/dL during period 1 and by −14.47±45.33 mg/dL during period 2, with an overall

Table 1 Baseline characteristics of study subjects

	Pioglitazone-placebo group=sequence AB	Placebo-Pioglitazone group=sequence BA	Total
	N=39	N=41	N=80
Race/ethnicity: N (%)			
Hispanic	16 (41)	16 (39)	32 (40)
Caucasian	9 (23)	9 (22)	18 (22.5)
African American	9 (23)	8 (19.5)	17 (21.25)
Asian	4 (10)	5 (12)	9 (11.25)
Multi	0 (0)	1 (2)	1 (1.25)
Unknown	1 (2.5)	2 (5)	3 (3.75)
Female: N (%)	33 (84.6)	37 (90.2)	70 (87.5)
Male: N (%)	6 (15.4)	4 (9.8)	10 (12.5)
Age (years) mean (SD)	46.03 (13.79)	45.32 (10.41)	45.66 (12.1)
Disease duration (years) mean (SD)	13.59 (11.64)	12.59 (10.46)	13.08 (10.99)
BMI mean (SD)	28.52 (5.76)	30.49 (7.5)	29.53 (6.74)
SLEDAI 2K mean (SD)	5.13 (2.75)	5.07 (3.04)	5.1 (2.88)

Descriptive statistics were used to characterise patients for continuous variables using mean and SD. For categorical variables frequencies and (%) percentages were used.
BMI, body mass index; SLEDAI 2K, Systemic Lupus Erythematosus Disease Activity Index 2000.

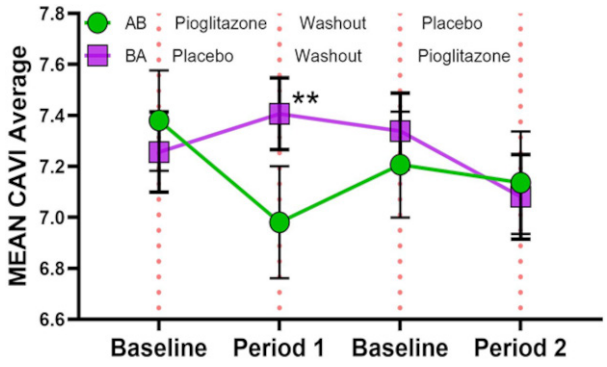

Figure 2 PGZ improves vascular stiffness in SLE. Mean Cardio-Ankle Vascular Index (CAVI) average of right and left side in subjects randomised to sequence AB (N=39; PGZ-wash-out-placebo) and sequence BA (N=41; placebo-wash-out-PGZ). The CAVI values decreased by 0.32 points (95% CI 0.10 to 0.54, p=0.005) in the pioglitazone group compared with the placebo. All data presented as mean+SD. **p≤0.01.

reduction by −18.09 mg/dL (95% CI −30.52 to −5.67, p=0.005) with PGZ use compared with placebo. We also noted a PGZ-mediated decrease in triglyceride-rich lipoproteins (TRLs) of −16.03 (95% CI −26.98 to −5.07, p=0.01), another subset of lipoproteins considered to be causal for atherosclerotic CVD (figure 3D). There were no significant changes in cholesterol efflux capacity with PGZ use (table 2).

There was reduction in circulating alanine with the PGZ use by −33.35 (95% CI −51.29 to −15.4, p=0.0004) (figure 4A). There were 37 subjects (51.4%) with evidence of IR (Homoeostasis Model Assessment of IR (HOMA IR cut-off >1.9) at the beginning of the trial. With PGZ use, 18 (48.6%) of these subjects had normalisation in HOMA IR. Baseline insulin and HOMA IR levels were 16.86+9.65 mcU/mL and 2.13±1.21, respectively. While on PGZ, serum insulin and HOMA IR levels decreased by −4.02±9.9 and −0.51±1.28 during period 1; by −5.46±8.59 and −0.69±1.08 during period 2 (p=0.003 and p=0.0003), respectively; figure 4B,C). Overall, serum insulin and HOMA IR levels decreased by-3.77 (95% CI −6.22 to −1.3, p=0.003) and −0.23 (95% CI −0.35 to −0.11, p=0.0003), respectively, with PGZ use compared with placebo. Serum glucose was not significantly modified with PGZ use (p=0.13). All metabolic parameters returned to baseline values during wash-out and placebo phases. Overall, short-term use of PGZ resulted in significant improvements in lipoprotein profiles, a significant shift in LDL particle number from a high to lower pro-atherogenic form and improved IR in mild-to-moderate SLE.

Pioglitazone does not modify interferon-stimulating genes but decreases NET levels

PGZ use did not alter interferon-stimulating genes (ISGs), as assessed by Nanostring (online supplemental figure 2). While PGZ use was not associated with changes in LDG levels, it was associated with lower levels of circulating NET remnants (p=0.026; online supplemental figure 3). There were no significant changes in soluble markers of endothelial cell activation (sL-selectin, sICAM-1 and sVCAM-1) with the use of PGZ. As the targeted analysis of inflammation-related genes showed no effect when subjects were treated with PGZ, we performed unbiased screening to detect potentially other effects of this drug on immune phenotype. Whole blood transcriptomic analysis and high parameter cytometry phenotyping of peripheral blood mononuclear cells was done on a subset of patients who had demonstrated the greatest improvements in CAVI when treated with PGZ, but no changes in either could be attributed to PGZ (online supplemental material). Overall, short-term use of PGZ did not modify ISGs and other immune related parameters but did lower the levels of circulating NETs.

Table 2 Summary of vascular and metabolic variables by sequence and period

	Sequence AB (pioglitazone/placebo) N=39				Sequence BA (placebo/pioglitazone) N=41					
	Period 1 (pioglitazone)		Period 2 (placebo)		Period 1 (placebo)		Period 2 (pioglitazone)		Treatment effect*	
Variable (mean±SD)	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change	Estimate (95% CI)	P value
CAVI average	7.38±1.23	−0.37±0.90	7.21±1.25	−0.07±0.66	7.26±1.01	0.11±0.65	7.34±0.92	−0.27±0.56	−0.32 (-0.54, - 0.10)	0.005
Log-transformed RHI	0.72±0.37	0.07±0.35	0.71±0.29	0.02±0.36	0.65±0.36	0.02±0.48	0.64±0.54	−0.05±0.35	−0.007 (-0.123 0.110)	0.91
PWV m/s	6.59±1.82	−0.31±2.06	7.15±1.22	−0.25±0.94	6.73±1.52	0.03±2.03	7.01±2.44	−0.31±1.86	−0.18 (-0.57,0.21)	0.37
Augmentation Index	23.59±16.33	−2.25±11.84	26.28±15.90	−1.75±10.01	26.24±10.9	−0.43±10.32	23.61±9.64	0.47±12.33	−1.36 (-4.11, 1.39)	0.33
Aortic Arch TBR*†	1.47±0.16	0.09±0.12			1.53±0.19	−0.05±0.27			0.01 (-0.16, 0.18)	0.84
Global TBR*†	1.68±0.14	0.03±0.20			1.66±0.19	0.04±0.30			0.09 (-0.04, 0.23)	0.17
Cholesterol	174.97±30.04	0.06±14.74	177.42±31.48	−2.44±15.83	169.71±30.18	1.35±21.73	171.58±36.69	−0.44±21.39	0.38 (-5.17, 5.92)	0.89
LDL mg/dL	90.9±29.38	0.14±14	93.58±28.43	−0.47±13.05	88.29±26.53	−0.88±18.64	93.32±31.95	−6.19±24.33	−1.65 (-6.38,3.09)	0.91
Triglycerides mg/dL	101.67±40.52	−20.94±39.57	97.19±44.85	−2.72±36.24	110.35±52.1	6.54±47.97	109.97±67.84	−14.47±45.33	−18.09 (-30.52, -5.67)	0.005
HDL mg/dL	63.79±20.37	4.14±12.29	64.33±18.29	−1.36±7.91	59±20.91	1.28±10.42	59.34±22.93	5.42±11.53	4.72 (1.27, 8.18)	0.008
HDL particle no mcmol/L	31.2±6.6	−1.58±3.83	31.13±7.41	0.18±2.62	31.36±6.51	0.11±3.45	31.61±6.65	−1.76±4.23	−1.84 (-2.90,- 0.78)	0.0009
HDL size nm	9.79±0.66	0.27±0.42	9.84±0.63	−0.04±0.27	9.58±0.63	0.02±0.35	9.6±0.65	0.29±0.37	0.28 (0.18, 0.39)	<0.0001
LDL particle no nmol/L	958.1±396.6	−83.4±234.1	977.3±401.5	17.4±192.2	1037.5±387.4	−7.9±201.6	1032.1±411.5	−140.6±268.6	−117.1 (-183.3, -51.0)	0.0006
LDL size nm	20.86±0.52	0.49±0.66	21.14±0.57	−0.15±0.42	20.85±0.68	−0.11±0.48	20.87±0.63	0.43±0.74	0.51 (0.38, 0.71)	<0.0001
Cholesterol efflux value	0.92±0.17	0.03±0.16	0.88±0.19	0.04±0.18	0.88±0.19	0.01±0.17	0.89±0.2	0.07±0.16	0.03 (-0.02, 0.08)	0.28
Glucose mg/dL	89.74±14.74	−3.69±16.08	89.92±10.26	−1.72±6.44	88.66±9.07	−0.65±6.85	88.92±9.99	−3.17±9.15	−1.91 (-4.39, 0.58)	0.13
Insulin Pmol/L	17.26±10.66	−4.02±9.9	18.98±19.26	−2.85±9.02	17.63±11.71	1.18±6.21	17.74±10.19	−5.46±8.59	−3.77 (-6.22,- 1.31)	0.0031
Homa2-IR	2.18±1.34	−0.51±1.28	2.36±2.15	−0.33±0.98	2.22±1.41	0.14±0.73	2.24±1.25	−0.69±1.08	−0.23 (-0.35,- 0.11)	0.0003

Data are mean±SD. Change is defined as the post baseline value minus the baseline value during the period: that is, M3 – D1 for period 1, M8 – M5 for period 2.
*Linear mixed effects models were used to calculate the estimated treatment effect (the treatment group difference in the change score between pioglitazone and the placebo), its 95% CI and the p value.
†These measures are based on 18F-FDG/PET CT scans. For these two variables measured in period 1 only, the treatment effect and the p value are calculated based on analysis of covariance.
CAVI, Cardio-Ankle Vascular Index; ^{18}F-FDG/PET CT scan, 18fluoro-D-glucose positron emission tomography integrated with CT; HDL, high-density lipoprotein; HOMA2IR, Homoeostasis Model Assessment of Insulin Resistance; LDL, low-density lipoprotein; PWV, pulse wave velocity; RHI, Reactive Hyperaemia Index; TBR, target/background ratio.

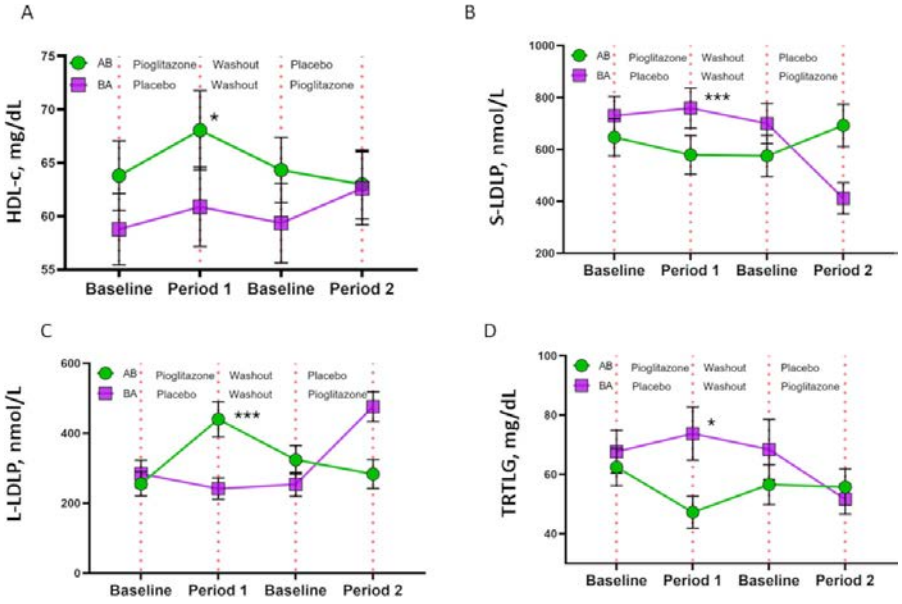

Figure 3 PGZ improves lipoprotein profiles in SLE. (A) Mean circulating HDL in subjects randomised to sequence AB (N=39; PGZ-wash-out-placebo) and sequence BA (N=41; placebo-wash-out-PGZ). The serum HDL levels increased by 4.72 mg/dL (95% CI: (1.27 to 8.18) with PGZ compared with placebo, with return to baseline by the end of wash-out period; p=0.008. (B) Mean circulating small LDL particles (s-LDLP) in subjects randomised to sequence AB (N=39; PGZ-wash-out-placebo) and sequence BA (N=41; placebo-wash-out-PGZ). The serum s-LDLP levels redcued by −254.76 (95% CI −354.13 to −155.39) with PGZ compared with placebo; p<0.0001. (C) Mean circulating large LDL particles (l-LDLP) in subjects randomised to sequence AB (N=39; PGZ-wash-out-placebo) and sequence BA (N=41; placebo-wash-out-PGZ). The serum l-LDLP levels increased by 236.68 (95% CI (182.47 to 290.89) with PGZ compared with placebo; p<0.0001. (D) Mean circulating triglyceride-rich lipoproteins (TRLs) in subjects randomised to sequence AB (N=39; PGZ-wash-out-placebo) and sequence BA (N=41; placebo-wash-out-PGZ). The serum TRL levels redcued by −16.03 (95% CI (−26.98 to −5.07) with PGZ compared with placebo; p=0.01. All data presented as mean+SD. *P≤0.05; ***p≤0.001. HDL, high-density lipoprotein; PGZ, pioglitazone; SLE, systemic lupus erythematosus.

Safety and tolerability

PGZ was well tolerated and did not affect disease activity in mild-to-moderate SLE. SLE disease activity, as measured by SLEDAI-2K, remained stable during the trial (online supplemental table 3). Two subjects developed moderate lupus flares during the wash-out period and withdrew from trial, as

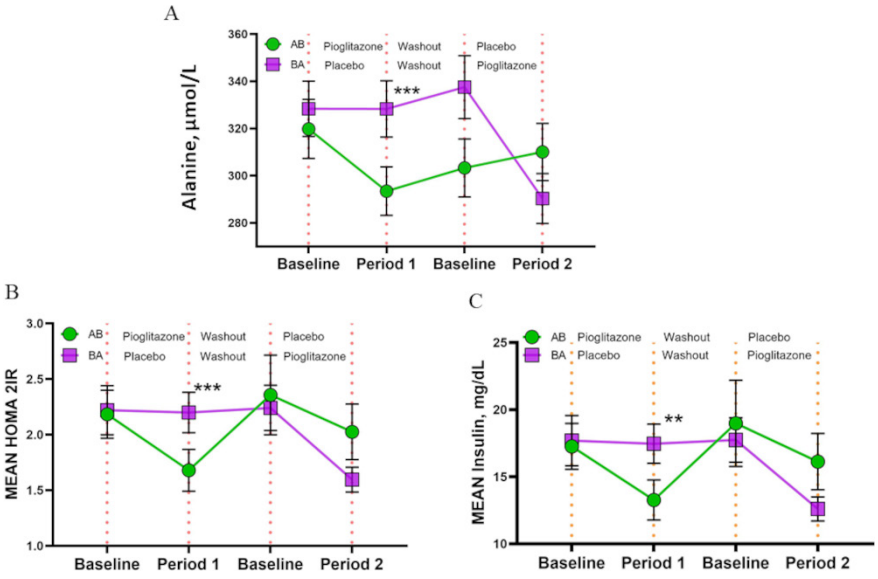

Figure 4 PGZ reduces serum alanine and improves insulin resistance in SLE. (A) Mean circulating serum alanine levels in subjects randomised to sequence AB (N=39; PGZ-wash-out-placebo) and sequence BA (N=41; placebo-wash-out-PGZ). The serum alanine levels redcued by −33.35 (95% CI (−51.29 to −15.4), with PGZ compared with placebo; p=0.0004. (B) Mean homoeostasis model assessment of IR (HOMA2-IR) in subjects randomised to sequence AB (N=39; PGZ-wash-out-placebo) and sequence BA (N=41; placebo-wash-out-PGZ). HOMA IR levels decreased by −0.23 (95% CI (−0.35 to −0.1), p=0.0003), respectively PGZ a compared with placebo. (C) Mean serum insulin levels in subjects randomised to sequence AB (N=39; PGZ-wash-out-placebo) and sequence BA (N=41; placebo-wash-out-PGZ). Overall, the serum insulin levels decresased by-3.77 (95% CI (−6.22 to −1.31), p=0.003) with the use of pioglitazone as compared with placebo. All data presented as mean+SD; **p≤0.01; ***p≤0.001. PGZ, pioglitazone; SLE, systemic lupus erythematosus.

Table 3 Adverse events (AE) by body system and treatment

Body system preferred term severity	Pioglitazone (N=77) n (%)	Placebo (N=77) n (%)
Blood and lymphatic system disorders	5 (6.5)	1 (1.3)
Cardiac disorders	4 (5.2)	1 (1.3)
Eye disorders	1 (1.3)	3 (3.9)
Gastrointestinal disorders	15 (19.5)	14 (18.2)
General disorders	8 (10.4)	5 (6.5)
Immune system disorders	0 (0.0)	1 (1.3)
Infections and infestations	15 (19.5)	27 (35.1)
Injury poisoning and procedural complications	0 (0.0)	1 (1.3)
Investigations*	15 (19.5)	7 (9.1)
Metabolism and nutrition disorders	4 (5.2)	1 (1.3)
Musculoskeletal and connective tissue disorders	4 (5.2)	4 (5.2)
Nervous system disorders	16 (20.8)	12 (15.6)
Psychiatric disorders	2 (2.6)	2 (2.6)
Renal and urinary disorders	5 (6.5)	2 (2.6)
Reproductive system and breast disorders	0 (0.0)	2 (2.6)
Respiratory thoracic and mediastinal disorders	6 (7.8)	9 (11.7)
Skin and subcutaneous tissue disorders	3 (3.9)	2 (2.6)
Surgical and medical procedures	1 (1.3)	0 (0.0)
Vascular disorders	1 (1.3)	2 (2.6)

n=number of subjects who had specific AE at least once; % of total number of subjects.
A total of 13 SAE were observed in 10 subjects. Eight SAEs while on placebo and five while on pioglitazone. All SAEs were followed until resolved.
*Abnormal lab values.
SAE, serous adverse event.

escalation of immunosuppressive therapy was not allowed while in the study. There was an increase in serum C4 levels associated with PGZ use (p=0.04) while the rest of the serological parameters (C3 and anti-ds-DNA antibody) did not show significant changes (online supplemental table 3). Self-reported disease outcomes, as measured by SF-36, showed a trend towards improvement with PGZ that was not statistically significant (p=0.08).

There were 249 AEs recorded during the study, with no significant difference in overall AEs between the two groups (52.6% of AEs on PGZ and 47.4% of AEs on placebo). The majority of AEs (67.5%) were mild and resolved without any intervention; there was one urinary tract infection requiring hospitalisation in a subject while on placebo. Overall, there were more infections while subjects were on placebo (table 3). No deaths occurred during the study (online supplemental table 4). Weight gain, fluid retention and mild transaminitis were noted in nine subjects on titrating up PGZ dose to 45 mg /day and these events either self-resolved or resolved after dose reduction to 30 mg/day. There were no cases of new onset hematuria, bladder cancer, congestive heart failure or fragility fractures during the study. Most laboratory tests remained stable, with changes that were not clinically significant but with some that were statistically significant and most likely due to volume overload (online supplemental table 5). Overall, PGZuse was well tolerated in SLE and was associated with an improvement in C4 complement proteins but no significant changes in disease activity in patients with mild-to-moderate SLE.

DISCUSSION

CVD due to accelerated atherosclerosis is a significant contributor of morbidity and mortality in SLE and the effect of drugs currently used to treat SLE on improving cardiometabolic parameters and CV risk in SLE has not been systematically demonstrated. Antimalarials may display a mild vasculo-protective role due to pleiotropic effects on the immune system[16] while some immunosuppressive roles may have mild protective effects that remain to be demonstrated in larger patient populations.[17] As such, finding interventions that can modulate lupus vasculopathy, modify cardiometabolic risk and not further immunosuppress these patients is an area of great need in this disease. In the current study, we showed that PGZ, when used in non-diabetic patients with mild to moderate SLE, improves arterial stiffness and various metabolic parameters associated with increased CVD risk. The results of the study support previous observations that TZDs have immunomodulatory and vasculo-protective roles in murine models of lupus and in patients with RA.[20–22]

Arterial stiffness, as measured by CAVI, was the main vascular parameter that improved during PGZ use. CAVI measures the stiffness of the arterial tree from the origin of the aorta to the ankle and has been shown to be an independent CVD risk factor and a putative surrogate end-point marker for vascular disease risk.[29] SLE patients have higher incidence of abnormal CAVI, and this may contribute to their increased CVD risk.[30] Supporting previous studies, the baseline CAVI values in SLE subjects in this study were significantly higher than the reference value for age and gender-matched healthy volunteers,[31] indicating that SLE subjects with mild to moderate disease display significant arterial stiffness that improves with short-term use of PGZ. In contrast, other vascular function measurements did not significantly change with PGZ. While the implications of these discrepancies using the different vascular function assessments remains to be determined, these results support the need for multimodal measurements of vascular function to better understand how different vascular territories are affected in SLE. Vascular inflammation that was measured in a subset of the subjects enrolled in the study did not show significant changes after 3 months of PGZ. The reasons for this lack of response may be related to the short duration of drug exposure that may not had been sufficiently long to lead to changes in inflammation of the vessel wall, in contrast to the metabolic effects that occurred within the timeframe of the study that could have benefited vascular function. In contrast, changes in systemic immune parameters that could have contributed to alter vascular wall inflammation were not modified during the trial, with the exception of NET levels. Another possibility for the lack of detected effect on arterial wall inflammation could have been that the FDG-PET-CT was performed only in a subset of patients in the study and the sample size may not have allowed to detect these differences. It is possible that the impact of PGZ on vascular function in SLE is not related to immune regulation but, rather, to modifications of metabolic parameters known to have significant impact on vascular disease.

In previous studies in non-lupus populations, PGZ was effective in primary and secondary CVD prevention and in modulating renal AEs in individuals with or at high risk to develop type 2 DM.[32] SLE patients have well described abnormalities in IR and lipoprotein profiles, with proatherogenic consequences.[33] In a previous small clinical trial, PGZ administration over 3 months led to improvements in HDL levels, IR and HDL size, while decreasing markers of inflammation such as C reactive protein and serum amyloid A.[34] In the current study, PGZ

use was associated with increases in HDL, HDL particle size and number, reduced triglycerides and TRLs, a switch from s-LDLP to less atherogenic larger ones, reduced alanine and improved IR. The decrease in circulating alanine levels with PGZ treatment may be due to previously reported effects of PPAR agonism on Alanine Aminotransferase activity, which converts alanine to pyruvate and glutamate.[35] The clinical significance of this finding is a subject for future investigation. As expected, the improvement in HOMA-IR was due to reduced serum insulin levels without a drop in serum glucose, which is important from a safety perspective in these non-diabetic patients. While cholesterol efflux capacity was not altered in this study, the changes in lipoprotein profile may confer additional antiatherogenic effects beyond this measurement. This remains to be determined in the future studies.

There are concerns with the use of pioglitazone in diabetics, such as fluid retention, increased risk of fracture and bladder cancer.[36–38] In SLE, short term PGZ use was well tolerated and the side effects were consistent with what has been described in the literature, including peripheral oedema and mild transaminitis in a small proportion of patients. There were no fractures, new onset of hematuria or bladder cancer during the study. However, whether longer exposure to this drug in SLE can promote these complications remains to be determined.

The subjects enrolled in this study had overall low SLE disease activity at enrollment. As such, the probability to observe any significant improvements in disease activity would be limited and the study was not designed or powered to assess the role of this drug in disease activity. Subjects were kept on standard of care and any escalation in dose or addition of new medication for SLE would result in withdrawal from the study, which further precluded establishing immunomodulatory roles of the drug in this disease. Of note, C4 levels increased significantly while on PGZ, indicating some potential role in normalising biomarkers of disease activity in SLE. This should be explored in future studies. The lack of significant changes in disease activity was paired to the observation that use of this drug for a limited period of time did not result in significant modulation of the type I IFN response, or changes in cytokine levels. Therefore, it is possible that the favourable effect on arterial stiffness promoted by this drug was secondary to the effects on lipoprotein parameters and IR. However, NET levels decreased while SLE patients were on PGZ, indicating a putative immunomodulatory effect on dysregulated neutrophil biology previously described in SLE. Whether this decrease in NETs contributed to improving vascular stiffness remains to be determined in follow-up studies, given that NETs have been found to be linked to vascular disease in lupus and other chronic inflammatory conditions.

Limitations of this study come from the relatively short duration of the study and the inclusion of only of mild-to-moderate SLE patients, which precluded our ability to further investigate how the drug modulated disease activity and more severe vascular disease. As mentioned above, the ability to check vascular inflammation only in a subset of the patients, limited the ability to evaluate the role of this drug in this specific parameter.

In summary, PGZ was well tolerated during short-term use in SLE, and was associated with significant improvements in arterial stiffness and various cardiometabolic parameters considered to be CVD risk factors. Exploring whether PPAR-γ modulation, with PGZ or other newer generation drugs, can mitigate organ damage and disease manifestations in SLE while maintaining an adequate safety profile should be explored in future studies.

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

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Reason: Listed as below name of ethics committee: National Institutes of HealthOffice of Human Subjects Research ProtectionsOffice of IRB Operations (IRBO) Reference Number: 15AR0060 Participants gave informed consent to participate in the study before taking part.

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EPIDEMIOLOGICAL SCIENCE

SARS-CoV-2 Omicron escapes mRNA vaccine booster-induced antibody neutralisation in patients with autoimmune rheumatic diseases: an observational cohort study

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ABSTRACT

Objectives This study investigates whether COVID-19 vaccines can elicit cross-reactive antibody responses against the Omicron variant in patients with autoimmune rheumatic diseases (ARDs).

Methods This observational cohort study comprised 149 patients with ARDs and 94 healthcare workers (HCWs). Blood samples were obtained at enrolment, a median of 15 weeks after the second vaccine dose or 8 weeks after the third dose. The functional cross-neutralisation capacity of sera was measured using the Omicron variant receptor-binding domain-ACE2 binding inhibition assay. We assessed the incidence of breakthrough infections and the potential correlation with neutralising responses in participants after receiving third doses. The association of time-from-vaccine and neutralising responses in sera was predicted using linear regression analysis.

Results The mean cross-neutralising responses against the Omicron variant developed after the second dose was 11.5% in patients with ARDs and 18.1% in HCWs (p=0.007). These responses were significantly lower in patients with ARDs than in HCWs after the third dose (26.8% vs 50.3%, p<0.0001). Only 39.2% of the patient sera showed functional neutralisation capacity to the Omicron variant and cross-neutralising responses were shown to be poorly correlated with anti-spike immunoglobulin G titres. Within 6 weeks of immunological assessments, significantly lower Omicron-neutralising responses were detected in sera from patients with ARDs who developed breakthrough infections compared with those who did not (p=0.018). Additionally, a relative decline was implied in neutralising responses against the Omicron variant as a reference to the wild-type virus during 120 days since the third vaccination, with a predicted decay rate of −0.351%/day (95% CI, −0.559 to −0.144, p=0.001).

Conclusions Striking antibody evasion manifested by the Omicron variant in patients with ARDs and current vaccine-induced immunity may not confer broad protection from Omicron breakthrough infection, highlighting the need for further research on vaccine effectiveness in patients with immune dysfunctions.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Antibody neutralisation of the Omicron variant of SARS-CoV-2 was potently induced by the third dose of an mRNA vaccine in the general population. However, real-world data evaluating the impact of the SARS-CoV-2 Omicron variant on vaccine-induced immunity in patients with autoimmune rheumatic diseases are sparse.

WHAT THIS STUDY ADDS

⇒ This study shows that, while the third dose of an mRNA vaccine is immunogenic in patients with autoimmune rheumatic diseases, at least half of the patients with measurable neutralising responses against the wild-type virus failed to generate cross-neutralising responses against the Omicron variant. Further, sera from vaccinated patients with confirmed breakthrough infections showed lower cross-neutralising responses, suggesting a significant correlation between the functional cross-variant neutralisation capacity and protection from breakthrough infection.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Given the limited correlation between cross-neutralising responses against the Omicron variant and the ancestral anti-spike immunoglobulin G titres elicited by the third dose of an mRNA vaccine in patients with autoimmune rheumatic diseases, quantifying the functional cross-variant neutralisation capacity may be a precise approach for determining the immunological benefit conferred to them by booster immunisations.

INTRODUCTION

SARS-CoV-2—the aetiological agent of COVID-19—has caused substantial morbidity and mortality in patients with autoimmune rheumatic diseases (ARDs).[1 2] Rapid development of successful vaccines has enabled their widespread administration.[3] Nevertheless, some patients with ARDs reportedly have higher breakthrough infection rates.[4] Given the absence of a definitive immune correlates indicating the clinical benefits of COVID-19 vaccines, neutralising antibody titres remain highly predictive of protection from symptomatic SARS-CoV-2 infection.[5] After the initial

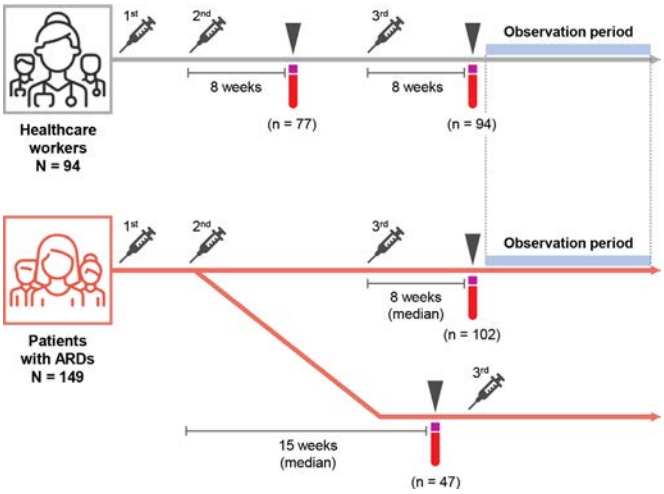

Figure 1 Schematic representation of the study flow diagram. SARS-CoV-2 spike-specific antibody concentrations and neutralisation responses against the wild-type virus and the Omicron variants were measured in serum samples from vaccinated healthcare workers and patients with autoimmune rheumatic diseases (ARDs). Grey triangles indicate the timing of sample collections for immunological assessments, and the blue shading illustrates the observation period for tracking breakthrough cases. The numbers in the brackets denote the number of participants in each group.

authorisation in Israel, many public health authorities stated that a third dose of the vaccine must be mandatory. This was under the presumption that recall responses led by booster doses increase the neutralising antibody responses and consequently induce protective immunity.[6–9] Unfortunately, patients with ARDs undergoing immunomodulatory therapies are excluded from COVID-19 vaccination trials, and there is limited data on immunogenicity of vaccines for the circulating SARS-CoV-2 variants of concern (VOCs).[10]

The highly mutated SARS-CoV-2 Omicron (B.1.1.529) variant has rapidly replaced the Delta strain and virtually all the circulating strains in the community.[11] Omicron's spike mutations are concentrated in the receptor-binding domain (RBD), which results in the variant escaping from vaccine-induced antibody neutralisation,[12–16] while vaccines elicit highly conserved cellular immunity between the Omicron and ancestral spikes.[17–20] To this end, a large-scale epidemiologic study suggested that the third dose of an mRNA vaccine provides exceptional protection from symptomatic SARS-CoV-2 infection, despite lesser protection against the Omicron variant.[21] In immunocompetent individuals, three consecutive exposures with spike antigen resulted in the maturation of antibody responses required to increase avidity, which may be critical for highly potent neutralisation for counteracting VOCs with immune evasion capabilities such as SARS-CoV-2 Omicron.[22–24] However, the susceptibility of the Omicron variant to vaccine-elicited neutralisation in patients with ARDs employing a myriad of immunomodulators remain unresolved.

The primary objective of this study was to provide a deeper understanding of the cross-neutralising antibody responses in patients with ARDs induced by third COVID-19 vaccine doses and whether the magnitude of neutralisation would be comparable to that observed in healthy recipients. To this end, we measured ancestral spike-specific binding antibody and neutralising antibody titres against the Omicron variant as well as the wild-type virus in a coordinated manner. The secondary objective was to determine the incidence of COVID-19 breakthrough infection and to further elucidate the relationship between the functional neutralisation capacity and the protection from COVID-19 in patients with ARDs.

METHODS

Study design

In January 2022, we initiated the study at the beginning of the unprecedented COVID-19 pandemic surge caused by the Omicron variant in Korea, which peaked on 16 March (online supplemental figure S1). Patients with ARDs (including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), ankylosing spondylitis (AS), Behçet's disease (BD), adult-onset Still's disease (AOSD), antineutrophil cytoplasmic antibody-associated vasculitis, systemic sclerosis, IgG_4-related disease) were asked to participate in the study during their regular outpatient visits if they had received a second or third dose of COVID-19 vaccine at least 3 weeks prior. Individuals diagnosed with COVID-19 or those who had received anti-CD20 therapy or chemotherapy were excluded. Patients taking methotrexate, mycophenolate mofetil or Janus kinase inhibitors were instructed to withhold the drug for 1 week after the vaccination. Blood samples were collected at enrolment between 12 January 2022 and 11 March 2022, and the cohort was followed-up for the development of COVID-19 breakthrough infections until the study end date of 6 April.

Healthy control participants included in the study were voluntarily recruited from healthcare workers (HCWs) and were followed longitudinally to study the immune responses to COVID-19 vaccination. They were not treated with immunosuppressants for any indication. Samples for analysis in this study were assessed post hoc (after booster immunisation), and breakthrough cases were identified during the same observation period. All participants were aged 18 years or older and had been vaccinated with mRNA (BNT162b2 and mRNA1273) or viral vector (AZD1222 and Ad26.COV2.S) vaccines according to the approved schedules. All study participants provided written informed consent prior to enrolment.

Assessment of SARS-CoV-2 spike-specific IgG

We performed the Euroimmun (Lübeck, Germany) anti-SARS-CoV-2 ELISA intended for the detection of the ancestral anti-spike IgG antibodies in all serum samples obtained from patients with ARDs and HCWs (figure 1), as previously described.[25 26] The microplate wells were coated with recombinant S1 domain of SARS-CoV-2 spike protein and the results were evaluated by measuring optical density (OD) at 450 nm, with responses expressed as arbitrary units per millilitre (AU/mL). Antibody titres greater than 1.1 AU/mL were considered to be seropositive.

Examination of virus neutralisation response

We used the GenScript (Piscataway, New Jersey, USA) cPass surrogate virus neutralisation test to specifically detect neutralising antibodies, which was granted emergency use authorisation by the US Food and Drug Administration and has been applied in several published studies.[27–31] This test mimics the interaction between the virus and host cell by using the recombinant components of the RBD of the SARS-CoV-2 spike protein and human ACE2 receptors. Assays are typically ELISA-based, and the percentage neutralisation can be calculated as (1 − OD of sample/OD of negative control)×100. The test has been validated for high sensitivity and specificity (with a recommended positive threshold of 30%), and strongly correlated with the

plaque reduction neutralisation test and the focus reduction neutralisation test.[32 33] The test was modified to detect SARS-CoV-2 neutralising antibodies against the Omicron RBD by replacing the horseradish peroxidase-conjugated recombinant RBD fragment according to the manufacturer's specifications.

Assessment of SARS-CoV-2 spike-specific cellular responses

We determined SARS-CoV-2–specific T cell responses by measuring interferon-gamma (IFN-γ) production on stimulation with SARS-CoV-2 S1 peptide pool using the Euroimmun Interferon Gamma Release Assay (IGRA). The response was defined as IFN-γ concentration in peptide stimulated minus that in unstimulated, in international units per millilitre (IU/mL). IFN-γ responses above 200 mIU/mL were interpreted as positive, according to the manufacturer's recommendations. This test has been proven useful in identifying individuals with post-vaccination cellular immunity.[34 35] SARS-CoV-2 IGRA test was conducted in the first and second weeks and the sixth and seventh weeks during the sampling period.

Identification of breakthrough infections

South Korea has conducted rigorous and extensive epidemiological field investigations regarding COVID-19. This process includes active, population-based surveillance of COVID-19-like illnesses and case-based contact tracing regardless of the symptoms. All suspected cases are confirmed by a reverse transcriptase-PCR (RT-PCR) assay. As part of the Korean government's COVID-19 response, rapid antigen tests were conducted by medical personnel and symptomatic individuals who tested positive for the period starting on 14 March were considered COVID-19 cases. Semi-structured, in-depth telephonic interviews conducted on 6 and 7 April were used for the identification of breakthrough cases among patients with ARDs during the observation period in the study. In parallel, all HCWs with compatible symptoms or exposure to confirmed cases were tested for COVID-19 using an RT-PCR assay as per the hospital's infection control policies.

Statistical analysis

The demographics of the study participants are summarised as medians with IQRs for quantitative variables and were compared using the Mann-Whitney U test or as percentages for qualitative variables and compared using the χ^2 test or Fisher's exact test. For virus neutralisation responses, the inhibition percentages are displayed and were compared using paired or non-paired t-tests when appropriate. Differences in the proportion of participants were evaluated using the chi-square test or Fisher's exact test. Anti-spike antibody titres were $\log_{10}$-transformed for visualisation and modelling. Linear regression models were applied to assess the potential decay in neutralising responses against the wild-type virus and the Omicron variant in immune sera as a factor of time elapsed from the third dose. Because of the small sample size, the IGRA results were expressed as medians with IQRs and compared using the Mann-Whitney test. Statistical tests were two tailed, and values of $p<0.05$ were considered significant. All analyses were performed using the GraphPad Prism V.9.0. and SPSS Statistics V.26.

RESULTS

Cohorts of vaccinated individuals

To characterise COVID-19 vaccine-induced immune responses on the domination of the SARS-CoV-2 Omicron variant, 149 patients with ARDs and 94 HCWs participated in this study (figure 1). Among the enrolled patients, 102 (68.5%) received the third dose of an mRNA vaccine (BNT162b2 or mRNA-1273) before enrolment. The median time from the date of the third vaccination to the date of sampling was 7.9 weeks (IQR, 5.6–9.8). Details of the patient characteristics are shown in table 1. The enrolled HCWs ranged from 24 to 64 years old (median: 38.5 years) and composed of both males (29.8%) and females (70.2%), with a similar sex distribution to the enrolled patients ($p=0.535$).

Vaccine-induced neutralisation responses

Neutralising antibody responses were quantified by testing the serum against purified RBD from the wild-type virus and the Omicron variant.[36] We found that two doses of COVID-19 vaccines induced strong neutralising responses against the wild-type virus in both HCWs and patients with ARDs (72.1% and 76.2%, respectively; $p=0.329$; figure 2A). However, the mean neutralising response against the Omicron variant was 18.1% in HCWs and 11.5% in patients with ARDs ($p=0.007$). Following administration of the third dose of an mRNA vaccine, HCWs developed a mean of 97.2% wild-type virus-specific neutralising responses, which decreased to 88.1% in patients with ARDs ($p<0.0001$, figure 2B). Meanwhile, the third dose elicited a mean of 50.3% cross-neutralising responses to the Omicron variant in HCWs, with a majority (72.3%) of sera demonstrating Omicron-neutralisation capacity (neutralising response ≥30%). By comparison, a significantly lower mean cross-neutralising response of 26.8% was observed in patients with ARDs ($p<0.0001$), and only 39.2% of sera were capable of neutralising the Omicron variant, despite a significant increase in responses compared with that in two-dose recipients ($p<0.001$). Specifically, patients with ARDs had intrinsically diminished neutralisation capacity against the Omicron variant, as indicated by the relative ratio of the Omicron- over the wild-type virus-neutralising response of 0.29, which was significantly lower than the 0.52 observed in HCWs ($p<0.0001$, figure 2C).

Correlation between Omicron-neutralisation and anti-spike IgG

The seropositivity rate regarding the ancestral anti-spike IgG (≥1.1 AU/ml) was 94.8% and 87.2% after the second dose in HCWs and patients with ARDs, respectively, which increased to 100% and 96.1% after the third dose. Following the third vaccination, a positive correlation between the ancestral anti-spike IgG titres and the Omicron-neutralising responses was identified by linear regression analysis for the HCWs (figure 2D, blue line), with a calculated slope of 122 (95% CI 64.3 to 180, $p<0.0001$, $R^2=0.160$). However, this association was far less relevant in patients with ARDs, with a slope of 24.3 (95% CI 8.43 to 40.2, $p=0.003$, $R^2=0.085$; figure 2D, red line). Indeed, only 40.8% of sera from IgG seropositive patients showed neutralisation capacity against the Omicron variant, and 93.5% of patients who did not demonstrate serum neutralisation of the Omicron variant were seropositive.

Differential neutralisation capacity against omicron variant

We subsequently evaluated the functional neutralisation capacity against the Omicron variant stratified by clinical and biological profiles. Among the third-dose recipients, 52.0% of individuals with SLE, 25.0% with RA, 37.5% with AS, and 33.3% with BD, while 100% with AOSD had measurable Omicron-neutralisation capacity in their sera (figure 3A,B). Sera from a fraction of SLE patients solely on hydroxychloroquine (70.0%)

Table 1 Characteristics of vaccinated patients according to neutralisation against the SARS-CoV-2 Omicron*

	2X vaccine (N=47)		3X vaccine (N=102)		
	Omicron neutralisation (+)	Omicron neutralisation (–)	Omicron neutralisation (+)	Omicron neutralisation (–)	
	N=3	N=44	N=40	N=62	P value†
Age (years)	62.0	45.5 (37.0; 56.3)	57.0 (46.0; 66.8)	62.0 (54.0; 69.5)	0.211
Male	1 (33.3)	10 (22.7)	7 (17.5)	20 (32.3)	0.099
Disease entities					
SLE (n=43)	–	18 (40.9)	13 (32.5)	12 (19.4)	0.019
RA (n=62)	2 (66.7)	16 (36.4)	11 (27.5)	33 (53.2)	
AS (n=11)	–	3 (6.8)	3 (7.5)	5 (8.1)	
BD (n=10)	1 (33.3)	–	3 (7.5)	6 (9.7)	
AOSD (n=6)	–	1 (2.3)	5 (12.5)	–	
Others (n=17)	–	6 (13.6)	5 (12.5)	6 (9.7)	
Comorbidities					
Asthma (n=5)	1 (33.3)	1 (2.3)	2 (5.0)	1 (1.6)	0.559
Cancer (n=18)	–	3 (6.8)	4 (10.0)	11 (17.7)	0.281
Cardiovascular disease (n=35)	1 (33.3)	10 (22.7)	7 (17.5)	17 (27.4)	0.249
Diabetes (n=21)	–	7 (15.9)	3 (7.5)	11 (17.7)	0.142
Thyroid disorder (n=17)	–	6 (13.6)	5 (12.5)	6 (9.7)	0.748
Immunomodulators					
Steroid (n=62)	1 (33.3)	20 (45.5)	14 (35.0)	27 (43.5)	0.39
Steroid dose (mg, prednisone equivalent)	2.5	5.0(1.6; 6.3)	3.8(2.5; 6.6)	5.0(2.5; 5.0)	0.683
Hydroxychloroquine (n=42)	–	17 (38.6)	14 (35.0)	11 (17.7)	0.048
Methotrexate (n=58)	2 (66.7)	16 (36.4)	12 (30.0)	28 (45.2)	0.126
Leflunomide (n=29)	–	8 (18.2)	6 (15.0)	15 (24.2)	0.262
Sulfasalazine (n=2)	–	1 (2.3)	1 (2.5)	–	0.392
Mycophenolate mofetil (n=17)	–	8 (18.2)	4 (10.0)	5 (8.1)	0.735
Calcineurin inhibitors (n=23)	1 (33.3)	8 (18.2)	9 (22.5)	5 (8.1)	0.039
Azathioprine (n=23)	1 (33.3)	5 (11.4)	6 (15.0)	11 (17.7)	0.717
Cyclophosphamide (n=2)	–	2 (4.5)	–	–	–
JAK inhibitors (n=3)	–	2 (4.5)	–	1 (1.6)	1
TNF inhibitors (n=17)	–	3 (6.8)	4 (10.0)	10 (16.1)	0.380
Tocilizumab (n=3)	–	2 (4.5)	–	1 (1.6)	1
Belimumab (n=1)	–	–	–	1 (1.6)	1
Laboratory tests					
Neutrophils (10^6 /L)	3972.0	3051.5(2267.8; 3893.3)	3037.5(2013.0; 3811.5)	3197.5(2589.0; 4222.0)	0.138
Lymphocytes (10^6 /L)	1289.0	1693.0 (1112.3; 2200.5)	1909.0 (1314.5; 2395.5)	1724.0(1271.8; 2392.8)	0.435
ESR (mm/hour)	30	22 (12; 31)	23 (11; 36)	25(11; 43)	0.676
CRP (mg/L)	1.7	0.9 (0.4; 2.3)	0.9(0.4; 2.7)	0.9(0.5; 3.3)	0.624
Creatinine (mg/dL)	0.76	0.68 (0.57; 0.84)	0.67(0.59; 0.80)	0.74(0.61; 0.90)	0.058
eGFR (mL/1.73 m^2)	103.4	98.6 (82.2; 123.9)	94.8 (78.1; 108.5)	90.4(73.0; 108.4)	0.215
Vaccine type					
mRNA-mRNA (n=38)	1 (33.3)	37 (84.1)			
Ad-Ad (n=6)	1 (33.3)	5 (11.4)			
Ad-mRNA (n=3)	1 (33.3)	2 (4.5)			
mRNA-mRNA-mRNA (n=57)			27 (67.5)	30 (48.4)	0.114
Ad-Ad-mRNA (n=43)			13 (32.5)	30 (48.4)	
Ad-mRNA-mRNA (n=2)			–	2 (3.2)	

*Neutralisation (+), neutralising response ≥ 30%; neutralisation (–), neutralising response <30%.
†Qualitative variables were compared using the $\chi 2$ or Fisher's exact test, and quantitative variables were compared using the Mann-Whitney U test. Statistical analyses for two-dose recipients are not provided because of the small number of participants with Omicron-neutralising capacity.
Ad, adenoviral vector; AOSD, adult-onset Still's disease; BD, Behçet's disease; CRP, C reactive protein; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; JAK, Janus kinase; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

and patients taking calcineurin inhibitors for various indications (64.3%) were most likely to exhibit functional cross-neutralising responses (figure 3C,D). We observed a significant reduction in the proportion of Omicron-neutralisation capacity with a neutrophil-to-lymphocyte ratio (NLR) greater than 2.0 (25.6 vs 47.6%, p=0.027; figure 3F). No difference in the proportion of Omicron-neutralisation capacity was detected between those previously immunised with one or more doses of the viral vector vaccine and those without prior exposure to the viral vector vaccine (figure 3G). There was a significant interaction with time elapsed since the third dose (p=0.012), which raised questions regarding the durability of cross-neutralising

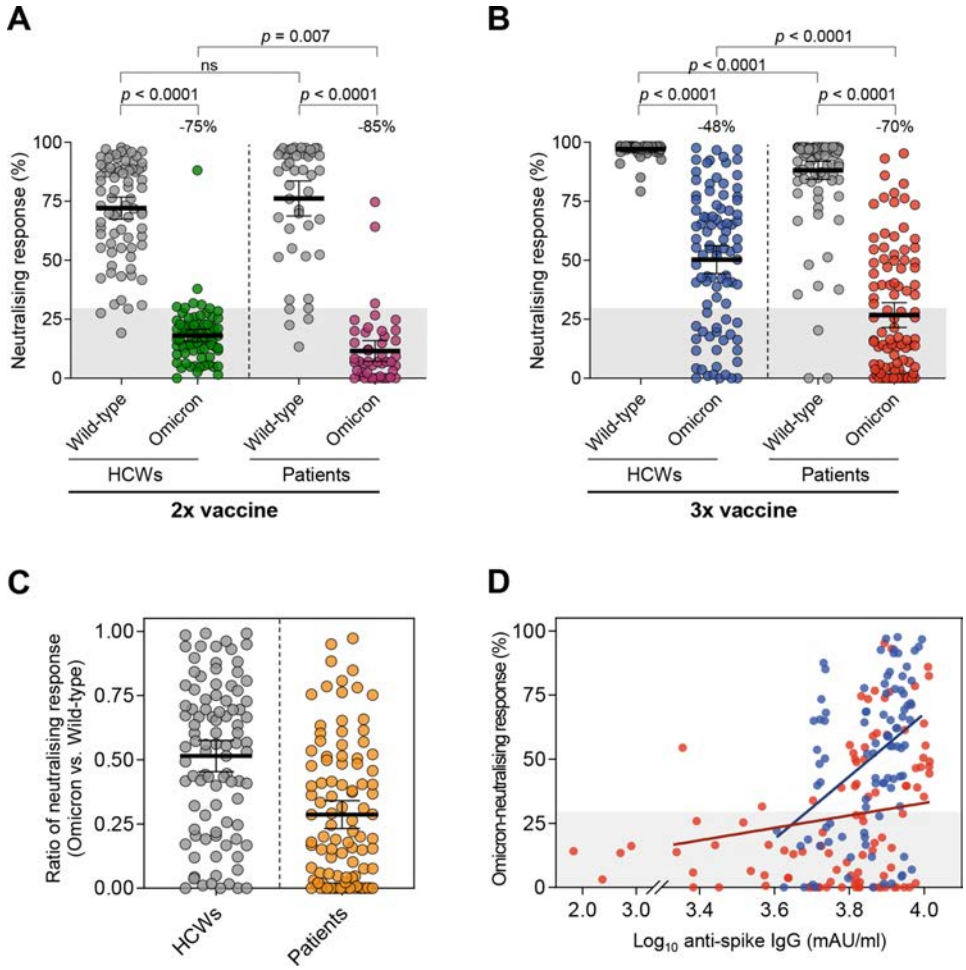

Figure 2 Cross-reactivity of neutralising antibody responses induced by COVID-19 vaccination. (A) Neutralisation responses against the wild-type SARS-CoV-2 and the Omicron variant were analysed for healthcare workers (HCWs) and patients with autoimmune rheumatic diseases vaccinated with primary series. (B) Neutralisation responses in HCWs and patients with autoimmune rheumatic diseases after the third dose of an mRNA vaccination. (C) The relative neutralisation capacity against the omicron variant compared with that against the wild-type SARS-CoV-2. (D) Results for neutralisation responses against the Omicron variant from study participants in (B) that received third vaccine doses were used for linear regression analysis of log-transformed ancestral anti-spike IgG titres in HCWs (blue) and patients with autoimmune rheumatic diseases (red). Dark horizontal lines for each group denote sample means, and the error bars and dotted lines indicate 95% CIs. NS, not significant.

antibody responses after immunisation with the third dose (figure 3H).

Vaccine breakthrough infections caused by SARS-CoV-2 Omicron

Of the 102 patients with ARDs who received the third dose, 99 responded to our interview survey (97.1% response rate) at the end of the follow-up. Throughout the observation period, 19.2% (19/99) of patients with ARDs and 33.0% (31/94) of HCWs developed breakthrough infections (online supplemental figure S2; log-rank test, p=0.710). Of note, the median time between the third dose vaccination and the date of confirmed breakthrough infection in patients with ARDs was significantly shorter compared with that in HCWs (93.0 days (IQR, 82.0–98.0) vs 122 days (IQR, 111–131); p<0.0001). Based on our findings, we postulated that limited neutralisation of the Omicron variant in sera have been implicated in the relatively short-lived protection from breakthrough infections in patients with ARDs.

Strikingly, 14 of the 19 breakthrough cases (73.7%) did not reach the threshold of Omicron-neutralisation capacity before SARS-CoV-2 infection (online supplemental table S1). Two vaccinated patients were hospitalised for COVID-19, and both had nil neutralising responses against the Omicron variant, despite high neutralising responses against the wild-type virus (96.9% and 94.3%, respectively). In our study cohort, patients with ARDs were stratified by the length of the observation time (the interval from the date of the immunogenicity assessment to the date of confirmed breakthrough infection or the end of the follow-up period) to better account for the difference in waning antibody responses over time (online supplemental figure S3). We found significantly lower Omicron-neutralising responses in sera from breakthrough-cases relative to those from non-cases (p=0.018), particularly within a 6-week interval from the immunogenicity assessments (figure 4A). These results suggest that levels of vaccine-induced cross-neutralising antibodies represented potential correlates of protection from breakthrough infections in patients with ARDs.

Next, we estimated the effect of the time elapsed from vaccination to neutralising responses against the wild-type virus and the Omicron variant during the initial 120 days after the third dose (figure 4B). As expected, sera from patients with ARDs efficiently neutralised the wild-type virus, showing a non-demonstrable decay in neutralising responses. In contrast, the same sera

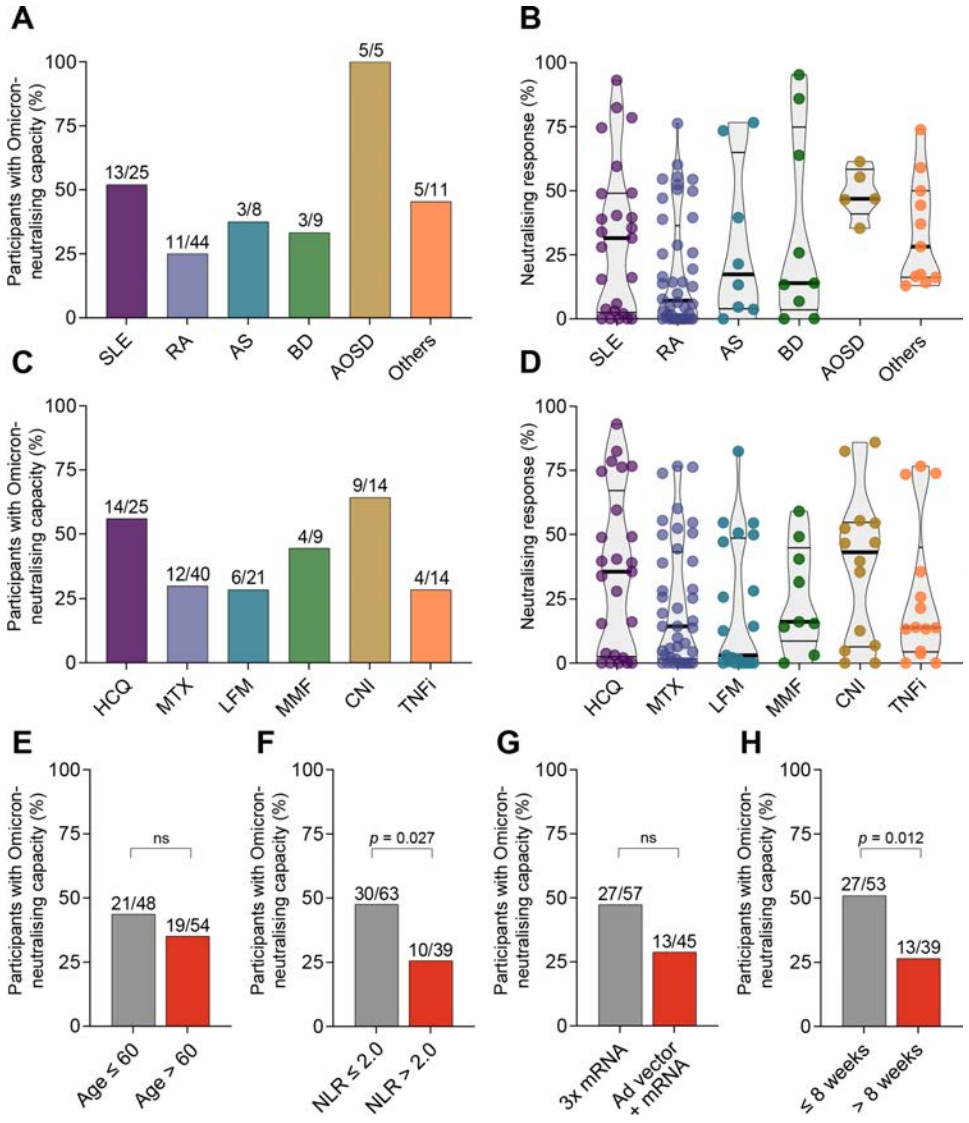

Figure 3 The functional neutralisation of the Omicron variant by immunised sera from patients with autoimmune rheumatic diseases. (A) percentages of sera from patients with autoimmune rheumatic diseases exhibiting Omicron-neutralising capacity defined by Omicron-specific neutralising responses ≥30% stratified by disease entity. (B) results for neutralisation responses against the Omicron variant from study participants in (A). (C) Percentages of sera from patients with autoimmune rheumatic diseases exhibiting Omicron-neutralising capacity stratified by immunomodulator use. (D) Results for neutralisation responses against the omicron variant from study participants in (C). (E–H) Percentages of sera from patients with autoimmune rheumatic diseases exhibiting Omicron-neutralising capacity stratified by age, neutrophil-to-lymphocyte ratio (NLR), vaccine type and time elapsed since the third dose. The numbers above the bar graph represent the number of participants in each group. The dark horizontal lines for each group denote sample medians, and the error bars indicate interquartile ranges. AOSD, adult-onset Still's disease; AS, ankylosing spondylitis; BD, Behçet's disease; CNI, calcineurin inhibitor; HCQ, hydroxychloroquine; LFM, leflunomide; MMF, mycophenolate mofetil; MTX, methotrexate; ns, not significant; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TNFi, tumour necrosis factor inhibitor.

neutralised the Omicron variant to a lesser extent, demonstrating a significant decline in cross-neutralising responses over time, with a predicted decay rate of −0.351% /day (95% CI −0.559 to −0.144, p=0.001), suggesting the potential for a substantial loss of the protection from breakthrough infection.

SARS-CoV-2-specific cellular immunity

A robust T cell responses likely play a role in prevention and resolution of severe SARS-CoV-2 infection. Hence, we examined SARS-CoV-2–specific T cell reactivity in patients with ARDs, at a median of 6.4 weeks (IQR 4.7–8.7) after receiving the third dose of an mRNA vaccine. Released IFN-γ levels in response to spike-based antigens declined slightly from a median of 324 mIU/mL (IQR 118–555) in HCWs to 203 mIU/mL(IQR, 37.5–470) in patients with ARDs, but the difference was not significant (p=0.262; figure 5A). A total of 53.5% of the participants had positive IGRA responses, and T cell reactivity in vaccinated individuals displayed similar patterns between the two cohorts (figure 5B), even if we could perform IGRAs only for some of the samples due to logistical issues at the time of study implementation (online supplemental table S1).

DISCUSSION

The immunogenicity of the COVID-19 vaccine in patients with ARDs is of concern.[37 38] However, most published data regarding immunocompromised patients do not consider VOCs, and thus offer limited real-world application. Although a few studies have reported neutralisation responses against alpha, beta and delta

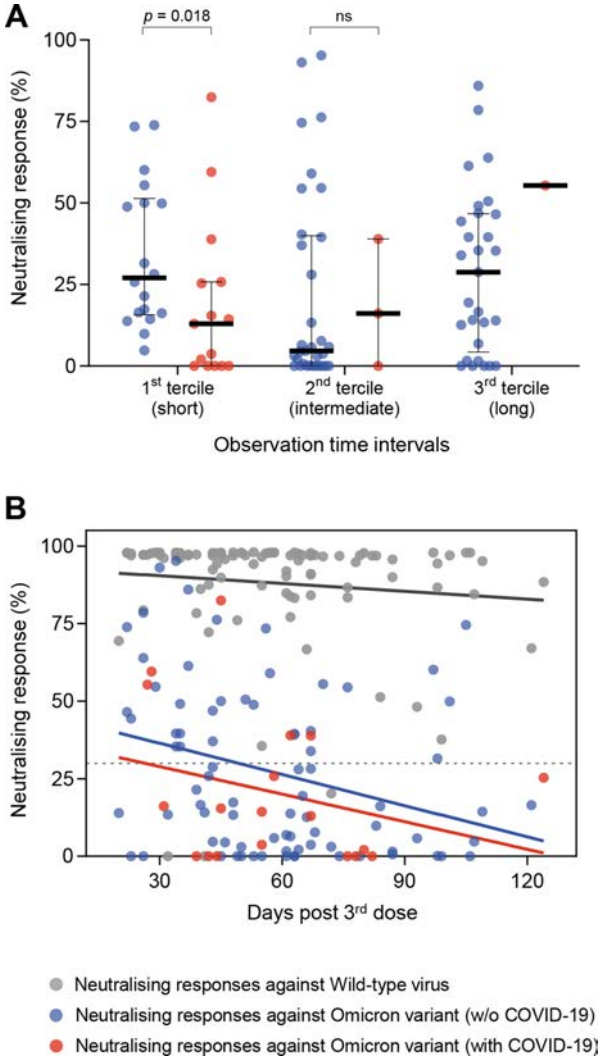

Figure 4 COVID-19 breakthrough infections in patients with autoimmune rheumatic diseases who received a third vaccine dose. (A) neutralisation responses in patients with autoimmune rheumatic diseases against the omicron variant are compared between those with (red) or without (blue) confirmed breakthrough infections in relation to the length of follow-up time. (B) Neutralisation responses against the wild-type SARS-CoV-2 (grey) and the omicron variant (blue and red) with regression lines are plotted over time elapsed since the receipt of the third dose. The dark horizontal lines for each group denote sample medians, and the error bars indicate interquartile ranges. NS, not significant.

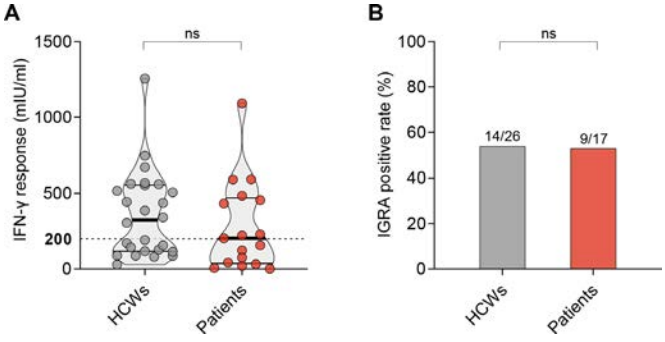

Figure 5 SARS-CoV-2-specific T cell responses after the third dose. Interferon gamma (IFN-γ) levels in plasma after whole blood stimulation with peptide pools spanning the SARS-CoV-2 spike protein. (B) Positivity rates of the interferon gamma release assay (IGRA). The IFN-γ response-positive cut-off was set at ≥200 mIU/mL. The dark horizontal lines for each group denote sample medians, and the error bars indicate interquartile ranges. The numbers above the bar graph represent the number of participants in each group. HCWs, healthcare workers; NS, not significant.

variants in solid organ transplant recipients[39 40] and a heterogeneous population of immunocompromised patients,[41] studies on patients with ARDs regarding the latest Omicron variant remain limited. Hence, we delineated the cross-reactivity of vaccine-induced humoral responses against the SARS-CoV-2 Omicron variant compared with that against the wild-type virus. Our findings suggested that neither primary series vaccinations nor booster doses are sufficient to induce Omicron-neutralising responses above the threshold in patients with ARDs, although responses were noticeably increased following the third dose of an mRNA vaccine. This impairment of cross-neutralisation responses across most of our patients contrasts starkly with a potent elicitation of the Omicron-neutralising responses after the third vaccination in healthy recipients. These differences could potentially be attributed to the nature of the patients undergoing immunomodulatory therapy, who typically exhibit profoundly blunted RBD-specific germinal centre B cell responses even after the third vaccination.[42 43]

High-throughput measurements of IgG antibodies that bind to the ancestral spike constitute a major part of immunogenicity assessments. Such analyses of an mRNA vaccine trial in the general population found that IgG titres correlated with the degree of vaccine efficacy, although this study precluded the assessment of SARS-CoV-2 VOCs.[44] Accordingly, considering that potent germinal centre B cell reactions are closely intertwined with efficient induction of neutralising antibodies, the poor correlation between anti-spike IgG and neutralising responses in patients with ARDs may be due to a relatively greater proportion of IgG recognising non-RBD spike epitopes and low-affinity IgG originating from extrafollicular B cells. Our results demonstrate that while booster doses may bring about an overall increase in total anti-spike IgG titres, such increases do not necessarily equate to improved neutralisation responses. Thus, quantifying the functional neutralisation capacity rather than the ancestral anti-spike IgG may be a more precise approach for determining the immunological benefit conferred by booster doses in patients with ARDs.

Protection against SARS-CoV-2 infection provided by third doses has now been well-demonstrated.[45] Such benefits are also conferred to immunosuppressed patients who exhibit greater risks of prolonged viral replication, potentially facilitating the emergence of new SARS-CoV-2 genetic mutations.[46 47] However, in our study, booster vaccination-induced Omicron-neutralising responses varied greatly between patients with ARDs, undoubtedly based on the properties of immunomodulators and patient demographics such as age and comorbidities. No clear trends were observed between the Omicron-neutralisation capacity and disease entities. While patients with ARDs have predictably diminished cross-neutralising responses to vaccination, the humoral reactivity of SLE patients solely on hydroxychloroquine therapy was less affected. Likewise, sera from patients treated with calcineurin inhibitors had an increased chance of exerting neutralisation responses, given that the inhibition of the nuclear factor of activated T cells does not necessarily hinder memory B cell expansion and differentiation into plasma cells, though the function of follicular helper T cells may be affected. Indeed, four of the five AOSD patients treated with calcineurin inhibitors

and the remaining patients who were treated with low-dose azathioprine demonstrated Omicron-neutralisation capacities. Furthermore, a strong association between NLR and Omicron-neutralising responses indicated a potentially skewed balance towards innate over adaptive immune responses.[48]

An initial report of breakthrough infections showed neutralising antibody levels in cases to be lower than that in uninfected controls.[49] We found similar results indicating limited protection from breakthrough infection in patients with poor cross-neutralising responses until 6 weeks following the immunological assessment. However, this may not be generalisable to settings with longer time intervals between the immunological assessment and the confirmation of breakthrough infection. The low breakthrough infection rate observed in patients with a prolonged follow-up period may be affected by the greater proportion of recently vaccinated individuals and the gradually decreasing trend in the incidence of COVID-19 during the post-peak phase of the pandemic (online supplemental figures S1 and S2).

Further, to account for variability in the duration of neutralising antibody-mediated protection from breakthrough infection, we calculated the rate of breakthrough infections according to the time elapsed since the third vaccination in both cohorts (online supplemental figure S2). Notably, our analysis indicated a tendency for a shorter duration of protection from the third dose in patients with ARDs than HCWs, although there was no statistically significant between-group difference in the overall incidence of breakthrough infections.

Taken together, as the magnitude of the Omicron-specific neutralising antibody responses induced by the third dose was markedly diminished and was suggested to decay quickly relative to the wild-type-specific neutralising antibody responses in patients with ARDs, this population is anticipated to be at an increased risk of developing breakthrough infections. Since the fourth dose is beginning to be administered, it remains to be determined whether such additional doses will provide improved neutralising responses in patients with exceptionally weak cross-neutralising responses. At the same time, more research into the potential benefits afforded by alternative Omicron-specific boosters may be necessary to effectively protect such immunologically vulnerable individuals.

This study had several limitations. First, neutralising antibody responses were assessed at once after the third dose vaccination. Thus, longitudinal antibody responses to the SARS-CoV-2 Omicron variant and whether and how the waning of immunity might affect breakthrough infection risks remain to be determined. Second, the enrolled patients were generally older than the recruited HCWs, and age-associated immunosenescence might have contributed to the deterioration in cross-neutralisation capacity. Third, our patient cohort was recruited from the outpatient clinic in a single academic hospital that comprises several distinct clinicopathological entities, making robust statistical analysis challenging. Fourth, SARS-CoV-2 Omicron-specific T cell responses were not examined; however, T cell responses are largely preserved against the Omicron variant.[50] Lastly, vaccine breakthrough cases in the patient cohort were identified by in-depth interviews. Despite a high response rate (97.1%) and our endeavours to obtain accurate information, the possibility of unidentified or unreported cases of SARS-CoV-2 infection during the observation period could not be ruled out.

In conclusion, the third dose of an mRNA vaccine could improve the cross-neutralisation of the SARS-CoV-2 Omicron variant in patients with ARDs, although more than half of the patients failed to generate Omicron-neutralising antibodies. Our study sheds light on the relative deficiency of the Omicron-specific neutralising responses in patients with ARDs and their anticipated vulnerability to breakthrough infection. As new SARS-CoV-2 variants are expected to circulate, further research on effective vaccination strategies for patients with immune dysfunction is urgently required.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval The study was approved by the institutional review board of the Chung-Ang University Hospital (2111-056-485). Participants gave informed consent to participate in the study before taking part.

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Data availability statement The data that support the findings of this study are available from the corresponding authors on reasonable request.

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EPIDEMIOLOGICAL SCIENCE

Immunogenicity induced by two and three doses of the BNT162b2 mRNA vaccine in patients with autoimmune inflammatory rheumatic diseases and immunocompetent controls: a longitudinal multicentre study

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ABSTRACT

Objectives To evaluate long-term kinetics of the BNT162b2 mRNA vaccine-induced immune response in adult patients with autoimmune inflammatory rheumatic diseases (AIIRD) and immunocompetent controls.

Methods A prospective multicentre study investigated serum anti-SARS-CoV-2 S1/S2 IgG titre at 2–6 weeks (AIIRD n=720, controls n=122) and 6 months (AIIRD n=628, controls n=116) after the second vaccine, and 2–6 weeks after the third vaccine dose (AIIRD n=169, controls n=45). T-cell immune response to the third vaccine was evaluated in a small sample.

Results The two-dose vaccine regimen induced a higher humoral response in controls compared with patients, postvaccination seropositivity rates of 100% versus 84.72%, p<0.0001, and 96.55% versus 74.26%, p<0.0001 at 2–6 weeks and at 6 months, respectively. The third vaccine dose restored the seropositive response in all controls and 80.47% of patients with AIIRD, p=0.0028. All patients treated with methotrexate monotherapy, anticytokine biologics, abatacept and janus kinase (JAK) inhibitors regained the humoral response after the third vaccine, compared with only a third of patients treated with rituximab, entailing a 16.1-fold risk for a negative humoral response, p≤0.0001. Cellular immune response in rituximab-treated patients was preserved before and after the third vaccine and was similar to controls. Breakthrough COVID-19 rate during the Delta surge was similar in patients and controls, 1.83% versus 1.43%, p=1.

Conclusions The two-dose BNTb262 regimen was associated with similar clinical efficacy and similar waning of the humoral response over 6 months among patients with AIIRD and controls. The third vaccine dose restored the humoral response in all of the controls and the majority of patients.

INTRODUCTION

Vaccination against SARS-CoV-2 is essential to mitigate the COVID-19 pandemic. Data on long-term vaccine-induced immunity are essential to optimise the vaccination policy, especially among the

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Data on the kinetics of vaccine-induced response following the second and third doses of the BNT162b2 mRNA vaccine in patients with autoimmune inflammatory rheumatic diseases (AIIRD) under different treatment regimens are limited.

WHAT THIS STUDY ADDS

⇒ This is the largest longitudinal study to report a similar rate of breakthrough COVID-19 infections and decline of antispike S1/S2 antibody titre following the two-dose BNT162b2 mRNA vaccine regimen over 6 months among patients with AIIRD and immunocompetent controls, restored by the third vaccine dose in all controls and the majority of patients, except for those treated with rituximab (RTX) in whom only a third exhibited restoration of the humoral response.

⇒ Treatment with methotrexate monotherapy, anticytokine biologics, abatacept and janus kinase inhibitors did not preclude the development of humoral response following the third vaccine, in contrast to treatment with RTX and glucocorticoids, both associated with a significantly impaired humoral response at all time points. A third of the RTX-treated non-responders after two vaccine doses seroconverted after the third vaccine dose.

⇒ Predicting factors for mounting an immunogenic response to the third vaccine dose in RTX-treated patients include higher serum total IgG level prior to last RTX course, a detectable CD19 cell count before and after the third vaccine dose, a low cumulative number of RTX courses and a longer interval between last RTX course and the third vaccine.

⇒ Cellular immune response, evaluated mainly in RTX-treated patients, was preserved prior to and after the third vaccine dose and was similar to controls.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These data suggest that among patients with AIIRD, the humoral immune response to vaccination with two doses of the BNT162b2 mRNA vaccine waned over 6 months, and a booster vaccine dose restored this response in most cases, thus supporting the policy of the booster vaccine in patients with AIIRD.

⇒ Treatment with RTX impairs the development of an adequate humoral response but seems not to affect the cellular response.

vulnerable population of patients with autoimmune inflammatory rheumatic diseases (AIIRD).

The waning of vaccine-induced immunity is well documented correlating with resurgent COVID-19 infection around the globe. The decline of antispike (S) antibody levels after the two-dose regimen BNT162b2 mRNA vaccine in infection-naive individuals was confirmed by several studies. A two-fold decrease in S antibody levels from a peak at 21–40 days to 84 days after receipt of the second BNT162b2 vaccine dose was observed among immunocompetent subjects (n=197).[1] A longitudinal study of vaccinated healthcare personnel showed a waning of humoral response over a 6-month period following the second BNT162b2 vaccine dose, especially among men, elderly and immunosuppressed participants, defined as organ transplant recipients, patients with HIV or patients treated with corticosteroids, biologic therapy, chemotherapy and postsplenectomy status (n=45).[2] These participants had a 65% decrease in IgG and 70% neutralising antibody levels compared with immunocompetent ones.[2] In a cohort of patients with chronic inflammatory diseases (CID) (n=23) and healthy controls (n=24), a decline in antispike IgG levels over 6 months after SARS-CoV2 vaccination was reported.[3] In another cohort of patients with rheumatic diseases, the rates of seropositivity remained largely stable at 3 months after completion of a two-dose mRNA SARS-CoV-2 vaccination.[4] In this cohort, antibody response against SARS-CoV-2 decreased 2.8-fold during 6-month follow-up but remained above the threshold of predicted neutralising capacity in the majority of patients.[5]

In view of the resurgent COVID-19 outbreak dated June 2021, Israeli authorities approved the administration of a third mRNA vaccine dose (booster dose) on 12 July 2021, first provided to high-risk populations, and on 30 July 2021, to persons aged 60 years or older. The booster vaccination proved to reduce the rates of both confirmed COVID-19 and severe COVID-19 in a large Israeli population of participants aged 60 years of age or older.[6] On 27 October 2021, the American College of Rheumatology recommended a booster vaccine dose for AIIRD patients receiving any immunosuppressive therapy other than hydroxychloroquine monotherapy,[7] also supported by the EULAR.[8] To date, emerging evidence suggests an augmented immunogenic response to the third SARS-CoV-2 vaccine dose among patients with AIIRD based on small-size studies[9–12] and a reduced risk of breakthrough COVID-19 infection compared with unvaccinated individuals.[13] Yet, there is a lack of longitudinal data on the kinetics of anti-S antibody titre following the second and third doses of COVID-19 vaccine in patients with AIIRD under different treatment regimens. Here, we report the results of an ongoing longitudinal study, including patients with AIIRD and control subjects conducted to investigate the kinetics of immunogenic response following the second and third doses of the BNT162b2 vaccine.

METHODS

This longitudinal observational exploratory multicentre study was conducted at the Rheumatology Departments of Tel Aviv Sourasky, Carmel, and Hadassah Medical Centers, Israel, between December 2020 and 30 October 2021.

Ethical approval information

The study was performed in accordance with the principles of the Declaration of Helsinki and approved by the research ethics committees of the three medical centres: TLV-1055–20, CMC-0238–20, HMO-0025–21, respectively. The participants signed an informed consent on recruitment into the study.

Endpoints of the study

The primary endpoint was long-term immunogenicity of the BNT162b2 mRNA vaccine in adult patients with AIIRD compared with immunocompetent controls assessed after the second and third vaccine doses.

Secondary endpoints included:

1. Effect of immunosuppressive treatments on vaccine immunogenicity.
2. Efficacy of vaccination in patients with AIIRD compared with controls.

Study population

Consecutive adult patients (≥18 years of age) with AIIRD were recruited into the study according to the previously reported inclusion criteria.[14] Patients were instructed to continue all medications during the vaccination period, except for rituximab (RTX) treatment, which was postponed after vaccination in certain cases on the physicians' discretion.

The control group included a sample of the general population, consisting mainly of healthcare personnel. Exclusion criteria for all groups were pregnancy, history of past vaccination allergy, previous COVID-19 infection and for controls—history of AIIRD and immunosuppressive treatment.

During the study, there was a drop out of patients as reported in the study flowchart (figure 1).

Due to an urgent decision by the Israeli Ministry of Health on the rollout of the third vaccine dose campaign for immunosuppressed patients, a limited number of patients participated in the serology testing adjacent to the third vaccine dose.

Vaccination procedure

All study participants received the two-dose regimen BNT162b2 mRNA vaccine (Pfizer-BioNTech), 30 µg per dose, 3 weeks apart. The third vaccine dose of BNT162b2 mRNA vaccine was offered to all study participants, at least 5 months after the first vaccine dose, as indicated by the national guidelines.

Humoral response assessment

The vaccine-induced humoral response was evaluated by serial measuring of the serum IgG neutralising antibody titre against SARS-CoV-2 trimeric spike S1/S2 glycoproteins, using the LIAISON (DiaSorin) quantitative assay, performed 2–6 weeks after the second vaccine dose, 6 months after the second vaccine dose and 2–6 weeks after the third vaccine dose (booster). This FDA-authorised assay has a clinical sensitivity and specificity above 98%.[15] A value above 15 binding antibody units (BAU)/

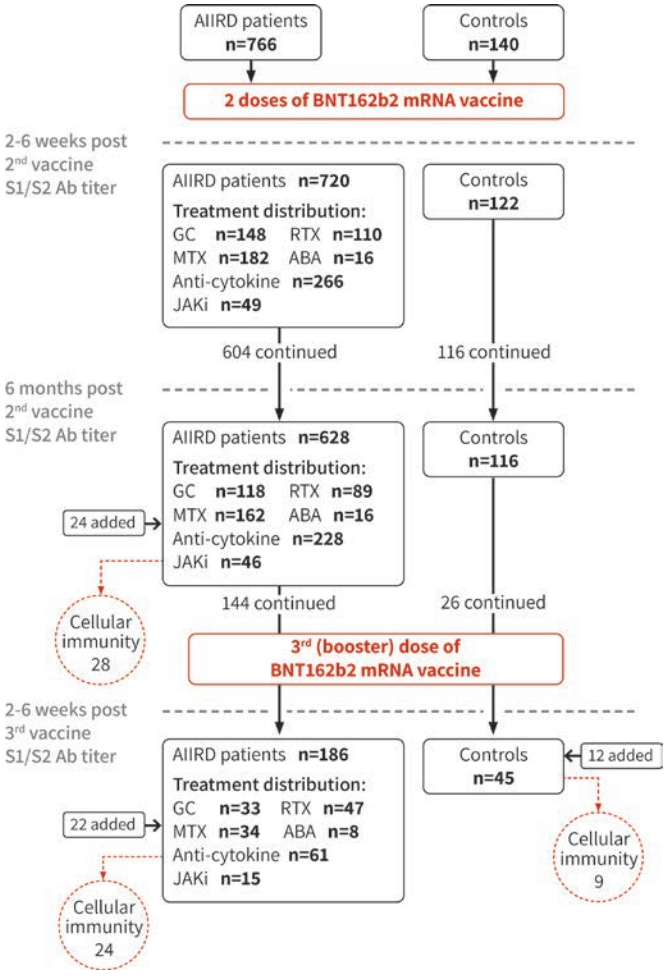

Figure 1 Flowchart of the study. Ab, antibody; ABA, abatacept; AIIRD, autoimmune inflammatory rheumatic diseases; GC, glucocorticoids; JAKi, janus kinase inhibitor; MTX, methotrexate; n, number; RTX, rituximab.

mL was considered as positive, according to the manufacturer's instructions.

Cellular immune response

T-cell immune responses and CD19 +cell count were measured in a subset of participants, prior to and after the third vaccine dose: 28 patients before the third dose vaccine dose, 24 patients after the third dose vaccine, including 20 paired samples of the same patients, and nine controls after the third vaccine dose. For this analysis, patients were selected according to treatments, focusing on RTX and abatacept.

T cell immune response was assessed by stimulating donor peripheral blood mononuclear cells (PBMC) with pooled complete S-peptide mix in the presence of protein transport inhibitor, followed by staining for the activation marker (CD40L) and intracellular cytokines, tumor necrosis factor (TNF)α and IFNγ.[16] For this purpose, we used SARS-CoV-2 T Cell Analysis Kits for human PBMCs (Cat#130-128-156, Miltenyi Biotec, Germany), and the assay was performed according to the manufacturer's instructions. Briefly, donor PBMCs were plated in a 96-well plate at a concentration of $0.5–1\times10^6$ PBMCs/100 uL and incubated at 37°C and 5% CO2 with 2 uL of either complete pooled S-peptide mix, CytoStimTM for positive control or 10% dimethyl sulfoxide in sterile water for negative control. After 2 hours, Brefeldin A was added to each well and cells were incubated for an additional 4 hours. Cells were then stained with viability dye, followed by fixation, permeabilisation and staining for surface markers (CD3, CD20, CD14, CD4, CD8, CD154) and intracellular cytokines, TNFα and IFNγ. Following staining, samples were acquired using BD FACSCanto II, and 20 000 CD4+ events were collected for each sample.

Analysis was performed on gated CD4+ T cells and the absolute number of activated TNFα or INFγ cells was recorded and normalised for 1×10^6 CD4+ T cells. In order to calculate the actual response rate, the absolute number of positive events in the unstimulated negative control was deducted from the absolute number of events in the S-stimulated samples, as shown in the following formula:

$$\left(\frac{1X10^6\,(\#S-stimulated\ Cytokine+CD4s)}{\#Total\ recorded\ CD4s} - \frac{1X10^6(\#\ Unstimulated\ cytokine+\ CD4s}{\#Total\ recorded\ CD4s}\right)$$

Efficacy of the vaccine

The participants were questioned if they contracted COVID-19 infection, confirmed by PCR, following each vaccine dose. In addition, up to the data cut-off, the patient files were reviewed for evidence of COVID-19 infection.

Patient and public involvement

The research question and outcome measures of this study were developed in collaboration with the representatives of patients with AIIRD based on a shared priority to investigate the long-term immune response to the mRNA BNT162b2 vaccine. Patients with AIIRD under the care of the medical centres conducting the trial were actively informed regarding the study and offered to participate. Due to the ongoing COVID-19 pandemic and related stringent restrictions, patients were not involved in the conduct of the study. The main study results will be disseminated to the participants and we will seek patient and public involvement in the development of an appropriate method of dissemination.

Statistical analysis

Categorical variables were reported as absolute and relative frequency. Continuous variables were reported with an arithmetic mean and SD or with median and range. Differences between continuous variables were tested for significance using the independent sample t test and the differences between categorical variables were tested for significance using the χ^2 test or Fisher exact test, as appropriate. Categorical predicting models based on logistic regression and numeric predicting models using linear regression were applied. Multivariate models were built with the backward method, with $p<0.15$ as a criterion for leaving and dropping covariates, unless specified otherwise in the model description. All tests applied were two-tailed, and a p value of 5% or less was considered statistically significant. Data were analysed using the R V.4.1.2 (R Development Core Team. Vienna, Austria).

RESULTS

A total of 729 patients with AIIRD and 122 controls participated in the first stage of this longitudinal multicentre study as reported previously.[14] Patients with AIIRD and controls had a similar predominance of the female patients. Patients with AIIRD were significantly older than controls, mean age 56.58±15.01 compared with 50.83±14.64 years, p=0.0001, respectively (online supplemental table S1)

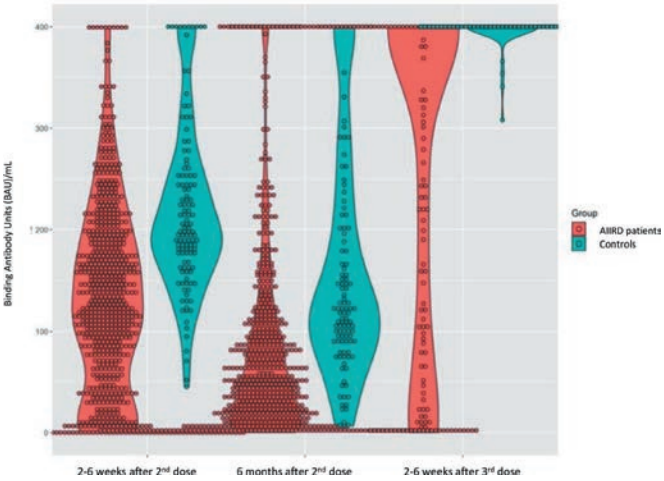

Figure 2 Kinetics of S1/S2 IgG titers following second and third BNT162b2 vaccine in AIIRD patients and controls. AIIRD, autoimmune inflammatory rheumatic diseases.

Figure 1 depicts the number of participants in each stage of the study. Six-month follow-up data were available for 87.23% (n=628) of patients with AIIRD and 95.09% (n=116) of controls. The main reasons for dropout included unwillingness to provide a serology blood test or loss to follow-up. A limited sample of patients and controls were enrolled for evaluation of the response to the third vaccine dose. The limited sample size of this subgroup was explained by an urgent and rapid rollout of the national vaccination campaign.

BNT162b2 vaccine-induced humoral response in patients with AIIRD and controls

The two-dose vaccine regimen induced a higher humoral response in controls compared with patients with AIIRD. Post-vaccination seropositivity rates at 2-to-6 weeks after the second vaccine were 100% for controls versus 84.72% (n=610) for patients, p<0.0001, and at 6 months, 96.55% (n=112) versus 74.36% (n=467), p<0.0001, respectively (online supplemental table S1). After receipt of the third vaccine dose, all of the controls (n=45) and 82.26% (n=153) of patients had detectable positive S1/S2 IgG titrers, p=0.0049.

At all timepoints, S1/S2 IgG antibody titres were significantly lower in patients compared with controls (online supplemental table S2, figure 2). Following the second vaccine dose, the decline of S1/S2 IgG titres within 6 months was similar in patients and controls, −56.72±77.39 and −55.4±84.26 BAU/mL, p=0.87, respectively, whereas the increase in S1/S2 IgG titres after the third vaccine dose was significantly higher in controls compared with patients with AIIRD, 284.09±76.58 versus 219.19±147.68 BAU/mL, p=0.0012, respectively.

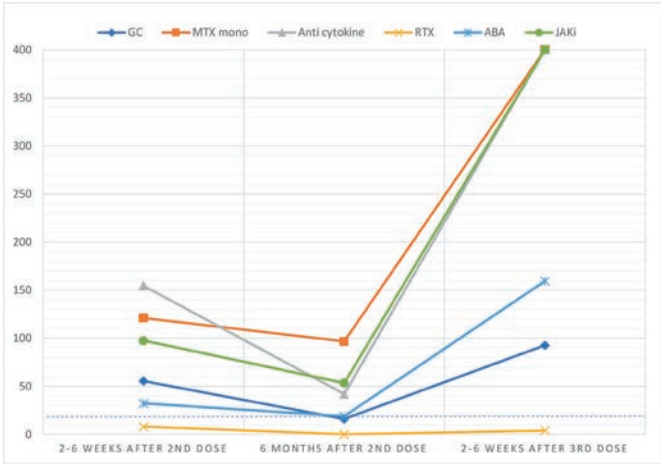

Figure 3 Kinetics of S1/S2 IgG titre in AIIRD patients according to immunosuppressive treatment. IgG titer is presented as median, BAU/ml. ABA, abatacept; AIIRD, autoimmune inflammatory rheumatic diseases; GC, glucocorticoids; JAKi, janus kinase inhibitor; MTX, methotrexate; RTX, rituximab.

Effect of immunosuppressive treatments on vaccine immunogenicity

We next analysed the effect of immunosuppressive treatments on vaccine-induced immunogenicity (online supplemental table S2, figure 3). Patients treated with methotrexate (MTX) monotherapy had a comparable to controls seropositive rate (93.75%, 30/32) at 6 months after vaccination, which increased to 100% after the third vaccine dose, with S1/S2 IgG titres similar to controls.

Patients treated with anticytokine biologics (TNFi, IL6i, IL17i) achieved a similar seropositivity rate, yet lower S1/S2 IgG titres after the second vaccine dose compared with controls. The decline of S1/S2 IgG titres over 6 months was steeper in patients compared with controls, −92.68±64.76 and −55.4±84.26 BAU/mL, p≤0.0001, respectively. At the 6-month timepoint, the rate of seropositivity was significantly lower in patients treated with anticytokine biologics compared with controls, 79.82% versus 96.55%, p=0.0001, respectively. Following the third vaccine dose, all patients treated with anticytokine biologics regained seropositivity similar to controls, and the increase in S1/S2 IgG titres was comparable to controls.

Although treatment with abatacept was associated with significantly lower seropositive rates and lower S1/S2 IgG titres at 2-to-6 weeks and at 6 months after the first two vaccines (62.5% seropositive rate at both timepoints, p≤0.0001 for both), all abatacept-treated patients regained a seropositive response after the third vaccine dose, with only mildly decreased S1/S2 IgG titres in patients compared with controls, 260.41±159.37 BAU/mL, p=0.049, respectively.

Treatment with janus kinase (JAK) inhibitors (JAKi) was associated with lower S1/S2 IgG titres at all timepoints compared with controls. Seropositive rates were mildly decreased following the second vaccine dose and similar after the third vaccine dose compared with controls.

Treatment with RTX was associated with the lowest seropositivity rate and low S1/S2 IgG titres at 6 month after the second vaccine dose compared with controls, 29.21% (26 of 89) and 25.89±62.65 BAU/mL, p≤0.0001, respectively. After the third vaccine dose, 40.43% (19 of 47) patients mounted a positive S1/S2 IgG response, with a significantly lower S1/S2 IgG titre compared with controls, 62.02±119.32 BAU/mL, p≤0.0001. Fourteen patients received RTX between the second and third vaccine doses, achieving comparable rates of seropositive response and S1/S2 titres to other RTX-treated patients.

The assessment of the impact of glucocorticoids (GC) alone on vaccination immunogenicity was limited, as only 12 patients were treated with prednisone as a monotherapy. A total of 117 patients were treated with GC in combination with other treatments, with a low daily GC dose of 6.6±6 mg/day. Compared with controls, patients with AIIRD treated with GC had a lower probability to achieve and retain a seropositive status at all the

Table 1 Cellular immunity and CD19 +cell count in a subset of participants measured prior to and after the third vaccine

	CD19% mean±SD		TNFα expression/10^6 CD4 cells mean±SD		IFNγ expression/10^6 CD4 cells mean±SD	
	Prior third vaccine	Post third vaccine	Prior third vaccine	Post third vaccine	Prior third vaccine	Post third vaccine
Controls n=9	NA	6.99±4.75	NA	1505.32±1386.07	NA	986.53±864.73
AIIRD patients*	2.522±3.74 n=28	3.453±3.9† n=24	1041.1±1148.63 n=28	1334.11±1207.56 n=24	553.05±545.71 n=28	774.82±731.59 n=24
RTX	0.579±1.06 n=21	1.638±2.85 n=17	1185.197±1277.02 n=21	1460.744±1308.7 n=17	563.509±596.6 n=21	858.486±827.79 n=17
ABA	8.273±1.947 n=3	5.63±2.645 n=2	321.242±276.06 n=3	284.695±194.7 n=2	430.338±533.795 n=3	130.5±176.07 n=2
TNFi	8.41±3.114 n=4	8.756±2.39 n=5	827.477±474.625 n=4	1323.295±951.411 n=5	590.202±314.447 n=4	748.081±301.065 n=5

*Twenty samples of the same patients before and after the third dose vaccine were included.
†p<0.05 for the comparison between AIIRD patients and controls.
ABA, abatacept; AIIRD, autoimmune inflammatory rheumatic disease; CD19%, CD19 per cent of total lymphocytes; IFNγ, interferon γ; n, number; NA, not available; RTX, rituximab; TNFi, tumour necrosis factor inhibitors; TNFα, tumour necrosis factor α.

three timepoints, 62.16%, 92/148 at 2–6 weeks post second dose, 50%, 59/118 6 months post second dose and 69.7%, 22/33 at 2–6 weeks after the third dose, p=0.0003 compared with controls for the latter. Consistently, S1/S2 IgG titre was significantly lower among GC-treated patients compared with controls at all time-points.

In order to assess the impact of medications on the kinetics of S1/S2 IgG titres over 6 months following the second vaccine dose, we conducted a linear regression analysis (online supplemental table 1). In the multivariate analysis adjusting for the absolute S1/S2 IgG titre and the initial serologic response (positive vs negative) to the first two vaccine doses, older age (−0.549 BAU/mL for every 1 year of age, p=0.0028), GC (−17.625 BAU/mL, p=0.0129) and TNFi (−56.786 BAU/mL, p<0.0001) treatment were associated with a decline, and MTX monotherapy (44.971 BAU/mL, p=0.0003) and mycophenolate mofetil (33.697 BAU/mL, p=0.0246) were associated with an incline of S1/S2 IgG titre.

Cellular immunity

Overall, patients with AIIRD had significantly lower CD19 levels compared with controls after the third vaccine dose (table 1). This was mainly driven by the low CD19 levels in RTX-treated patients.

The cellular immunity subset of patients consisted mostly of patients treated with RTX (n=21). The cellular response reflected by the expression of TNFα and IFNγ from CD4-positive lymphocytes after the third vaccine dose was comparable between patients and controls, although it was numerically lower in abatacept-treated patients (table 1).

Prediction of a positive immunogenic response to the third BNT162b2 vaccine dose

To identify the predictors for a positive immunogenic response to the third vaccine dose, we performed a univariate analysis comparing responders and non-responders (table 2). Younger age, positive response to the second vaccine dose and detectable CD19 cell count after the third vaccine dose were predictive of an immunogenic response to the third vaccine, whereas treatment with RTX and GC was predictive of a negative response. Monotherapy with csDMARDs, MTX, anticytokine biologics, TNFi, IL6i, IL17i, abatacept and JAKi were all associated with 100% positive response to a third vaccine dose.

In a multivariate model adjusting for age, S1/S2 IgG titre at 2–6 weeks following the second vaccine dose was predictive of a positive immunogenic response, OR 1.025 (95% CI 1.007 to 1.044), p=0.0032, implicating that for each unit of S1/S2 IgG, the probability of a positive immunogenic response after the third vaccine dose increased by 1.025. Treatment with RTX was predictive of a negative immunogenic response to the third vaccine dose, OR 0.062 (95% CI 0.017 to 0.224), p<0.0001, indicating a 16.1-times increased risk of a negative response.

Univariate analysis of RTX-related variables associated with a seropositive response to the third BNT162b2 vaccine dose

The subset of RTX-treated patients (n=47), including rheumatoid arthritis (RA) (n=20), connective tissue disease (n=13), antineutrophil cytoplasmic autoantibodies (ANCA)-associated vasculitis (n=10), other vasculitides (n=3) and SLE (n=2), participated in the analysis of the response to the third vaccine dose.

Of 47 patients, 18 patients had a concomitant treatment with GC and 4 patients were treated with MTX. All RTX-treated patients with a positive serology at 6 months after the second vaccine dose had a positive response after the third dose. Importantly, a third (n=12) of non-responders (n=36) to the initial vaccination seroconverted after the third vaccine dose (online supplemental table S5).

A univariate analysis found that S1/S2 IgG titres at 2–6 weeks following the second vaccine dose (OR, 95% CI 1.021 (1.001 to 1.041) for each unit, p=0.025), persistence of the seropositive response at 6 months after the second vaccine dose, and total serum IgG level prior to last RTX course (OR 1.003 (95% CI 1.001 to 1.005) for 1 md/dL, p=0.0473), were associated with a positive immunogenic response following the third vaccine (online supplemental table S4). CD19 cell count was numerically higher in the responders than non-responders, yet this difference did not reach a statistical significance. The interval between last RTX course and the third vaccine dose was longer in responders compared with non-responders (435.68±95.95 vs 358.2±140.93 days, OR 1.005 (95% CI 0.999 to 1.011), p=0.053, respectively) (figure 4). No significant impact of concomitant medications (CS or MTX) on the immune response to the third dose vaccine was found in this subset of patients.

Efficacy of BNT162b2 vaccine

Of a total of 906 participants, 1.77% (n=16) were diagnosed with COVID-19 by a nasopharyngeal swab for SARS-CoV-2 PCR: 1.83% of patients with AIIRD (14/766, 11 cases after two vaccine doses and 3 cases after the third vaccine dose) and 1.43% of controls (2/140, both after two vaccine doses). Two patients with AIIRD were hospitalised due to severe COVID-19; one of them, a patient with inflammatory myositis died of COVID-19. He was treated with RTX and did not develop an antibody response after two vaccine doses.

Compared with patients with AIIRD who did not contract SARS-CoV-2, patients with AIIRD who had a breakthrough COVID-19 infection had lower S1/S2 IgG titres (69.6±74.1 and 131.4±92.3, p=0.02; 27.9±37.5 and 78.7±90.8 BAU, p=0.0003, respectively) and were less likely to be seropositive

Table 2 Unadjusted logistic regression analysis to predict a positive immunogenic response following the third BNT162b2 vaccine dose in AIIRD patients

	Seropositive pts n (%) or mean±SD/median (range)	Unadjusted OR (95% CI)	P value
Age	60.17±13.56	0.955 (0.924 to 0.987)	p=0.0079
Age >65 n=83	61 (73.49)	0351 (0.155 to 0.769)	p=0.0122
Male n=49	39 (79.59)	0.858 (0.372 to 1.978)	NS
Seropositive 2–6 w post second vaccine n=119	114 (95.8)	23.689 (8.334 to 67.335)	p<0.0001
S1/S2 IgG titre 2–6 w post second vaccine	117.91±89.99	1.042 (1.022 to 1.062)	p<0.0001
Seropositive 6 m post second vaccine n=87	86 (98.85)	43.95 (5.75 to 336.12)	p=0.0003
S1/S2 IgG titre 6 m post second vaccine	64.35±86.15	1.207 (1.075 to 1.355)	p=0.0015
Cellular immunity			
CD19% after second vaccine n=20	2.94±3.857	1.522 (0.837 to 2.767)	NS
CD19% after third vaccine n=23	6±4.119	2.391 (1.118 to 3.061)	p=0.0168
TNFα expression per 10^6 CD4 after second vaccine n=20	1317.03±1503.75	1 (1 to 1)	NS
TNFα expression per 10^6 CD4 after third vaccine n=23	1347.95±1394.72	1 (1 to 1)	NS
IFNγ expression per 10^6 CD4 after second vaccine n=20	612.59±668.93	1.001 (0.999 to 1.003)	NS
IFNγ expression per 10^6 CD4 after third vaccine n=23	773.739±788.051	1 (0.998 to 1.002)	NS
AIIRD treatments			
No medication n=17	14 (82.35)	1.007 (0.272 to 3.72)	NS
GC n=33	23 (69.7)	0.407 (0.171 to 0.966)	p=0.0416
csDMARDs mono n=19	19 (100)	NA	NA
csDMARDs combo n=4	3 (75)	0.624 (0.064 to 6.354)	NS
MTX n=34	30 (88.24)	1.768 (0.577 to 5.415)	NS
MTX.mono n=6	6 (100)	NA	NA
MMF n=6	4 (66.67)	0.416 (0.073 to 2.371)	NS
biologics mono n=86	67 (77.91)	0.574 (0.268 to 1.23)	NS
biologics+csDMARDs n=22	16 (72.73)	0.526 (0.189 to 1.465)	NS
Anti-cytokine biologics n=61	61 (100)	NA	NA
TNFi n=22	22 (100)	NA	NA
IL6 i n=13	13 (100)	NA	NA
IL17i n=26	26 (100)	NA	NA
RTX n=47	19 (40.43)	0.025 (0.009 to 0.074)	<0.0001
Abatacept n=8	8 (100)	NA	NA
JAKi n=15	14 (93.33)	3.222 (0.408 to 25.428)	NS
JAKi mono n=8	8 (100)	NA	NA
JAKi +csDMARDs (MTX) n=6	6 (100)	NA	NA
RTX related variables			
Serum total IgG n=46	1046.41±389.56	1.002 (1 to 1.004)	NS
RTX cumulative dose n=48	6500 (2000–24000)	1 (1 to 1)	NS
RTX total courses n=34	5 (1–12)	0.85 (0.703 to 1.029)	NS
Last RTX course dose, mg	2000 (500–2000)	1 (1 to 1)	NS
Days since last RTX course n=48	429.048±110.212	1.005 (1.001 to 1.009)	NS
More that 180 days since last RTX course n=48	21 (47.73)	NA	NA

Anti-cytokine includes TNFi, IL-6 and IL-17 inhibitors.
ABA, abatacept; AIIRD, autoimmune inflammatory disease; CD19%, CD19 percent of total lymphocytes; csDMARDs, conventional synthetic disease modifying antirheumatic drugs; GC, glucocorticoids; IFNγ, interferon γ; IL, interleukin; JAKi, janus kinase inhibitors; m, months; MMF, mycophenolate mofetil; mono, monotherapy; MTX, methotrexate; n, number; pts, patients; RTX, rituximab; TNFi, tumour necrosis factor α inhibitors; TNFα, tumour necrosis factor α; w, weeks.

at 2–6 weeks and 6 months after the second vaccine dose, 50% and 85.3%, p=0.005; 46.15% and 74.96%, p=0.03, respectively (table 3).

DISCUSSION

To the best of our knowledge, this is the largest prospective multicentre controlled study to report on the kinetics of the immune response induced by two and three doses of the BNT162b2 mRNA vaccine in patients with AIIRD under different treatment regimens. During 6 months after two vaccine doses, 74.26% of patients with AIIRD maintained a detectable antibody response compared with 96.55% of immunocompetent controls, with an overall similar decline of antispike antibody titre in both groups. The findings of this study endorse the recommendation for administration of the third vaccine dose,[7 8] as a seropositive antibody response was restored in the majority of patients with AIIRD (80.47%) and all of the controls. After the third vaccine dose, all patients treated with MTX monotherapy, anticytokine biologics, abatacept and JAKi restored the antibody response compared with only a third of RTX-treated patients. Treatment with RTX was associated with a 16.1-fold risk for a negative antibody response. Notably, cellular immune response in RTX-treated patients was preserved prior to and after the third vaccine dose and was comparable to controls. Breakthrough COVID-19 infection occurred mainly after the two vaccine doses and was low in both patients with AIIRD (1.83%) and control (1.43%) groups. Yet, two patients developed a severe COVID-19 with a lethal outcome in one case, compared with none among controls. Overall, the study results are reassuring, providing robust evidence of sustained vaccination efficacy in a heterogeneous

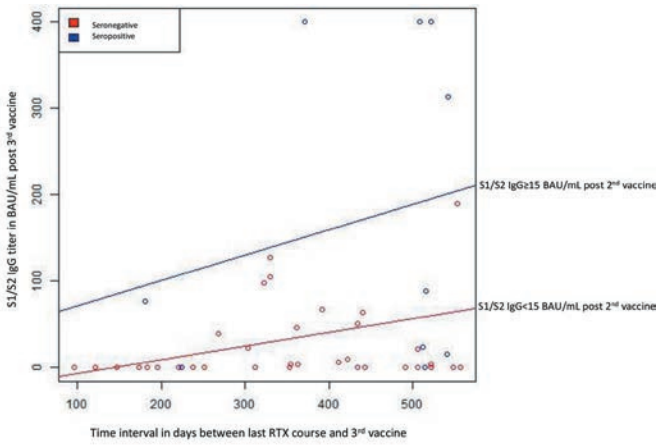

Figure 4 S1/S2 IgG titer following a third BNT162b2 vaccine dose in rituximab-treated patients grouped by immunogenic response to the second vaccine dose and according to the interval between last rituximab course and the third vaccine. AIIRD, autoimmune inflammatory disease; BAU, binding antibody units; RTX, rituximab

cohort of patients with AIIRD treated with various antirheumatic therapies, which were continued throughout the study period, without holding treatments prior to or after vaccination.

Previous studies have shown a waning of the antibody response to COVID-19 vaccination in the general population,[2 17] providing an epidemiologic basis for the booster (third dose) COVID-19 vaccine for persons vaccinated at least 5 months previously. In our study cohort, waning of the two-dose BNT162b2 vaccination-induced antibody response, measured by anti-spike S1/S2 antibody titres over 6 months, ranged between 15% and 20%. Frey *et al* reported a higher prevalence of antibodies to receptor binding domain (RBD) of the spike protein 6 months after vaccination with mRNA vaccines in 96% of patients with rheumatic diseases.[5] This difference might be explained by different treatment patterns used in both studies, with a higher prevalence of RTX use in our study, 12.7% versus 4.6%, respectively. Interestingly, a faster decline of S1/S2 antibody levels in our study was noted in patients treated with anticytokine biologics (TNFi, IL6i and IL17i) than in controls. Consistently, Geisen *et al* reported a pronounced decline of antispike antibody levels and, even more so, of neutralising antibody levels 6 months after mRNA vaccination in patients with CID compared with those treated with

Table 3 Characteristics of AIIRD patients with and without breakthrough SARS-CoV-2 infection

	Breakthrough COVID-19 patients (n=14)	Patients who did not contract COVID-19 (n=752)	P value
Age, mean±SD, years	54±15.5	56.6±15.01	0.549
Female n (%)	9/12 (75)	502/728 (70.11)	1
S1/S2 IgG titre 2–6 w after second vaccine mean±SD, BAU/ml	69.6±74.1 n=12	131.4±92.3 n=708	0.0214
Positive serology 2–6 w after second vaccine n (%)	6/12 (50)	604/720 (85.31)	0.0047
S1/S2 IgG titre 6 m after second vaccine mean±SD, BAU/ml	27.9±37.5 n=13	78.7±90.8 n=615	0.0003
Positive serology 6 m after second vaccine n (%)	6/13 (46.15)	461/628 (74.96)	0.0264
S1/S2 IgG titre 2–6 w after third vaccine, mean±SD, BAU/ml	344.3±96.4 n=3	266.3±167.9 n=183	0.424
Positive serology 2–6 w after the third vaccine	3/3 (100)	150/183 (81.97)	1
AIIRD diagnoses			
RA	5 (35.71)	276 (37.6)	0.04
PsA	1 (7.14)	170 (23.16)	
AS	2 (14.29)	70 (9.54)	
SLE	1 (7.14)	109 (14.85)	
CTD	4 (28.57)	33 (4.5)	
LVV	0	21 (2.86)	
AAV	1 (7.14)	29 (3.95)	
Other vasculitis	0	26 (3.54)	
AIIRD treatments			
Any medication	12 (85.7)	683/752 (90.82)	0.3781
GC	4 (28.6)	146/752 (19.41)	0.4924
csDMARDs combination	1 (7.1)	29 (3.86)	0.4312
MTX combined with other treatments	3 (21.4)	182/752 (24.2)	1
MMF	2 (14.29)	28 (3.72)	0.1008
Biologic mono	4 (28.6)	282 (37.5)	0.5867
Biologic +csDMARD	3 (21.4)	99 (13.16)	0.4155
Anti-cytokine biologic	4 (28.6)	264 (35.11)	0.7802
TNFi	4 (28.6)	176 (23.4)	0.7498
Anti IL-6	0	39 (5.19)	1
Anti IL-17	0	49 (6.52)	1
RTX	4 (28.6)	107 (14.23)	0.1314
ABA	0	18 (2.39)	1
JAKi mono	1 (7.1)	21 (2.79)	0.3374
JAKi+csDMARD	1 (7.1)	25 (3.32)	0.3859

Anti-cytokine biologics include includes TNFi, IL-6 and IL-17 inhibitors.
AAV, ANCA associated vasculitis; ABA, abatacept; AIIRD, autoimmune inflammatory disease; AxSpA, axial spondyloartthritis; BAU, binding antibody unit; csDMARDs, conventional synthetic disease modifying antirheumatic drugs; CTD, connective tissue disease; GC, glucocorticoids; JAKi, janus kinase inhibitors; LVV, large vessel vasculitis; m, months; MMF, mycophenolate mofetil; mono, monotherapy; MTX, methotrexate; n, number; PsA, psoriatic arthritis; RA, rheumatoid arthritis; RTX, rituximab; SLE, systemic lupus erythematosus; TNFi, tumour necrosis factor α inhibitors; w, weeks.

other immunosuppressants and healthy controls.[3] Chen *et al* found that patients with CID treated with TNFi monotherapy (n=11) had not only lower inhibitory titers but also greater decreases in antibody Fc effector functions following BNT162b2 mRNA vaccination compared with other therapeutic.[18] The significance of these findings in relation to COVID-19 prevention remains uncertain. In our study, despite the faster decline of S1/S2 antibody level, only four patients treated with TNFi contracted mild COVID-19 and none of patients treated with other anticytokine biologics. Timewise, three TNFi-treated patients contracted COVID-19 infection about 6.8 months after the second vaccination dose, and the other patient—closely after the booster vaccine, correlating with a low S1/S2 antibody titres at 6 months follow-up.

The booster dose resulted in a positive antibody response in the majority of patients and all of the control group. We further analysed the factors predicting a positive antibody response after the booster dose. Expectedly, treatment with RTX was the predominant factor for the lack of antibody response at all time points. This finding stands in line with a recent meta-analysis that included 1342 patients treated with anti-CD20 therapies.[19] Treatment with RTX within 6 months prior to vaccination and B-cell depletion indicated a high risk for the lack of an antibody response to vaccination.[19] In our study, a third of the RTX-treated non-responders following the first two doses of vaccination seroconverted following the booster vaccine. Consistently, Jyssum *et al* reported that only 21.8% of RTX-treated patients with RAcompared with 98.4% of controls developed an antibody response after two vaccine doses, while the third vaccine elicited an antibody response in only 16.3% of patients.[20] A longer time interval since last RTX treatment to vaccination was associated with a better chance of a positive antibody response in this study. Simon *et al* showed that only 20% patients with AIIRD treated with RTX seroconverted after two doses of SARS-CoV-2 vaccine compared with 80% of patients not exposed to RTX.[11] Hadjadj *et al* provided novel data on the lack of neutralising activity against Alpha and Delta variants after two doses of BNT162b2 vaccine in RTX-treated patients (n=22) compared with 100% neutralising response in controls.[21] In this cohort, 50% of patients seroconverted after two vaccine doses, and the third vaccine dose did not elicit an antibody response among non-responders.[21] Taken together, these results support the rationale for administering the booster vaccine in patients with AIIRD, particularly in non-responders to the first two vaccine doses, as up to 30% of RTX-treated non-responders are expected to develop an antibody response after the booster dose. Consistently with the previous literature,[19] the time interval between RTX administration and vaccination was shown to be significant factors for the development of a humoral response to vaccination, and, thus, should be considered in vaccination planning. While B-cell repopulation represents another significant factor for a positive response to vaccination in RTX-treated patients,[22 23] in our study, there was only a numeric difference in the CD19 cell counts in favour of responders. This is most likely related to a small size of this subgroup. Thus, for non-responders to the booster vaccine, a fourth vaccine dose may be considered, supported by emerging evidence on positive antibody response in patients with a previous negative response to COVID-19 vaccination[24] or a newly emerged passive vaccination.[25]

In light of the blunted antibody response to vaccination in RTX-treated patients, the role of cellular response becomes of particular interest. Mrak *et al* reported SARS-CoV-2-specific T cell response elicited by two-dose mRNA vaccination in 58% of RTX-treated patients with various rheumatic diseases, independent of a humoral immune response.[22] Jyssum *et al* detected CD4+T cell and CD8+T cell responses in 53% and 74% of RTX-treated patients with RA after two vaccine doses, achieving a cellular response in all patients following the booster dose.[17] Simon *et al* and Hadjadj *et al* reported an increase in T cell responses following the booster dose in RTX-treated patients.[11 21] Our results are in line with the previous observations of an independent cellular response to vaccination in patients with impaired or absent antibody response to vaccination, potentially improving the efficacy of vaccination. To date, the role of cellular response on long-term persistence of protective immunity remains unclear.

Our study provides important insight into the divergent impact of antirheumatic therapies on the long-term immune response to the BNT162B2 vaccination. We previously demonstrated a preserved short-term immunogenicity for most DMARDs, including MTX, anticytokine biologics and JAKi following the two-dose regimen BNT162b2 mRNA vaccine.[14] Consistently, treatment with MTX monotherapy, anticytokine biologics, abatacept and JAKi did not preclude the development of a humoral response following the third vaccine, whereas GCs were associated with a significantly impaired humoral response at all time points. Consistent with the study by Frey *et al*, patients on DMARD monotherapy had higher antibody titres than those on combination therapy.[5] A recent study by Mandl *et al* also showed that treatment with GCs in combination with other treatments had a negative impact on the seroconversion rate and the overall antibody level following vaccination.[26]

Despite significant progress in the field of SARS-CoV-2 vaccination, no consensus exists regarding reliable correlates of protection against COVID-19 after vaccination, a particularly important issue in immunosuppressed patients. SARS-CoV-2 vaccination induces both humoral and cellular responses, but it is widely thought that vaccine-induced neutralising antibodies to the RBD of the SARS-CoV-2 S protein are plausible mechanism of protection.[27] Two studies by Khoury *et al*[28] and Earle *et al*[29] demonstrated a significant correlation between vaccine efficacy and vaccine-induced neutralising antibody activity, further supported by clinical studies.[30] Breakthrough infections were observed in 7.4% of the cohort, with anti-RBD antibody response at 1 month postvaccination, identified as a significant predictor of breakthrough infection.[31] In our study, breakthrough infection rate was low and comparable in patients with AIIRD and controls, although the course of COVID-19 was different between the groups, with two cases of severe disease among patients with AIIRD. Notably, patients with a breakthrough infection had lower antibody titers than other AIIRD participants.

Our study has several limitations. Due to a rapid rollout programme of the third vaccine dose, there was a significant drop in the number of subjects participating at this stage. The study did not include neutralisation assays, and cellular immunity was tested in a limited sample of subjects. As the study took place prior to the emergence of the consequent B.1.1.529 (Omicron) variant in December 2021, data on the vaccination efficacy do not apply to this strain, which became dominant later.

In summary, this study is the largest longitudinal study to report a similar rate of breakthrough COVID-19 infections and decline of antispike S1/S2 antibody titre following the two-dose BNT162b2 mRNA vaccine regimen over 6 months among patients with AIIRD and immunocompetent controls, restored by the third vaccine dose in the majority of patients and all controls. Treatment with RTX precludes the development of an antibody response to all three vaccine doses while the cellular response remains preserved. The results of our study support the policy of booster vaccine administration in all patients with AIIRD. As the majority of patients had an overall adequate antibody response to vaccination, holding specific medications, such

as MTX, at the time of vaccination remains debatable, and more epidemiologic evidence is warranted to evaluate the impact of this measure on the efficacy of vaccination. As the study was conducted prior to the emergence of the Omicron variant, further studies are needed to evaluate the efficacy of vaccination in this setting. Strategies to prolong host immunity need to be evaluated in order to protect the population of patients with AIIRD against SARS-CoV-2 and its variants.

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Contributors The study was designed, directed and coordinated by Prof Ori Elkayam, the principle investigator and the guarantor of the study, and Dr Devy Zisman. Drs Victoria Furer, Tali Eviatar, Devy Zisman, Hagit Peleg, the sub-investigators, were in charge of the study conduct at all stages. Dr David Hagin and Tal Freund, were responsible for the assessment of cellular immunity. All the MD co-authors recruited participants into the study. Mrs Pel and Mrs Nevo served as main study coordinators. Prof Ori Elkayam, Dr Victoria Furer, and Dr Tali Eviatar had a full access to the study's data and wrote the article, which was critically reviewed by Prof Daphna Paran, Dr Devy Zisman, Dr Hagit Peleg, and Dr David Hagin.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval This study involves human participants and was approved by Tel Aviv Sourasky Medical Center TLV-1055-20, Carmel Medical Center CMC-0238-20, Hadassah Medical Organization HMO-0025-21. Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available upon reasonable request. The data used to support the findings of this study are included within the article

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TRANSLATIONAL SCIENCE

Behçet's disease risk-variant HLA-B51/ERAP1-Hap10 alters human CD8 T cell immunity

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ABSTRACT

Objectives The endoplasmic reticulum aminopeptidase (*ERAP1*) haplotype *Hap10* encodes for a variant allotype of the endoplasmic reticulum (ER)-resident peptide-trimming aminopeptidase ERAP1 with low enzymatic activity. This haplotype recessively confers the highest risk for Behçet's diseases (BD) currently known, but only in carriers of *HLA-B*51*, the classical risk factor for the disease. The mechanistic implications and biological consequences of this epistatic relationship are unknown. Here, we aimed to determine its biological relevance and functional impact.

Methods We genotyped and immune phenotyped a cohort of 26 untreated Turkish BD subjects and 22 healthy donors, generated CRISPR-Cas9 *ERAP1* KOs from *HLA-B*51*$^{+}$ LCL, analysed the HLA class I-bound peptidome for peptide length differences and assessed immunogenicity of genome-edited cells in CD8 T cell co-culture systems.

Results Allele frequencies of *ERAP1-Hap10* were similar to previous studies. There were frequency shifts between antigen-experienced and naïve CD8 T cell populations of carriers and non-carriers of *ERAP1-Hap10* in an *HLA-B*51* background. *ERAP1* KO cells showed peptidomes with longer peptides above 9mer and significant differences in their ability to stimulate alloreactive CD8 T cells compared with wild-type control cells.

Conclusions We demonstrate that hypoactive ERAP1 changes immunogenicity to CD8 T cells, mediated by an HLA class I peptidome with undertrimmed peptides. Naïve/effector CD8 T cell shifts in affected carriers provide evidence of the biological relevance of *ERAP1-Hap10/HLA-B*51* at the cellular level and point to an HLA-B51-restricted process. Our findings suggest that variant ERAP1-Hap10 partakes in BD pathogenesis by generating HLA-B51-restricted peptides, causing a change in immunodominance of the ensuing CD8 T cell response.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ *ERAP1-Hap10* encodes for a hypoactive endoplasmic reticulum aminopeptidase (ERAP1) resembling a functional KO, and recessively confers the highest risk for Behçet's disease (BD) in the presence of *HLA-B*51* (epistasis).

WHAT THIS STUDY ADDS

⇒ ERAP1-Hap10/HLA-B51 skews frequencies and phenotypes of human antigen-experienced versus naïve CD8 T cells in vivo, pointing to the biologic relevance of this variant and suggesting its importance in HLA-B51-restricted CD8 T cell activation.

⇒ Knock-out of *ERAP1*—modelling hypofunctional ERAP1-Hap10—alters immunogenicity, mediated through an HLA class I-bound peptidome which is characterised by longer, that is, less trimmed peptides above 9mer.

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

⇒ The study provides rationale for the development of ERAP1 activity modulating therapy targeted to BD patient subsets defined by genotype as opposed to disease phenotype alone.

⇒ The findings have relevance to understanding, risk stratifying and treating other, clinically distinct HLA class I-associated diseases in whom epistasis between *ERAP1* haplotypes and disease-associated *HLA class I* alleles has been shown to be linked to risk and protection, such as ankylosing spondylitis and psoriasis.

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INTRODUCTION

Over the past decade, high-quality genome-wide association studies (GWAS) have identified several genes with potential impact on our understanding of mechanisms driving Behçet's disease (BD). These studies confirmed *HLA-B*51* as a major risk factor.[1 2] In addition, they revealed other risk-conferring loci within and outside of the *HLA* region.[2 3] Most of the latter, however, are shared with recurrent aphthous stomatitis (RAS), a common disease worldwide.[4] While RAS—which consists of oral ulcers only—is almost universally present in BD, BD phenotypes are far more complex and must include additional manifestations such as skin lesions, genital ulcers, uveitis and pathergy for the disease to be diagnosed.[5] Vision-threatening uveitis occurs in more than half of the subjects with BD, and the disease can have significant morbidity and mortality through central nervous system (CNS) and large vessel involvement, signifying a sharp contrast between RAS and BD in terms of its nature, severity and burden.[6–8] This has dampened enthusiasm for some of the shared hits on BD and RAS GWAS as potential mechanistic

research targets for BD. A notable exception to this relative lack of specificity is the discovery of epistasis between *HLA-B*51* and the *ERAP1-Hap10* variant of *ERAP1:* both gene variants together profoundly increase the risk for BD (11-fold) over that conferred by *HLA-B*51* alone (about 4-fold) in an interdependent relationship not shared with RAS.[9 10]

ERAP1 encodes for endoplasmic reticulum aminopeptidase 1 (ERAP1), an endoplasmic reticulum (ER)-resident enzyme that trims peptides to a length of around nine amino acids (9mer), which is ideal for loading into the tight binding groove of HLA class I molecules, such as HLA-B51.[11–13] These peptide-HLA complexes (p-HLA) then translocate to the cell surface where they are recognised by cognate T cell receptors (TCRs) on $CD8^+$ T lymphocytes for immune surveillance, which may or may not result in an immune response, depending on the p-HLA complex seen by a specific TCR. Peptides presented via this 'cytosolic', or 'HLA class I' pathway are either derived from intracellular proteins or are extracellular antigens introduced through intracellular infections, or are cross-presented by dendritic cells. It is apparent that the risk-associated interdependence of two polymorphisms in genes of the HLA class I antigen presentation pathway strongly suggests its mechanistic relevance to BD. To date, however, it has remained unclear how this risk is mediated immunologically. In fact, any evidence for its possible impact on cellular immune phenotypes or function is lacking. This has hampered the mechanistic understanding of BD pathogenesis in subjects affected by the variant. In a larger context, it has prevented progress in our understanding of possible mechanistic contributions of HLA-B51 to BD pathogenesis, which has remained an enigma for almost 50 years.

To tackle this problem, and under consideration of knowledge gained from previous work which determined a low, almost absent, enzymatic activity of ERAP1-Hap10,[14–17] we hypothesised that a hypoactive ERAP1 leads to the loading of 'under-trimmed', longer peptides onto HLA class I, resulting in an aberrant CD8 T cell response that partakes in driving the disease.

To address this question, we genotyped a cohort of untreated, active patients with BD and healthy subjects, assessed potentially variant-dependent immune phenotypes in those subjects, and created an in vitro model system using CRISPR-Cas9 genome editing, which allowed us to determine the effect of low ERAP1 activity on peptide lengths in the HLA class I peptidome and its effects on immunogenicity as assessed through CD8 T cell effector function. The work presented here represents the results of our attempts to generate early evidence for the immunological significance of *ERAP1-Hap10*/*HLA-B*51* epistasis as a potentially disease-driving factor in BD.

METHODS

Recruitment of study subjects

28 diseased and 22 presumably healthy, age and sex-matched subjects were recruited at the Behçet's Disease Research Center at Istanbul University—Cerrahpasa in Istanbul, Turkey. All subjects fulfilled the traditional International Study Group (ISG) criteria for the diagnosis of BD, had active disease, and were without immunosuppressive treatment for at least 3 months prior to inclusion. Recruitment of these patients was random but within 'severe' BD phenotypes, that is, ocular and major vascular BD, to minimise diagnostic ambiguity. Two subjects were excluded from sample processing and analysis; one subject because of a BD incompatible ocular phenotype determined by uveitis ophthalmology, and another subject because of incomplete clinical information. Demographic features of the study populations are provided in online supplemental table 1. Patients or the public were not involved in the design, conduct, reporting, or dissemination plans for this research. The study was approved by the IRB of Istanbul University—Cerrahpasa and informed consent obtained from all participants.

Biological specimens

Peripheral blood obtained through venipuncture was processed for peripheral blood mononuclear cells (PBMC) through Ficoll gradient centrifugation as described,[18] cryopreserved[19] and stored in liquid nitrogen until transport in N2 dewars to the US for subsequent cryostorage and experiments.

DNA extraction, amplification and sequencing

Genomic DNA was extracted per manufacturer's instructions (Qiagen Kit) from thawed PBMCs. *ERAP1* exons (2/5/6/11/12/15) containing the haplotype defining missense single nucleotide polymorphisms (SNPs) previously described[10] and *HLA-B* exons (2/3) were amplified, and single-band products of the correct size, identified through agarose gel electrophoresis, sequenced by Psomagen (Rockville, Maryland). Gene sequence alignment and SNP analysis were done using gene blast and DNASTAR Lasergene 16 software. Forward and reverse sequences were aligned to test for concordance in exon sequence and all SNPs. Comparison with the previously published missense SNPs was done to predict coding haplotypes and zygosity. *HLA class I* typing was available from clinical care or obtained through anti-HLA-B5 staining with confirmation through Sanger sequencing as described above. Additional information is provided in online supplemental table 2 and figure 1.

Flow cytometry

Flow cytometry was performed on thawed PBMC, cultured immortalised lymphoblastoid cell lines (LCL), or sorted CD8 responder T cells using our standard published protocols.[20] Cells were acquired using an LSR II.UV cytometer.

Genome editing and culture of cell lines

The LCL line GM23090 (Coriell), which carries *HLA-B*51*, *ERAP1-Hap2*/*Hap7*, was edited using CRISPR-Cas9 exploiting the non-homologous end joining pathway in a two-step lentiviral transduction approach as described.[21] Briefly, Cas9 expressing *HLA-B*51*$^+$ LCL were generated through lentiviral transduction, cloned in limiting dilution and then functionally vetted for stable Cas9 expression using the pXPR_011 GFP lentiviral system.[21] Clones with high Cas9 activity were selected for additional lentiviral transduction with a gRNA targeting *ERAP1* (KO condition) at exon 2 or non-sensical gRNA (wilde type (WT) control condition). Complete, previously published *HLA* typing results for LCL GM23090 as well as genotyping results for *ERAP1*, generated as described above, are found in online supplemental table 3.[22]

Immunoprecipitation and mass spectrometry

LCL (at least 100×10^6/LCL condition) were harvested and washed x 3, lysates were generated and immunoprecipitation was performed using anti-HLA-ABC antibody (clone W6/32, Biolegend) ligated to Dynabeads. HLA class I-associated peptides (HAPs) were eluted using 50 mM Glycine at pH 2.8. For mass spectrometry, the peptide mixture was desalted on C18 SepPak columns and aliquots were loaded onto an EASY-Spray analytical column coupled to a Thermo Fisher Scientific Orbitrap Fusion Lumos Mass Spectrometer. The mass spectrometry raw data were deposited in MassIVE,[23] searched against the human reference proteome obtained from the human protein database Uniprot (June 2017), supplemented with a list of common contaminant proteins, using the search engine Byonic

(V.2.13.2). Peptide lengths were plotted. HLA-B51:01 binders were determined computationally using HLArestrictor with NetMHCpan V.2.4 applying a 2% rank and an IC50 of 500 for weak, and a 0.5% rank with an IC50 of 50 for strong binders. HLA-B51:01 binders selected by peptide length of the non-overlapping KO peptidome were subjected to microbial homology analyses as described by Luzka *et al* and in online supplemental table 4.[24]

CD8 T cell purification, LCL cell irradiation and cell stimulation assays

Human CD8 T cells were isolated from cryopreserved PBMC through magnetic sorting (human CD8 isolation kit, STEMCELL, Catalogue # 17953) according to the manufacturer's instructions. LCL were cocultured with allogeneic CD8 T cells. In long-term stimulation assays, LCL were irradiated to prevent outgrowth of T cells in the culture.

Intracellular cytokine staining and degranulation assay

Intracellular staining for interferon (IFN)-gamma, granzyme B and perforin was performed following surface staining for CD3 and CD8, fixation in 4% PFA and cell permeabilisation with brefeldin as described.[25] Degranulation assays were performed separately, using a CD107a-APC antibody (BD Biosciences, catalogue# 560664, clone H4A3) at the manufacturer-recommended concentration, which was incubated with the cells over the entire stimulation period. After 1 hour of stimulation, monensin (BD Biosciences, catalogue # 554724) was added and cells incubated for another 4 hours as described, harvested, stained with titrated viability dye (fixable blue), CD3, CD8 antibodies and acquired using an LSRII. UV cytometer.[26]

ELISA

LCL/CD8 cell coculture supernatants were harvested and IFN-gamma concentration was assessed with ELISA (Biolegend, Catalogue# 430107) as per manufacturer's instructions.

Raw data processing, computation and statistics

Compensation for flow cytometry experiments was performed at the time of sample acquisition on an LSR II using standard BD acquisition software. For targeted analyses, Flow Cytometry Standard (FCS) files were imported into FlowJo and analysed through gating on pertinent lineage markers (CD3, CD8) in bivariate plots followed by gating over fluorescence minus one controls for the quantification of antigens expressed on a spectrum. Results for stimulated over unstimulated conditions were analysed using Prism software after calculating the mean of intrinsic triplicates for each condition with t test and Mann-Whitney test. A minimum of three independent experiments were performed for each readout. For unbiased analyses, FCS files were loaded into Cytobank and subjected to the CITRUS algorithm under application of a partition around medoids (PAM) model with a false discovery rate (FDR) of 0.05 and visualised in CITRUS dimension reduction plots identifying differentially expressed cell populations classifying samples of the sample groups subjected to the analysis.

RESULTS

Genotypic profiles of diseased and healthy study subjects

In order to determine the allele frequencies of polymorphisms forming the *ERAP1-Hap10* haplotype in individual subjects of our cohort, we amplified the corresponding exons of genomic DNA at the *ERAP1* locus. Sanger sequencing of the amplified products revealed a frequency of *ERAP1-Hap10*$^{+/+}$ (homozygous) carriers (figure 1) in all subjects of 6.25% (3/48). 31.25% (15/48) were either heterozygous or homozygous for *ERAP1-Hap10*. Within the group of BD subjects, the frequency of heterozygotes was 30.77% (8/26) and 7.70% (2/26) for homozygous carriers of *ERAP1-Hap10*. BD subjects who carried *HLA-B*51* (73%) were heterozygous in 31.58% (6/19) and homozygous for *ERAP1-Hap10* in 5.26% (1/19) of cases. A large (GWAS) of 1876 Turkish BD cases and 1761 controls had previously determined the frequency of homozygous *HLA-B*51* BD carriers of *ERAP1-Hap10* at 4.9% through imputation.[10] This is in line with our data (5.26%) obtained through direct Sanger sequencing of human genomic DNA. Our findings, therefore, confirm the relatively low frequency of homozygous carriers of *ERAP1-Hap10* in an *HLA-B*51* background in Turkish subjects with BD. They also indicate that heterozygotes are far more common.

A specific, highly differentiated CD8 T effector cell population distinguishes carriers of *ERAP1-Hap10* from non-carriers in an *HLA-B*51* background

To examine if and, if so, how the *ERAP1-Hap10* genotype affects the immune phenotypes of its carriers in an *HLA-B*51* background within the human T cell compartment, we employed an 11-colour staining panel containing major T cell lineage and activation markers on genotyped PBMCs collected from the subjects of the cohort. Unbiased sample classification analysis of $CD45^+$ $CD3^+$ gated T cells using the cluster identification, characterisation and regression (CITRUS) algorithm under application of a PAM clustering model determined $CD8^+CD57^+CD28^-CCR7^-$ cells as a major, significant discriminator between samples from carriers versus non-carriers of *ERAP1-Hap10* in *HLA-B*51*$^+$subjects (figure 2A–C). As $CD8^+CD57^+CD28^-CCR7^-$ cells represent a matured, highly differentiated, typically oligoclonally expanded, cytotoxic phenotype of CD8 T cells,[27 28] this finding strongly suggests a link of the risk genotype with oligoclonal expansions of HLA class I-restricted human $CD8^+$ T cells that have effector function.

ERAP1-Hap10 shifts frequencies of antigen-experienced versus naïve CD8 T cell populations in carriers of *HLA-B*51*

Next, given the result of the CITRUS analysis for a specific cell population, we aimed to determine whether the carrier status of *ERAP1-Hap10* in *HLA-B*51*$^+$subjects alters cell frequencies of antigen-experienced (memory, effector-memory, and TEMRA) versus antigen-inexperienced (naïve) CD8 T cells in general. We performed targeted flow cytometric analyses using CD3 (T cell), CD8 (CD8 T cell), CD45RA versus CCR7 and/or CD27 versus CD28 (naïve versus effector/effector memory) to address this question. There were significant, reciprocal shifts in the frequencies of naïve versus effector-memory cells between *ERAP1-Hap10* carriers and non-carriers that were *HLA-B*51*$^+$ (figure 3A). Effect sizes were large. For carriers and non-carriers of *ERAP1-Hap10* among *HLA-B*51*$^+$subjects with BD, there were significant changes in frequencies of effector-memory $CD8^+$ T cells with large effect sizes (figure 3B). Changes in frequencies of naïve CD8 T cells in this group did not reach statistical significance, but still had moderate effect sizes and identical direction of effect as in the larger group comparisons for BD and healthy subjects combined, therefore likely representing an underpowered comparison.

When comparing subjects with BD versus healthy donors (HD) in an *HLA-B*51* background but with *ERAP1-Hap10* expression in BD and in the absence thereof in HD, inverse changes of differential expression of CD28 and CD27 in the CD8 T

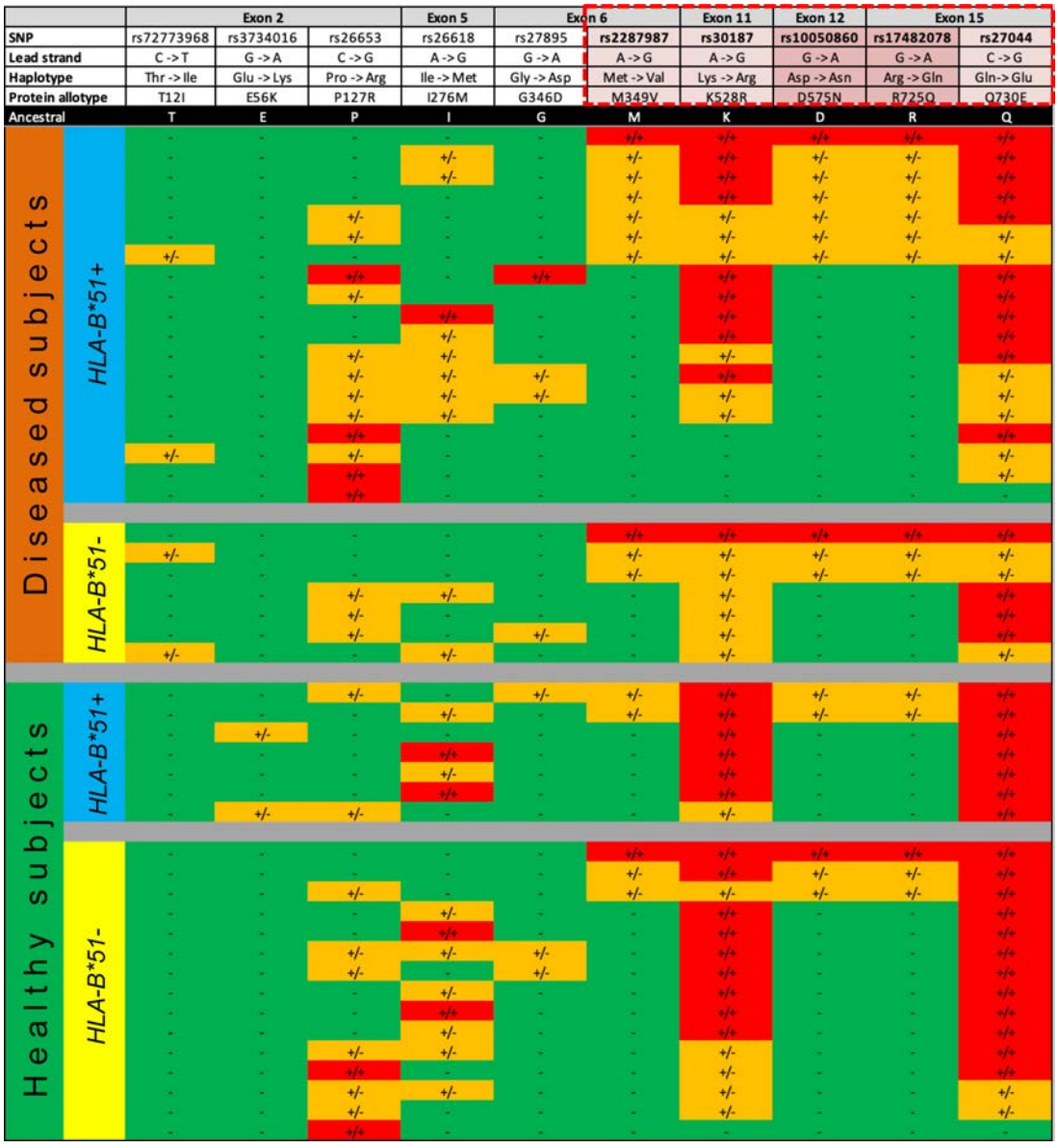


Figure 1 Frequency distribution of *ERAP1-Hap10* in 26 BD and 22 HD recruited in Turkey is consistent with the published allele frequencies determined through imputation in the Turkish population. Dotted red lines show the SNPs forming the core risk haplotype. +/+=homozygous. +/−=heterozygous. *ERAP1-Hap10* confers risk for BD in epistasis with *HLA-B*51*, but protects from ankylosing spondylitis and psoriasis.

cell compartment in between these two groups were significant despite low n, and had very large effect sizes (figure 3C). BD subjects who were carriers of the risk genotype also clearly had significantly different fractions of CD57 expressing CD8 T cell frequencies when compared with *HLA-B*51*$^+$ healthy subjects (figure 3D).

An important recent study experimentally assessed trimming activities for the 10 most common ERAP1 allotypes, allowing trimming activity estimates for ERAP1 allotypes encoded by homozygous and compound heterozygous carriers of most *ERAP1* haplotypes.[15] Applying these estimates, we performed a data simulation excluding subjects whose trimming activity trended towards the mid-range, which increased effect sizes further (online supplemental figure 3).

Combined, these results strongly suggest that the ERAP1-Hap10 allotype in the presence of HLA-B51 globally alters frequencies of antigen-experienced versus naïve CD8 T cells in the peripheral blood, likely reflecting significant migration of activated CD8$^+$ T cells to inflamed tissues. Importantly, it also suggests that HLA-peptide recognition by cognate CD8 TCRs may be controlled and modulated by allotypic ERAP1 in HLA-B51 restriction. The global modulation of naive/memory CD8$^+$ T cell frequencies may reflect the fact that the hypomorphic *ERAP1-Hap10* has a global effect on peptide trimming and antigen presentation, thereby profoundly altering TCR repertoire and T cell activation.

Loss of ERAP1 function results in longer peptides above 9mer

In order to assess for consequences of loss of ERAP1 function mechanistically, we knocked out *ERAP1* using a CRISPR-Cas9 approach in an LCL line derived from an *HLA-B*51*$^+$ human carrier (online supplemental table 3). As ERAP1-Hap10 has been shown to possess low enzymatic activity that resembles that of a functional KO,[14 15] this represents a human in vitro model system that approximates ERAP1 activity in the risk variant. Following our hypothesis that the low enzymatic activity of ERAP1-Hap10 alters the HLA-B51-bound peptidome with a propensity for

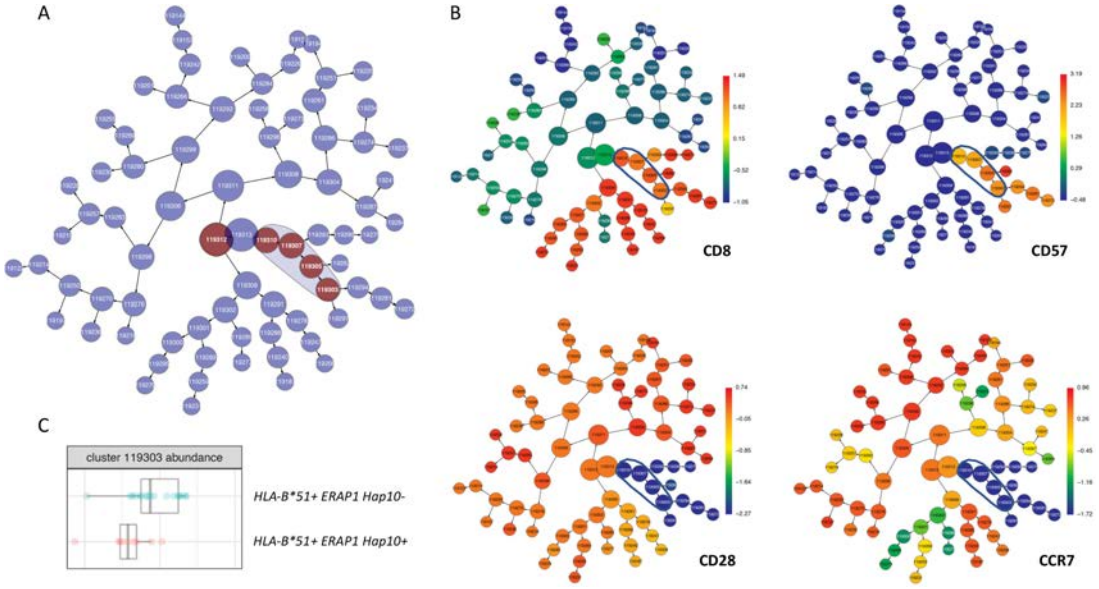
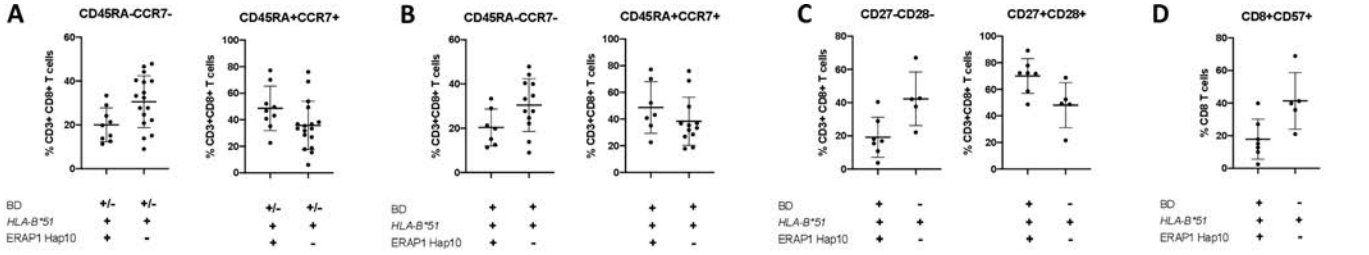


Figure 2 CITRUS analysis T cell panel. Data were pre-processed by gating on singlets, live CD45$^+$CD3$^+$ lymphocytes (T cells) prior to computation using the CITRUS algorithm. (A) Feature plot indicates nodes which represent cell populations that discriminate with statistical significance in between *Group 1*: HLA-B51$^+$ERAP-Hap10$^-$ subjects (n=17) and *Group 2:* HLAB-51$^+$ERAP1-Hap10$^+$ (n=9). (B) Marker plots (one each for CD8, CD57, CD28, and CCR7) indicate the phenotypical identity of the nodes marked in A), that is, CD8$^+$CD57$^+$ T cells that have lost CCR7 and CD28 expression indicating maturation, oligoclonal expansion, terminal differentiation and high cytotoxicity. (C) Box plot indicating abundance for a significant cluster on a log 10 scale. FDR set to 0.05. PAM model applied. PBMC stained for viability, CD45, CD3, CD4, CD8, CD57, CD45RA, PD1, CCR7, CD28, CD27. The complete analysis with abundance of all markers used is shown in online supplemental figure 2.

longer, that is, less efficiently trimmed peptides, we immunoprecipitated HLA I-peptide complexes from isogenic, *HLA-B*51$^+$ ERAP1* competent (WT control) and *ERAP1* KO LCL, performed peptide sequence identification by mass spectrometry, and compared peptide length frequencies (PLF) of the respective HLA class I peptidomes. Peptidomes contained overlapping and non-overlapping fractions in between WT and KO (figure 4A). PLF differed significantly (figure 4B) between WT and KO. PLF of the KO peptidome peaked at 9mer increasing relatively and disproportionally to the KO up until 9mer, but then inverted with longer peptides becoming relatively more abundant in the KO than in the WT (figure 4B). 9mer is the ideal peptide length for fit into the tight binding groove of most HLA class I molecules. As ERAP1 trims to around 9mer, our results indicate that absent ERAP1 impairs this process and leads to the loading of longer peptides onto HLA class I molecules.

To assess the relevance of this mechanism for HLA-B51 binding specifically, we computationally deconvoluted the non-overlapping KO ('undertrimmed') and WT ('properly trimmed') for predicted HLA-B51:01 binders and then reanalysed peptide frequencies (figure 4C). Again, WT control PLF was relatively outperformed by peptides derived from the KO above nine mer, indicating that



Group	A	B		A	B
N	9	17		9	17
Median	19.8	32.4		48.59	35.72
P-value	0.0287			0.0495	
Cohen's d	1.1			0.74	
Glass's δ	1.42			0.77	

Group	A	B		A	B
N	7	12		7	12
Median	19.8	30.5		42.1	34.4
P-value	0.0793			0.1673	
Cohen's d	0.98			0.55	
Glass's d	1.21			0.55	

Group	A	B		A	B
N	7	5		7	5
Median	16.9	42.1		72.2	49.2
P-value	0.0177			0.0354	
Cohen's d	1.6			1.5	
Glass's d	1.9			1.7	

Group	A	B
N	7	5
Median	17.9	41.3
P-value	0.0366	
Cohen's d	1.5	
Glass's d	1.9	

Figure 3 Inverse frequencies of CD8$^+$ naïve and effector-memory CD8 T cells in between risk variant (*HLA-B*51$^+$*and *ERAP1-Hap10$^+$*) carriers and non-carriers (*HLA-B*51$^+$ERAP1-Hap10$^-$*) in (A) all donors regardless of disease status, and (B) within BD subjects and (C, D) between *HLA-B*51$^+$ERAP1-Hap10$^+$* BD and *HLA-B*51$^+$ERAP1-Hap10$^-$* HD. Mann-Whitney U test. Two different effect size measures are provided. Values >0.8 mark large, >0.5 medium and <0.5 small effect sizes for Cohen's and Glass's estimates. These shifts in early naïve (CD45RA$^+$CCR7$^+$ or CD27$^+$CD28$^+$) and late memory, highly aggressive antigen-experienced CD8$^+$ effector T cells (CD8$^+$CD57$^+$;CD27$^-$ CD28$^-$; CD45RA$^-$CCR7$^-$) suggest their antigen-specific, HLA-restricted activation and migration to diseased tissues. See online supplemental figure 3 for data simulations that escalate trimming activity differences through the exclusion of mid-range trimmers across all genotypes.

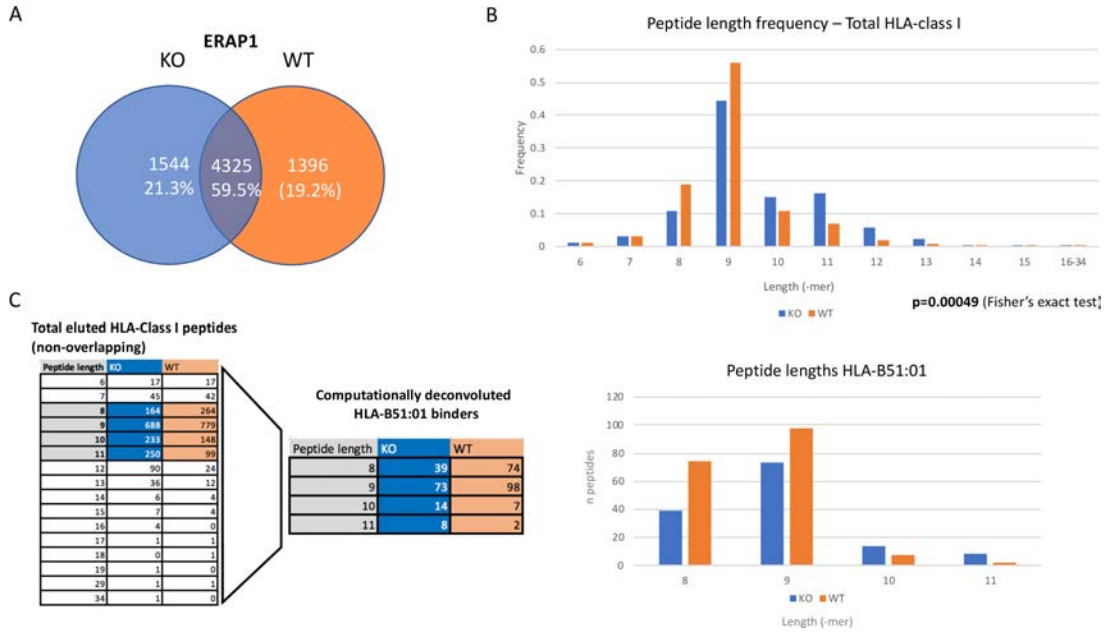


Figure 4 Shifts in peptide length frequencies towards longer (less trimmed) peptides in the absence of ERAP1 at 9mer, the ideal peptide length for HLA class I binding (A, B). This holds true after computational deconvolution for HLA-B51-binding peptides (C). Mass spectrometry of the HLA class I-bound peptides eluted from 1.25×10^8 LCL in each condition (genome-edited or not). One representative experiment out of 3 is shown. *Fisher's exact test.* p=0.00049. See online supplemental table 4 for microbial homology analyses of the 10-mer and 11-mer peptides from the non-overlapping peptidome deconvoluted for HLA-B51:01 in 4C.

peptide undertrimming in the absence of ERAP1 leads to the loading of elongated peptides onto HLA-B51 and that dysfunctional ERAP1 alters the HLA-B51-bound peptidome. Therefore, low or absent ERAP1 activity has a pronounced effect on the length of HLA-presented peptides, likely affecting T cell recognition and modifying the antigenicity of selected peptides.

To determine whether long peptides generated in the absence of ERAP1 activity may resemble human pathogen sequences, we subjected HLA-B51:01 deconvoluted 10 and 11-mer peptides from the non-overlapping KO peptidome to a microbial homology analysis.[24] Fourteen out of 22 peptides displayed degrees of homology to linear microbial epitopes that had previously been determined to be immunogenic experimentally (online supplemental table 4).

Loss of ERAP1 function modulates CD8-mediated immunogenicity

Finally, we strived to assess whether the absence of functional ERAP1 in antigen-presenting cells would alter CD8 immune responses, given that changes in the HLA class I-bound peptidome are likely to change recognition by cognate TCR, that is, change immunodominance and immunogenicity. To this end, we co-cultured *ERAP1* KO and WT LCL with allogeneic human HD PBMC-derived CD8 T cells and assessed their responses through intracellular cytokine staining (ICS, figure 5A, B, F and G), ELISA (figure 5C) and proliferation of carboxyfluorescein succinimidyl ester (CFSE) labelled cells (figure 5D,E). The primary readout was IFN-gamma secretion from responder CD8 T cells in the coculture system as a surrogate for CD8 T cell-mediated immunogenicity. The frequency of IFN-gamma producing $CD8^+$ T cells was significantly different when cocultured with KO versus WT control LCL (figure 5A,B). Differences in the proliferation of IFN-gamma producing cells (figure 5D,E) and IFN-gamma in the supernatant (figure 5C), likewise, reached statistical significance with comparable, large effect sizes. As cytotoxic CD8 T cells possess an entire armamentarium of molecules mediating immunogenicity in addition to IFN-gamma, we also assessed the frequencies of granzyme B and perforin-producing CD8 T cells and, again, detected significant differences between KO and WT control-stimulated CD8 T cells (figure 5F,G). Finally, we performed degranulation assays measuring CD107a expression on CD8 T cell membrane-fused and reinternalised granule membranes as a surrogate for cytotoxicity,[26 29–31] which, likewise, showed significant differences between KO and WT effects (online supplemental figure 4). These findings clearly indicate that diminished ERAP1 function changes immunogenicity and suggest that this is mediated through a change in the HLA class I-bound peptidome.[32 33]

DISCUSSION

GWAS have identified immunogenetic risk factors for BD both within and outside of the *HLA* locus and recently unveiled an epistatic relationship between *HLA-B*51* and a haplotype of *ERAP1*, which encodes for the hypoactive enzyme allotype ERAP1-Hap10. This genotype confers the strongest and most BD-specific risk known to date. The overarching objective of our work was to initiate a process of understanding how this risk is mediated biologically. As both HLA-B51 and ERAP1 are molecular constituents of the endogenous antigen presentation pathway which presents peptides on HLA class I molecules to the TCR of CD8 T cells, we saw compelling rationale in hypothesising that altered ERAP1 function would result in shaping the HLA-B51 peptidome to induce a change in CD8 T cell responses, that is, modulate immunogenicity and immunodominance.

At the gene level, we observed similar frequencies of the *ERAP1* variant encoding for *ERAP1-Hap10* in *HLA-B*51*$^+$carriers in our small sample cohort as uncovered in large GWAS of BD and HD in the Turkish population by imputation and confirmed those by direct Sanger sequencing of gDNA. It had been unclear, however, if the risk genotype had any post-translational biological relevance—immune-phenotypically and/or functionally—that is, whether there were any mechanistic, potentially disease-driving consequences at the cellular level. Here, we provide evidence that this is indeed the case by presenting data, which show that an altered HLA class I-bound peptidome, induced by reduced ERAP1 function, changes

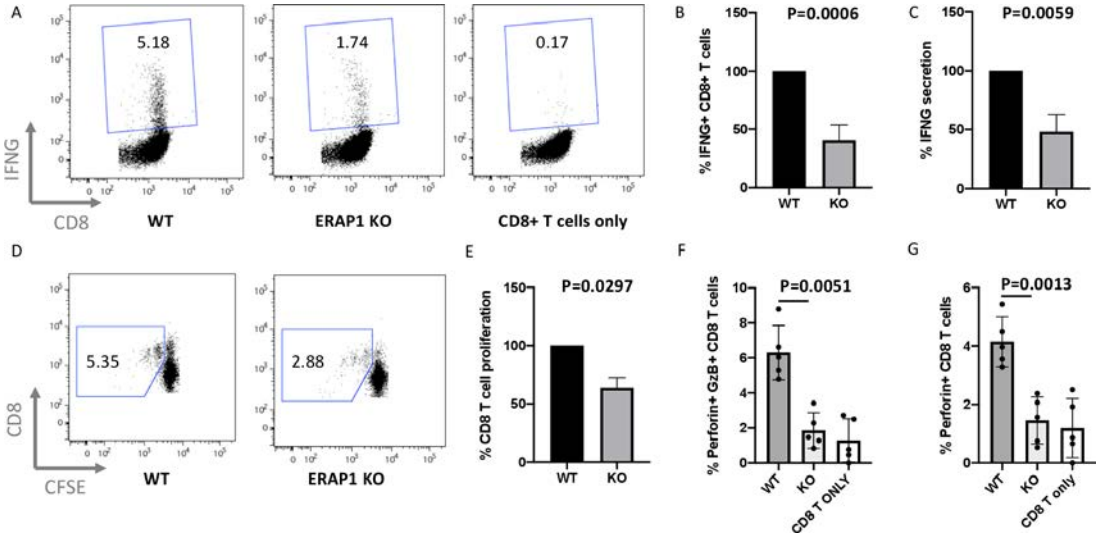


Figure 5 Loss of ERAP1 function shifts CD8 T cell immunodominance. *ERAP1* KO significantly alters immunogenicity of LCL when cocultured with allogeneic human CD8 T cells, assessed here by IFN-gamma production at the single cell (ICS, (A, B) and bulk (ELISA, (C) levels. Other effector readouts underpin this finding: CD8 proliferation on CD3-gated PBMC (CFSE, (D, E), perforin and granzyme B (F, G). CRISPR-Cas9 stable HLA-B*51 $^{+}$ LCL were transduced with *ERAP1*-targeting gRNA (KO) or non-sensical gRNA (WT), and co-cultured with allogeneic human CD8 T cells in 1:4 ratio. In the long-term stimulations (D–G), LCL were irradiated with 6000 rad. Eight independent experiments. Six (A–E) or five (F,G) different CD8 T cell donors, all in triplicates. Ratio-paired t-test. Normalised WT with gated examples (A,B; D,E). Raw data in (F, G) show distribution of data. See online supplemental figure 4 for results of degranulation assays.

immunogenicity. Perhaps equally important, we also show that the variant carrier genotype has an effect on the immune-phenotype in humans, including in those that have fully expressed the disease. Expectedly, these alterations in immune-phenotype localise to the CD8 T cell compartment and affect highly differentiated, antigen-experienced CD8 T cell populations that are HLA class I-restricted. Specifically, $CD57^{+}CD28^{-}CCR7^{-}$ $CD8^{+}$ T cells which are known as an oligoclonally expanded effector population that has undergone repetitive cycles of antigen stimulation in chronic inflammatory conditions, in particular, in those due to viral infections,[27 28] emerged as a population that distinguishes *ERAP1-Hap10* carriers from those that do not carry *ERAP1- Hap10*, in an *HLA-B*51* background—a finding that points to an HLA class I-restricted, ERAP1-modulated process. This is further substantiated by additional results we obtained from targeted analyses of antigen-experienced versus naive CD8 T cells, which indicated significant shifts of these subsets of CD8 T cells in an *HLA-B*51* background depending on ERAP1-Hap10 expression (figure 3).

Our results from microbial homology analyses show resemblance in a large fraction of long, HLA-B51:01-restricted peptides in the *ERAP1* KO LCL conditions (figure 4C) with experimentally proven immunogenic epitopes of infections that are pathogenic in humans (online supplemental table 4). While these are interesting observations, the potential relevance of those findings should be interpreted with great caution when thinking in the context of a suggested causal link of these pathogens for BD. Their conceptual significance here is merely to demonstrate that long peptides, generated in the absence of ERAP1 activity, may contain epitopes with some degree of sequence homology to those that elicit powerful CD8 T cell responses necessary for fighting many of these infections.

While our data, and reasoning based on well-established immunological facts, strongly point to a pathogenic role for CD8 T cells at least in risk variant carriers, there has been scarce evidence for potentially disease-driving CD8 T cell-mediated immunity in BD per se so far.[34–36] This may be due to a general paucity of investigation directed to address this question specifically and a lack of appropriate tools to do so until recently. Several important previous studies, however, did provide clear proof of the presence of CD8 T cells at an important effector site in BD, that is, the anterior chamber of the eye in patients with uveitis due to BD.[37–39] Others have demonstrated their presence and transmigration in the cutaneous pathergy reaction, a highly BD-specific phenomenon, and in nodular skin and other lesions associated with BD.[40–44] Those data, most of which were generated well before *HLA-B*51/ERAP1* epistasis was discovered, underline the significance of the findings we present here and augment the notion that the variant may play an important part in inducing or maintaining disease in BD, including organ-threatening disease.

Previous work with human cells has significantly contributed to our understanding of the constituents and biophysical properties of the HLA-B51-bound peptidome but did not address the potential effects of such changes on effector function.[45–48] Early work by Shastri *et al* showed alteration of CD8 immunogenicity in intersex adoptive transfer mouse models that induce a non-lethal rejection response and are, therefore, similar to our human mixed lymphocyte reaction system of LCL cocultured with allogeneic CD8 T cells. The CD8 response in the ERAAP deficient mice was diminished (but enhanced in an autologous system) on adoptive transfer, which is very much in line with our findings in the human allogeneic mixed lymphocyte reaction (MLR) system.[32 33] Combined, this implies aberrant immune function through loss of ERAP1/ERAAP: weakening of the physiologic allo-response to non-self, but induction of immunogenicity to self-derived intracellular proteins or those that have entered the HLA class I pathway through intracellular infection or cross-presentation.

The decades-long unsuccessful efforts to clearly link immune phenotypes and mechanisms in BD to *HLA-B*51*, its traditionally most prominent genetic risk factor, has entered a new stage through the discovery of its epistatic relationship with *ERAP1-Hap10*, which augments this risk profoundly. This has provided a strong conceptual rationale for the potential involvement of the endogenous antigen presentation pathway in this subset of BD. The findings we present here support, extend and sharpen this assumption by showing that (1) absence of ERAP1 function clearly alters the immunogenicity

to human CD8 T cells, likely induced by a propensity for longer 'under-trimmed' peptides and (2) ERAP1-Hap10—including in heterozygous subjects—induces shifts in antigen-experienced and naive CD8 T cell compartments in carriers of *HLA-B*51*, including in those afflicted with the disease. The data simulations shown in online supplemental figure 3 further underpin that these shifts very likely depend on ERAP1 trimming activity per se and, therefore, apply across a large portion of heterozygote *ERAP1-Hap10* carriers who, for the most part, fall into the low-trimming range according to the findings of a recent study.[15] Some caution interpreting these results is advised, however, given the small sample sizes after exclusion of mid-range trimmers and the understandably low number of peptides tested in that study, which may have left differences in substrate specificity unaccounted for which may alter trimming activity estimates.[49]

Alternate mechanisms mediating risk are conceivable and may include aberrancies of HLA assembly and folding in the ER, possibly with an associated stress response as postulated in *HLA-B*27*-associated diseases. HLA recognition by natural killer (NK) cell receptors with sensitivity to HLA-bound peptides is another testable possibility awaiting exploration in the future.

The observation that *ERAP1-Hap10* protects from *HLA-B*27*$^+$ ankylosing spondylitis and *HLA-C*06*$^+$ psoriasis also suggests HLA class I-restricted (tolerogenic) processes.[50 51] However, the effects of *ERAP1* KO in *HLA-B*27* transgenic rats seem more mechanistically diverse.[52–54] They may include a reduction of potentially ER stress-inducing unfolded HLA-B27 heavy chains and lower rates of disulfite-linked HLA-B27 that can bind to innate immune cell receptors.[54] Unique biophysical features of the HLA-B27 molecule, not shared with HLA-B51, make the conceptual extrapolation of these findings to HLA-B51 and ERAP1 in BD problematic, however, and leave HLA class I restriction as a potentially unifying theme.[55 56]

Combined, our findings provide evidence for the immunological relevance of the *HLA-B*51/ERAP1-Hap10* risk variant in humans, including in those with BD. This strongly suggests the modulation, initiation or termination of HLA-B51-restricted immune responses mediated by allotypic ERAP1-Hap10 in affected carriers through the aberrant generation and presentation of a finite, likely small, number of HLA class I-restricted peptides as a potentially disease-driving process. Further understanding the fine-tuning of this process through the identification of pathogenic HLA class I-restricted peptides and their cognate TCR will enable the rational testing and design of compounds and genetic strategies that modulate ERAP1 activity as a therapeutic means, which may be targeted to patients carrying the risk genotype in the future.

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CLINICAL SCIENCE

Management and outcome of native joint septic arthritis: a nationwide survey in French rheumatology departments, 2016–2017

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ABSTRACT

Objectives To describe current management and outcome of native joint septic arthritis (NJSA) in French rheumatology departments.

Methods For this retrospective, nationwide multicentric study, 127 French rheumatology departments were contacted to report up to 12 cases of NJSA that occurred between 1 January 2016 and 31 December 2017. Characteristics, diagnosis procedures, therapeutic management and outcome were recorded.

Results Overall, 362 patients were included (mean age 64.0±18.6 years, median Charlson comorbidity index 3.5 (0–14)). Knee was the most frequent site (n=160 (38.9%)), and *Staphylococcus* sp (n=185 (51.4%)), the most frequent pathogen. All patients received antibiotics for a mean duration of 46.8 (±22.0) days, including intravenous route for a mean of 17.2 (±15.4) days. Management was heterogeneous. Surgical procedure was performed in 171 (48.3%), joint immobilisation in 128 (43.8%). During follow-up, 91 (28.3%) patients have had serious complications and 28 (9.2%) of them died. Factors associated with 1-year mortality were age (OR 1.08, 95% CI 1.04 to 1.13; p<0.001), Charlson's index (OR 1.30, 95% CI 1.06 to 1.58; p=0.012), presence of bacteraemia (OR 4.02, 95% CI 1.35 to 11.99; p=0.008), antibiotic use in the previous 3 months (OR 3.32, 95% CI 1.11 to 9.87; p=0.029) and *Staphylococcus aureus* NJSA compared with *Streptococcus* sp. NJSA (OR 7.24, 95% CI 1.26 to 41.68, p=0.027). The complete recovery with no adverse joint outcome at 1 year was observed in n=125/278 patients (55.0%).

Conclusion Prognosis of NJSA remained severe with a high rate of morbimortality. Its management was very heterogeneous. This study highlights the importance of the new French recommendations, published after the completion of the study, in order to facilitate NJSA management.

WHAT IS ALREADY KNOWN ON THIS TOPIC?

⇒ Native joint septic arthritis (NJSA) is a rare condition that can lead to irreversible joint destruction, disability or death in the absence of appropriate treatment.
⇒ Diagnosis (ie, identification of microorganism) is sometimes challenging.
⇒ Uncertainty remains on the optimal therapeutic management, such as antibiotic type and/or duration or usefulness and type of surgical procedures.

WHAT THIS STUDY ADDS?

⇒ Management of NJSA in rheumatology department was very heterogeneous reflecting insufficiency of evidence in literature and of recent guidelines.
⇒ Prognosis of NJSA remained severe with a 1-year mortality of 9.2%. Frailty patients and those with bacteraemia were more likely to die. Antibiotic use before microbiological samples was associated with a higher risk of negative culture NJSA and 1-year mortality.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Identification of the microorganism responsible of NJSA is crucial and every possible effort should be made to ensure identification of the microorganism before starting antibiotics.
⇒ Blood cultures not only help the microbiological diagnosis, but also the evaluation of the NJSA severity. They should be systematically done and even repeated at diagnosis.
⇒ Further studies on treatment are warranted to improve harmonisation of NJSA management.

INTRODUCTION

Native joint septic arthritis (NJSA) is considered as a medical emergency and a potentially life-threatening

condition. Absence or delay of appropriate treatment can lead to irreversible joint destruction, disability or death. In a previous French retrospective study, the risk of an adverse joint outcome after a NJSA occurred in about 30%.[1 2] Recently, a sixfold risk of knee arthroplasty within the 15 years following NJSA has been reported in patients who had undergone arthroscopic knee washout for NJSA compared with the age-matched general population controls.[3] Older age, comorbidities and underlying rheumatic diseases have been identified as predictor of poor outcome. Also, even in recent cohorts, mortality remained high, around 5%–9%, occurring mainly within the first 90 days,[1 3–5] and increased with age.[6]

NJSA is a rare condition, with an incidence ranging from 4 to 19/100 000 patient-year.[4 5 7 8] Also, diagnosis could be challenging, particularly when no microorganism is identified. The optimal treatment (antibiotic and surgical procedures) is debated and heterogeneous depending more on practitioner habits than on evidence-based medicine. In 2017, this study was launched by French Rheumatology Society Bone Joint infection working group for analysing current practice and serve as bases for the French guidelines established in parallel.[9] The previous guidelines dated back from 1991 in France and 2006 in Great Britain.[10 11] Since then, the management and antibiotic use have considerably changed, but no formal consensus existed on the way to manage these patients. Also, multiple audits suggested that too often patients with NJSA did not receive the most accurate care.[12]

We, therefore, conducted this retrospective national survey in rheumatology departments, to describe 'in real life', the current management of NJSA and identify possible factors associated with mortality and adverse joint outcomes.

METHODS

Study design and data sources

Between January 2019 and July 2019, the French Rheumatology Society Bone Joint infection working group conducted this retrospective, multicentric nationwide survey. One hundred and twenty-seven French rheumatology departments were contacted to report up to 12 consecutive cases of NJSA that occurred from 1 January 2016 to 31 December 2017. In each department, patients were identified through International classification of Diseases−10 codes: 'pyogenic arthritis' (M00) and 'direct infection of the joint, during infectious disease' (M01). All patients >16 years old, treated for a peripheral NJSA, were eligible for inclusion. NJSA with no organism identified could be included, unless diagnosis other than NJSA considered more likely by the investigators. Mycobacterial, fungal and viral infections were excluded as well as pyogenic vertebral osteomyelitis and septic arthritis occurring on prosthetic joint (online supplemental data S1). Ethical approval for this study was granted by the local ethics committee of Rennes University Hospital (advice n°19.03). Due to the deidentified and non-interventional nature of the study, it was determined to be exempt.

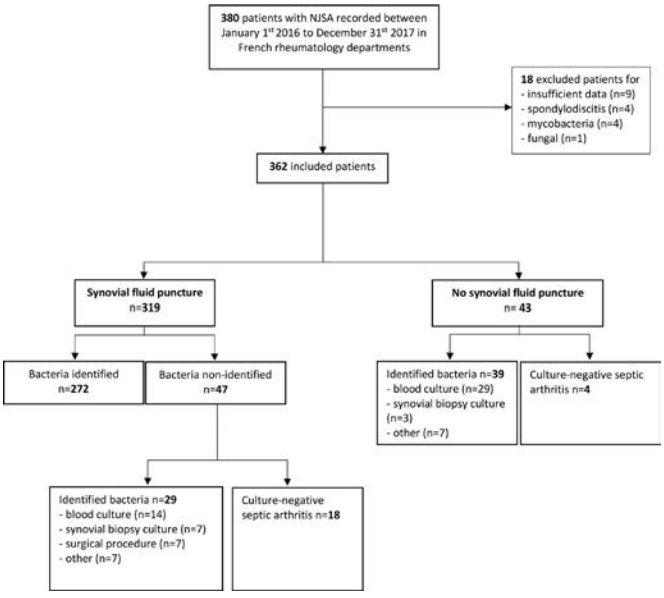

Figure 1 Flowchart and strategy for microbiological identification. NJSA, native joint septic arthritis.

Data collection and definition

Investigators completed an online questionnaire to collect the following data: patient demographic characteristics, comorbidities as well as clinical and microbiological characteristics, therapeutic management and outcomes of the NJSA. Culture-negative NJSA was defined by the absence of identified microorganism on any bacteriological samples, that is, synovial fluid analysis, blood cultures and surgical samples, but considered and treated as NJSA.

Outcomes

Since NJSA could be associated with poor outcomes such joint destruction, disability or death, the following outcomes and their risk factors were analysed:

- Adverse joint outcome at 1 year: defined by occurrence of poor functional joint outcome, radiographic deterioration, amputation, total joint replacement of the affected joint or persistent postinfectious arthritis.
- Complete joint recovery at 1 year: defined by the absence of these items.
- One-year mortality: defined as death of any cause in the year following NJSA.

Statistical analyses

Results are reported as absolute number (percentage) for categorical variables, and as mean±SD or median (range) for continuous variables. Univariate and multivariate analyses were performed to identify factors associated with culture-negative NJSA, and with adverse joint outcome and mortality at 1 year. For each outcome, to identify factors associated with this outcome, univariate analyses were performed using Wilcoxon test for continuous variables and χ^2 test for categorical variables. Factors associated with the outcomes in the univariate analysis ($p<0.20$) and those considered as important based on expert opinion and literature review were included in a multivariate logistic regression model. For all statistical analyses, a p value<0.05 was considered statistically significant.

RESULTS

Patients

Fifty-two centres, 28 (53.8%) general hospitals and 24 (46.2%) tertiary care centres (university hospitals) reported 380 patients of which 362 were included in this study (figure 1 and online supplemental data S2-S3). Patients came to rheumatology department after direct admission in 280 cases (78.0%) or from other departments or medical institutions in 79 cases (22.0%).

Patient characteristics are summarised in table 1. A pre-existing arthropathy of the affected joint was observed in 97 (28.3%) patients. Only nine (2.6%) patients had an inflammatory

Table 1 Clinical and microbiological characteristics of the 362 patients with native joint septic arthritis

	Male gender n=243 (%)	Female gender n=119 (%)	All cohort n=362 (%)
Demographic characteristics and comorbidities			
Age (years)	62 (±18.6)	69 (±18.6)	64.0 (±18.6)
Charlson's comorbidity index*, median (range)	4 (0–14)	4 (0–9)	3.5 0–14)
Diabetes mellitus	61 (25.1)	28 (23.5)	89 (24.5)
Uncomplicated	36 (14.8)	20 (16.8)	56 (15.4)
End-organ damage	25 (10.3)	8 (6.7)	33 (9.1)
Body mass index >25 kg/m^2	65 (26.7)	24 (20.2)	89 (24.5)
Cardiovascular disease	59 (24.3)	22 (18.5)	81 (22.4)
Hypertension	15 (6.2)	8 (6.7)	23 (6.3)
Congestive heart failure	17 (7.0)	5 (4.2)	22 (6.1)
Myocardial infarction	19 (7.8)	4 (3.4)	23 (6.4)
Peripheral vascular disease	22 (9.0)	23 (19.3)	45 (12.4)
Moderate to severe renal disease†	29 (11.9)	13 (10.9)	42 (11.6)
Cancer <5 years or metastatic	32 (13.2)	16 (13.4)	48 (13.3)
Moderate to severe liver disease	20 (8.2)	2 (1.7)	22 (6.1)
Moderate to severe chronic pulmonary disease	19 (7.8)	2 (1.7)	21 (5.8)
Tobacco, alcohol addiction	54 (22.2)	12 (10.1)	66 (18.2)
Organ transplant	5 (2.1)	2 (1.7)	7 (1.2)
HIV	9 (3.7)	0 (0.0)	9 (2.5)
Intravenous drug use	6 (2.5)	2 (1.7)	8 (2.2)
Concomitant treatment at increased risk of infection			
Chemotherapy in the past 6 months before NJSA	8/231 (3.5)	5/110 (4.5)	13/337 (3.9)
bDMARDs, csDMARDs‡, immunosuppressive therapy	12/231 (5.2)	6/110 (5.4)	18/337 (5.3)
Corticosteroids in the past 3 months before NJSA	28/218 (12.8)	21/109 (19.3)	49/327 (15.0)
NSAIDs in the past 15 days before SA	64/215 (29.8)	30/103 (29.1)	94/317 (29.7)
Underlying rheumatic disease			
Osteoarthritis	38/236 (16.1)	40/116 (34.5)	78/353 (22.0)
Crystal arthropathy	28/236 (11.9)	12/116 (10.3)	40/353 (11.6)
Inflammatory rheumatic disease	13/243 (5.3)	13/119 (10.9)	26/362 (7.2)
Rheumatoid arthritis	9/236 (3.8)	6/116 (5.2)	15/353 (4.2)
Spondyloarthritis	3/236 (1.3)	0/116 (0.0)	3/353 (1.7)
Other§	1/236 (0.4)	7/116 (6.0)	8/353 (2.3)
Pre-existing arthropathy on septic joint	51/229 (22.3)	46/113 (40.7)	97/343 (28.3)
Osteoarthritis	29/229 (12.7)	36/113 (31.9)	65/343 (19.0)
Crystal arthropathy	18/229 (7.9)	10/113 (8.9)	28/343 (8.2)
Inflammatory rheumatic disease	4/229 (1.7)	5/113 (4.4)	9/343 (2.6)
Previous septic arthritis	1/229 (0.4)	0/113 (0)	1/343 (0.3)
Antibiotics use in the previous 3 months before NJSA	59/195 (37.1)	28/106 (26.4)	87/301 (28.9)
Clinical presentation of native joint septic arthritis			
Mean symptom duration before hospital admission (days)	18.7 (±38.7)	19.9 (±38.7)	19.1 (±38.7)
Median (IQR)	7(IQR: 3–17)	6.5(IQR: 5–17)	7(IQR: 4–17)
Joint effusion	194/228 (85.1)	93/114 (81.6)	287/342 (83.9)
Fever	101/228 (44.3)	48/114 (42.1)	149/342 (43.6)
Chills	42/228 (18.4)	13/114 (11.4)	55/342 (16.1)
Infective endocarditis	8 (3.2)	4 (3.3)	12 (3.3)
Articulation involved	271	140	411
One site	218/239 (9.1)	101/114 (8.9)	319/356 (89.6)
Multiple site	21/239 (8.8)	16/114 (14.0)	37/356 (10.4)
Large joints involvement	248 (91.5)	126 (90.0)	374 (91.0)
Knee	107 (39.5)	53 (37.9)	160 (38.9)
Gleno-humeral	35 (12.9)	15 (10.7)	50 (12.2)
Hip	19 (7.0)	13 (9.3)	32 (7.8)
Wrist	20 (7.3)	10 (7.1)	30 (7.3)
Tibio-talar	21 (7.7)	8 (5.7)	29 (7.1)
Sternoclavicular	14 (5.2)	6 (4.2)	20 (4.9)
Other¶	32 (11.8)	21 (15.0)	53 (12.9)
Small joints involvement	12 (4.4)	9 (6.4)	21 (5.1)
Hand joints	7 (2.6)	4 (2.6)	11 (2.7)
Foot joints	5 (1.8)	5 (3.6)	10 (2.4)
Other**	11 (4.1)	5 (3.6)	16 (3.9)
Micro-organisms identified	228 (93.8)	112 (94.1)	340 (93.9)
Staphylococcus sp.	127 (52.2)	58 (48.7)	185 (51.4)
Methicillin-susceptible Staphylococcus aureus	107 (44.0)	40 (33.6)	147 (40.8)
Methicillin-resistant Staphylococcus aureus	9 (3.7)	11 (9.2)	20 (5.6)
Coagulase negative Staphylococcus	11 (4.5)	7 (5.8)	18 (4.4)
Streptococcus sp.††	53 (2.2)	31 (26.0)	84 (23.3)
Enterobacterales	15 (6.2)	11 (9.2)	26 (7.2)
E.coli	11 (4.5)	8 (6.7)	19 (5.3)
Other‡‡	4 (1.6)	3 (2.5)	7 (1.9)
Enterococcus faecalis	7 (2.8)	1 (0.8)	8 (2.2)
Pseudomonas aeruginosa	7 (2.8)	2 (1.6)	9 (2.5)
Polybacterial infection	11 (4.5)	2 (1.6)	13 (3.6)
Other§§	6 (2.5)	6 (5.0)	12 (3.6)
No organism identified	15	7	22 (6.1)
Identified mode of contamination	132/243 (54.3)	60/119 (50.4)	192/362 (53.0)
Haematogenous	86/132 (65.1)	32/60 (53.3)	118/192 (61.5)
Contiguous	34/132 (25.8)	14/60 (23.3)	48/192 (25.0)
Direct iatrogenic inoculation	12/132 (9.1)	14/60 (23.3)	26/192 (13.5)
Osteoarticular surgery in the previous 6 months before NJSA	3/132 (2.3)	2/60 (3.3)	5/192 (2.6)
Corticosteroid intra-articular injection	8/132 (6.1)	8/60 (13.3)	16/192 (8.3)
Hyaluronic acid intra-articular injection	1/132 (0.8)	4/60 (6.7)	5/192 (2.6)

Results are shown on 362 patients unless otherwise stated and presented as mean±SD and number (%), unless indicated.
*Charlson's index comorbidity weighted by mean age.
†Defined by creatinine clearance inferior to 60 mL/min.
‡Biological disease-modifying antirheumatic drugs, conventional disease-modifying anti-rheumatic drugs.
§Connective tissue disorders, polymyalgia rheumatica, seronegative rheumatism, not available.
¶Elbows, acromioclavicular, symphysis pubis, sacroiliac, midfoot.
**No reported indication.
††Detailed in online supplemental data S4.
‡‡Other unspecified species of enterobacterales.
§§*Acinetobacter Iwoffi, Neisseria meningitidis, Pasteurella multocida, Corynebacterium Striatum, Nocardia, Pantoea Ananatis, Kingella Kingae, Propionibacterium acnes.*
bDMARDs, biological disease-modifying anti-rheumatic drugs; csDMARDs, conventional disease-modifying anti-rheumatic drugs; NJSA, native joint septic arthritis.

rheumatic disease involving the joint affected by NJSA. Of note, 87 (28.8%) patients had received antibiotics in the 3 months prior to hospitalisation, including 39 (12.9%) after NJSA onset and before bacteriologic investigations.

Clinical, biological and radiological presentation

Joint effusion was present in the vast majority of cases: 287 (83.9%), while fever was observed in only 149 (43.6%). Median joint pain visual analogue scale (0 to 10) at diagnosis was 7 (0–10) (table 1).

The most frequent presentation of NJSA was monoarticular arthritis (n=319, 89.6%) and mainly affected the knee (n=160, 38.9%) (table 1). Raised C reactive protein levels were observed in 351 (99.4%) patients, with a mean of 208.0 (±124) mg/L. Of the 312 patients with an initial radiography, only 50 (16.0%) had radiological features of NJSA.

Diagnosis and microbiological characteristics

Among the 362 patients (figure 1), a microorganism was identified in 340 patients (93.9%), by either synovial fluid analysis (n=272, 80.0%) or another bacteriological sample (n=68, 20.0%). PCR of ribosomal DNA 16s (PCR rDNA,16s) performed on 47 synovial fluids was positive in only nine cases (19.1%), including eight patients for whom other microbiological samples were positive. Thus, microbiological identification relies solely on a positive PCR in only one patient. Synovial fluid puncture was not performed in 43 patients because microbiologic diagnosis was obtained either by another bacteriologic sampling (n=24; 55.8%), by a surgical procedure (n=2; 4.7%) or because the involved joint could not be aspirate (n=17; 39.5%).

Microbiological species identified are listed in table 1. *Enterobacterales* NJSA were older compared with other NJSA (mean age in years 75 (±15) vs 63 (±19), p<0.001). Of note, 37 patients (14.7%) had concomitantly a crystal and a microorganism identified in the synovial fluid. Blood culture was performed in 346 patients (95.6%) and was positive in 156 (45.2%) patients. In the 90 patients for whom microorganism was not isolated by joint puncture (puncture not performed or with negative culture), microorganisms were most frequently detected by blood cultures in 43 (47.8%) (figure 1). The most common mode of contamination was haematogenous (n=118, 61.5%), while direct iatrogenic inoculation was recorded in 26 cases (13.5%) (table 1). Echocardiography was performed to detect infective endocarditis (IE), in 257 patients (80.0%)), with finally confirmed infective endocarditis (IE) in only 12 (3.3%). Ten patients had concomitant bacteraemia. *Staphylococcus aureus* was the most common pathogen involved in IE (n=7, 58.3%). No risk factor for IE based on age, comorbidities and microbiological features as been identified (data not shown).

Therapeutic management

In 356 patients (63.2%), antibiotic therapy was not started until the microorganism was identified either by Gram stain or a positive culture on synovial fluid or in the blood cultures. An infectious specialist advice was requested in 275 cases (75.9%), either initially (n=224, 61.9%) or subsequently because of worsening (n=51, 14%).

Antibiotic therapy was very heterogeneous in terms of antibiotic type, duration and route of administration (table 2). For example, among the 147 methicillin-susceptible *Staphylococcus aureus* NJSA, 19 different antibiotic combinations were used as the first-line treatment after microbiological findings, and 165 (45.6%) patients had to change at least two times antibiotic treatment. Use of antibiotic as monotherapy at initiation was more frequent in case of *Streptococcus* sp compared with *Staphylococcus* sp (52.4% vs 34.8%, p=0.006). Only 25 (6.9%) patients were initiated with orally administered antibiotic therapy. Among them, 12 subsequently switched for parenteral antibiotics because of microbiological findings (n=7), clinical or biological worsening (n=4), poor adherence (n=1) or infectious specialist advice (n=1).

Overall, the mean duration of antibiotics was 46.8 (±22.0) days including a mean of 17.2 (±15.4) days of intravenous route (table 2).

Among all, 171 patients (48.4%) underwent a surgical procedure of 227 joints (table 2), mainly for knee NJSA (n=104). The most frequently used procedure was surgical lavage (n=162, 94.7%) associated or not with a synovectomy. Primary surgical management was decided at the diagnosis start of NJSA management in n=111 (65.7%) or after failure of initial conservative approach or worsening in n=58 (34.3%). Joint immobilisation was performed in n=129 (43.8%).

Outcomes

Mean length of hospital stay was 22.6 days (±16.3) (table 2). During this period, 91 patients (28.3%) had complications including 19 transfers in intensive care unit (online supplemental data S5).

One-year mortality was 9.2% (28/305 patients) rising to 21.3% (17/80 patients) in patients over 80 years old (online supplemental data S6). No association with mortality was observed based on microbiological characteristics or site of NJSA. Antibiotic duration was shorter in patients who died (p=0.029). However, this parameter was not included in the multivariate analysis since shorter duration was linked to the early deaths (ie, before the end of antibiotic treatment). In multivariate analysis, older age (adjusted OR (aOR): 1.08 [95% CI: 1.04 to 1.13], for each additional year), higher Charlson's index (aOR: 1.30 (95% CI 1.06 to 1.58), for each additional point), presence of bacteremia (aOR: 4.02 (95% CI 1.35 to 11.99)), use of antibiotics in the 3 months prior to hospitalisation (aOR: 3.32 (95% CI 1.11 to 9.87)) were associated with 1-year mortality (table 3). Among patients having had antibiotics in the 3 months prior to hospitalisation, only those for whom antibiotics were started after NJSA onset and before microbiological investigations had a higher risk for mortality (alternative model, p<0.002). *Staphylococcus aureus* were associated with an increased risk of mortality, compared with *Streptococcus* sp (aOR: 7.24 (95% CI 1.26 to 41.68)). Adverse joint outcome at 1 year, evaluated in 278 patients, was observed in 125 patients (45.0%) (Description of local joint outcome in online supplemental data S5). No factor was associated to adverse joint outcome in multivariate analysis (table 4).

Culture-negative septic arthritis

Overall, 22 (6.1%) were considered culture-negative NJSA despite careful assessment (table 5). Compared with bacteriological proven NJSA, only use of antibiotic in the previous 3 months (aOR: 2.67 (95% CI 1.02 to 6.95)) was significantly associated with culture-negative NJSA in multivariate model adjusted on age (table 5).

DISCUSSION

We here report the results of nationwide multicentric survey including a large number of patients with NJSA. Even in this recent study, NJSA remains a serious condition with high rate

Table 2 Therapeutic management of NJSA in all patients and according to the identified micro-organism

	All patients n=362 (%)	Patients with *Staphylococcus* sp. NJSA n=185 (%)	Patients with Streptococcus sp. NJSA n=84 (%)	Patients with culture-negative NJSA n=22 (%)
Antibiotic therapy				
Mean duration to antibiotic initiation, days				
From admission	3.1 (±5.2)	2.7 (±4.1)	1.8 (±2.2)	32.3 (±46.0)
From first symptoms	21.2 (±39.1)	20.6 (±38.7)	12.5 (±18.1)	6.7 (±10.4)
Mean antibiotic duration, days				
Total duration	46.8 (±22.0)	48.5 (±20.7)	44.4 (±17.5)	44.5 (±8.6)
Intravenous	17.2 (±15.4)	14.9 (±11.6)	17.8 (±11.8)	15.9 (±14.2)
Oral route	29.0 (±22.1)	33.5 (±21.3)	25.7 (±18.3)	27.9 (±39.4)
Modalities of initiation				
Before microbiologic documentation	129/355 (36.3)	54/180 (30.0)	27/83 (32.5)	22 (100)
After microbiologic documentation	225/355 (63.3)	126/180 (70.0)	56/83 (67.5)	–
Monotherapy				
Introduction as monotherapy	151/358 (42.2)	64/184 (34.8)	44/83 (52.4)	13/21 (61.9)
Antibiotic prescribed as monotherapy	Amoxicillin 37/151 (24.5) Cefazolin 33/151 (11.9)	Cefazolin 28/64 (43.8) Penicillin M 21/64 (32.8)	Amoxicillin 27/44 (61.4) Cephalosporins 9/44 (20.4)	Amoxicillin 3/13 (23.1) Cefazolin 2/13 (15.4)
Monotherapy during all treatment	65/151 (43.0)	8/64 (12.5)	32/44 (72.8)	4/13 (30.8)
Most frequently prescribed antibiotics	–	Fluoroquinolone (25.0) Cefazolin (15.1) Rifampicin (14.6)	Amoxicillin (51.1) Gentamicin (10.6)	Fluoroquinolone (53.3) Amoxicillin (10.6)
Joint drainage	189 (52.2)	93 (50.3)	50 (59.5)	10 (50 .0)
Needle aspiration for joint drainage, n	63/351 (18.0)	31/170 (18.2)	20/77 (26.0)	2/22 (9.1)
Median number per patient (range)	3(1-10)	2.5(1-9)	3(1-10)	3(2-4)
Surgical procedure	171/354 (48.3)	84/178 (47.2)	45/83 (54.2)	10/22 (45.5)
Arthroscopic lavage/synovectomy, n	95/171 (55.6)	43/84 (51.2)	28/45 (62.2)	5/10 (50.0)
Arthrotomy lavage/synovectomy, n	67/171 (39.2)	38/84 (42.9)	18/45 (40.0)	4/10 (40.0)
Unspecified lavage or synovectomy, n	8/171 (4.7)	4/84 (4.8)	0/45 (0.0)	1/10 (10.0)
Joint replacement	3/171 (1.8)	1/84 (1.2)	1/45 (2.2)	0.10 (0.0)
Other*	5/171 (2.9)	2/84 (2.4)	0/45 (0.0)	0/10 (0.0)
Primary surgical management decided from NJSA diagnosis	111/169 (65.7)	54/82 (65.9)	27/45 (60.0)	9/10 (90.0)
Joint immobilisation	128/292 (43.8)	65/149 (43.6)	35/64 (54.7)	7/20 (35.0)
Duration of immobilisation, days	21,7 (±14.1)	23,4 (±14,9)	21.0 (±12.9)	28.6 (19.6)
Functional rehabilitation	131/281 (46.6)	56/136 (41.1)	40/67 (59.7)	8/22 (36.4)
Mean duration of hospitalisation stay, days	22.6 (±16.3)	22.5 (±17.2)	24.0 (±13.6)	17.2 (±9.7)

Results are presented as mean±SD and number (%), unless indicated.
*Abscess drainage, amputation, osteotomy, arthrodesis.
NJSA, native joint septic arthritis.

of 1-year mortality (9.2%), or adverse joint outcome (45.0%). Management of this condition was very heterogeneous, reflecting the lack of evidence or consensus and the absence of recent guidelines at the time we conducted this study. We identified age, comorbidities, bacteraemia, antibiotic use before bacteriological sampling and *Staphylococcus aureus* (compared with *Streptococcus* sp) as being associated with mortality. Antibiotic use before bacteriological sampling was also associated with a higher risk of culture-negative NJSA. But, none of these factors was associated to adverse joint outcome.

In the literature, case-definition of septic arthritis usually relies on modified Newman criteria.[13] However, we intentionally chose broader inclusion criteria, in line with our main objective, being more representative of the real-life NJSA management. To our knowledge, multicentric surveys are unusual in the field of NJSA, studies being mostly monocentric and retrospective. Thus, our study was more representative of the nationwide variety of management of NJSA.

Clinical and microbiological characteristics of our patients were closed to those found in other studies.[1 4] Of note, 28.3% of NJSA had pre-existing arthropathy on the affected joint, mainly osteoarthritis (18.9%), and crystal arthropathy (8.2%). Rheumatoid arthritis (RA), commonly described as a risk factor for NJSA,[14 15] was found in only 4% of patients, and was even lower if we considered only cases where RA affected the same joint as the NJSA. This result is line with a 2%–4% rated observed in more recent cohorts[1 4 5 16] could be explained by a better management of RA with less articular damage.

The most frequent causative organisms were *Staphylococcus* sp and *Streptococcus* sp as previously described in literature.[1 17]

Diagnotic strategy to obtain microbiological documentation was very homogenous and congruent with guidelines.[9 11 18] Our study supports the systematic implementation of joint aspiration and blood cultures. Interestingly, blood cultures were very effective in obtaining bacteriological diagnosis, being positive in almost half of all the patients, including those with no organism identified in the joint or for whom joint aspiration could not be performed. In previous studies, frequency of bacteraemia was rarely reported but accounted for 24% to 46% of NJSA.[1 15 19–21] Likewise, there was limited data on frequency of infective

Table 3 Univariate and multivariate analysis of factors associated with mortality in the following year

	Survivor n=276 (%)	Dead n=28 (%)	Univariate analysis P	Adjusted OR (95% CI)	Multivariate analysis P
Demography and comorbidities					
Age, years	62.4 (±17.7)	78 (±13.8)	<0.001	1.08 (1.04 to 1.13)	<0.001
Sex male	90 (67.4)	20 (71.4)	0.823	1.49 (0.53 to 4.14)	0.441
Body mass index >25 kg/m^2	132/228 (57.9)	11/22 (50.0)	0.625	–	–
Charlson's index	1.5 (±2.1)	3.0 (±2.4)	0.001	1.30 (1.06 to 1.58)	0.012
Concomitant treatment					
Corticosteroids in the previous 3 months	35/252 (13.9)	8/26 (30.7)	0.037	–	–
NSAIDs in the previous 15 days	76/247 (30.8)	3/24 (12.5)	0.100	2.98 (1.00 to 8.88)	0.053
Drug increasing infectious risk*	23/259 (8.9)	5/25 (20.5)	0.084	–	–
Pre-existing arthropathy on septic joint	71/260 (25.7)	12/25 (42.9)	0.052	–	–
Osteoarthritis†	44 (16.0)	9 (32.1)	0.041	–	–
Crystal arthropathy	22 (8.0)	4 (14.3)	0.276	–	–
Inflammatory rheumatic disease	8 (2.9)	1 (3.5)	1.000	–	–
Antibiotics in the previous 3 months	62/233 (26.6)	12/22 (54.5)	0.012	3.32 (1.11 to 9.87)	0.029
NJSA characteristics					
Micro-organisms					
Streptococcus sp.	70 (25.4)	2 (7.1)	0.052	Ref	Ref
Staphylococcus aureus	122 (44.2)	17 (60.7)	0.250	7.24 (1.26 to 41.68)	0.027
Others	84 (30.4)	9 (32.1)	1.000	5.01 (0.83 to 30.39)	0.080
Culture-negative NJSA	17/276 (6.2)	3/28 (10.7)	0.406	_	–
Knee involvement	123 (44.6)	17 (60.7)	0.151	–	–
Hip involvement	24 (8.7)	2 (7.1)	1.000	–	–
Multiple joint involvement	27/271 (10.0)	3/27 (11.1)	1.000	–	–
Bacteraemia	111/262 (42.4)	20/27 (74.1)	0.003	4.02 (1.35 to 11.99)	0.008
Infective endocarditis‡	5/198 (2.5)	4/22 (18.2)	0.007	–	
Antibiotic therapy					
Delay from first symptoms to antibiotic initiation, days	20.5 (±34.4)	13.5 (±17.4)	0.289	–	–
Total duration, days§	48.0 (±21.8)	38.3 (±23.0)	0.029	–	–
Intravenous duration, days	16.7 (±15.8)	22.4 (±13.9)	0.007	–	–
Oral route, days	30.8 (±21.4)	15.8 (±20.0)	<0.001	–	–
Joint drainage					
Needle aspiration	49/254 (19.3)	6/25 (24.0)	0.597	–	–
Surgical management	133/271 (49.0)	13/27 (48.1)	1.000	–	–
Primary arthroscopic lavage/synovectomy	48/270 (17.8)	3/27 (11.1)	0.444	–	–
Primary arthrotomy lavage/synovectomy	34/270 (12.5)	4/27 (14.8)	0.756	–	–

Results are presented as mean±SD and number (%), unless indicated.
The main model of multivariate analysis reported in this table included age and Charlson index.
*Chemotherapy, csDMARDS, bDMARDS, immunosuppressive therapy, treatment for organ graft, in the previous 6 months.
†Not included in the multivariate model, since collinear/correlated with age.
‡Not included in the multivariate model, since highly collinear/correlated with bacteremia.
§Not included in the multivariate model, since shorter duration was linked to the early deaths (ie. before the end of antibiotic treatment).
bDMARDs, biological disease-modifying anti-rheumatic drugs; csDMARDs, conventional disease-modifying anti-rheumatic drugs; NSAIDS, non-steroidal anti-inflammatory drugs.

endocarditis until a recent study reporting a rate of 3.7%,[22] consistent with our findings.

The frequency of culture-negative NJSA observed here (5.5%) was in the lowest values of literature (4% to 17%),[4 23–25] may be explained by the extensive microbiological searches performed in our patients. The only risk factor identified being antibiotic use in the previous 3 months, this highlights the importance of not giving antibiotics prior to microbiological sampling when NJSA is suspected.

By contrast, therapeutic management was very heterogeneous and ensues from the insufficiency of evidence in literature and the absence of recent guidelines at the time of the study. The widely variable length of admission to hospital after disease onset highlights that NJSA may be misrecognised or underappreciated by primary physicians. Antibiotic treatment was mostly started after knowledge of microbiological findings and had an average overall duration of 6 weeks, in line with recent studies in large joint NJSA reporting an average duration of 5 to 7 weeks.[1 5] Even if the trend is to shorten antibiotic duration, optimal duration for antibiotic treatment in NJSA is uncertain. For uncomplicated hand small joint NJSA with surgical management, Gjika *et al*[16] reported that 2 weeks were sufficient compared with 4-week antibiotic therapy. But, we caution against generalisation of these results outside small joint NJSA.[26] Our study population was very different with 91% of large joint involvement, and occurring mostly after a haematogenous seeding. Nevertheless, long-term exposure to antibiotics increases bacterial resistance. Currently, a French nationwide trial initiated by members of these study group in collaboration with infectious disease specialists aims to evaluate whether a shorter antibiotic treatment (3-week treatment)

Table 4 Univariate and multivariate analysis of factors associated to an adverse joint outcome

	Complete local recovery n=153 (%)	Adverse joint outcome n=125 (%)	Univariate analysis P	Adjusted OR (95% CI)	Multivariate analysis P
Demography and comorbidities					
Age, years	64.0 (±17.1)	61.8 (±17.8)	0.298	0.99 (0.98 to 1.01)	0.408
Sex male	108 (70.6)	81 (64.8)	0.368	0.88 (0.49 to 1.56)	0.662
BMI>25 kg/m^2	74/130 (56.9)	58/60 (58.0)	0.977	–	–
Charlson's index	1.6 (±2.2)	1.5 (±1.9)	0.547	0.96 (0.84 to 1.10)	0.595
Concomitant treatment					
Corticosteroid in the previous 3 months	23/148 (15.5)	13/108 (12.0)	0.539	1.23 (0.68 to 2.22)	0.500
NSAIDs in the previous 15 days	38/144 (26.4)	38/107 (35.5)	0.156	–	–
Drug increasing infectious risk*	15/145 (10.3)	9/116 (7.8)	0.615	–	–
Pre-existing arthropathy on septic joint	36/143 (25.2)	35/118 (29.7)	0.502	–	–
NJSA characteristics					
Micro-organisms					
Streptococcus sp.	37 (24.2)	32 (25.6)	0.944	–	–
Staphylococcus aureus	71/140 (50.7)	52/120 (43.3)	0.287	–	–
Others	45 (29.4)	41 (32.8)	0.522	–	–
Knee involvement	66 (43.2)	56 (44.8)	0.876	–	–
Hip involvement	10 (6.5)	15 (12.0)	0.170	–	–
Multiple joint involvement	12/151 (7.9)	15/121 (12.4)	0.310	–	–
Bacteremia	69/146 (47.3)	46/118 (39.0)	0.221	–	–
Infective endocarditis	4/106 (3.7)	1/93 (1.08)	0.378	–	–
Antibiotic therapy					
Delay before antibiotic initiation, days	16.9 (±28.0)	24.6 (±40.5)	0.069	1.00 (0.99 to 1.01)	0.497
Total duration	46.9 (±18.7)	49.4 (±25.1)	0.928	–	–
Intravenous duration	17.3 (±15.5)	16.2 (±16.0)	0.272	–	–
Oral route	29.7 (±18.8)	31.7 (±24.5)	0.633	–	–
Joint drainage					
Needle aspiration	26/140 (17.0)	23/114 (18.3)	0.871	–	–
Surgical management†	56/151 (37.1)	77/122 (63.1)	<0.001	–	–
Primary arthroscopic lavage/synovectomy	21/151 (13.9)	24/121 (19.8)	0.253	–	–
Primary arthrotomy lavage/synovectomy	14/151 (9.3)	21/121 (17.4)	0.072	2.05 (0.96 to 4.40)	0.064
Joint immobilisation	44/121 (36.4)	63/107 (58.9)	0.001	–	–

Results are presented as mean±SD and number (%), unless indicated.
*Chemotherapy, csDMARDS, bDMARDS, immunosuppressive therapy, treatment for organ graft in the previous 6 months.
†Since overall surgical management could reflect a more serious local condition, only primary surgical management decided from NJSA diagnosis was included in the multivariate model to minimise risk of indication bias.
bDMARDs, biological disease-modifying anti-rheumatic drugs; BMI, body mass index; csDMARDs, conventional disease-modifying anti-rheumatic drugs ; NSAIDS, non-steroidal anti-inflammatory drugs.

is safe and not inferior to the conventional 6-week treatment in NJSA (SHASAR, NCT03716921). Of note, in this trial, as in the OVIVA trial, the duration of antibiotic in the control arm is of 6 weeks, demonstrating that it is still the reference standard. Nevertheless, we now know from the OVIVA trial, that the duration intravenous course should be shorter than the 15 days, we here observed since even in patients with complex bone or joint infections to 7 days was sufficient.[27]

Overall, 45% of patient had adverse joint outcome within 1 year, in line with literature ranging from 24% to 49%.[1 15 16] Surgical drainage accounted for the half of our population, in line with the literature (37%–83%).[1 5 28] However, evidence regarding the need for a systematic surgical drainage or its best timing is scarce. A non-operative approach of NJSA management has not been yet prospectively studied. By contrast with Flores-Robles *et al* who did not observe worst outcomes with medical or surgical management,[29] in our cohort, patients undergoing surgery had higher rate of adverse joint outcome. However, we could not exclude an indication bias, since patients undergoing surgery probably had a more serious condition. Effectively, when focusing on systematic surgical joint drainage decided as a part of initial management, surgery was no more associated with worst outcome. Regarding surgical modalities, arthroscopy or arthrotomy, having the same effectiveness,[30–33] some authors support that arthrotomy could be associated to a worst joint outcome and a longer recovery.[30 31] We here found the same trend, even though non-significant.

Mortality raised to 9.2% in our study, in the same range of studies mainly including large joint NJSA,[3 5 19 22] but higher than in studies with different recruitments reports (around 6%).[1 4] Small NJSA have different epidemiology and lower mortality.[5] As expected,[3 15 19] age and Charlson's comorbidity index were independently associated with 1-year mortality. Also, patients with bacteremia had a fourfold higher risk of mortality, as previously reported.[19] Mortality raised to 18% in patients with IE. We are the first to report that *Staphylococcus aureus* NJSA had a worst prognosis with a nearly sevenfold higher risk of death as compared with *Streptococcus* sp. In contrast to previous report, polyarticular involvement was not associated with increased mortality.[2 15 19] For the first time, we report that use of antibiotics

Table 5 Univariate and multivariate analysis of factors associated to culture-negative native joint septic arthritis

	Culture positive NJSA n=340 (%)	Culture negative NJSA n=22 (%)	Univariate analysis P	Adjusted OR (95% CI)	Multivariate analysis P
Demography and comorbidities					
Age, years	64.5 (±18.5)	56.7 (±19.8)	0.102	0.98 (0.96 to 1.01)	0.230
Sex male	228/340 (67.1)	15/22 (68.2)	1.000	0.81 (0.29 to 2.25)	0.687
BMI>25 kg/m^2	157/276 (56.9)	8/18 (44.4)	0.432	–	–
Charlson's index	1.6 (±2.0)	1.8 (±2.6)	0.734	–	–
Inflammatory rheumatic disease	4/340 (1.2)	1/22 (4.5)	0.270	–	–
Crystal arthropathy	39/291 (13.4)	1/22 (4.5)	0.485	–	–
Concomitant treatment					
Corticosteroid in the previous 3 months	47/308 (15.3)	2/19 (10.5)	0.754	–	–
NSAIDs in the previous 15 days	87/298 (29.2)	7/19 (36.8)	0.654	–	–
Drug increasing infectious risk*	29/319 (9.1)	2/20 (10)	1.000	–	–
Antibiotics in the previous 3 months	76/281 (26.4)	11/20 (55.0)	0.016	2.67 (1.02 to 6.95)	0.044
NJSA characteristics					
Knee involvement	149/340 (43.8)	11/22 (50.0)	0.731	–	–
Hip involvement	31/340 (9.1)	1/22 (4.5)	0.705	–	–
Finger and toes involvement	17/340 (5.0)	4/22 (18.2)	0.033	3.46 (0.55 to 21.93)	0.187
Multiple joint involvement	34/334 (10.2)	3/22 (13.6)	0.716	–	–
Maximal CRP (mg/L)	206 (±131.0)	198.2 (±118.6)	0.013	0.99 (0.99 to 1.00)	0.055
Crystal detection on synovial fluid	36/236 (15.2)	1/16 (6.3)	0.479	–	–
Microbiological investigations					
Synovial fluid puncture	300/339 (88.2)	18/22 (81.9)	0.488	–	–
Blood cultures	326/340 (95.9)	20/22 (90.9)	0.249	–	–
Synovial biopsy	42/339 (12.1)	10/22 (45.5)	<0.001	–	–
PCR DNAr 16s	39/292 (11.5)	8/18 (36.3)	0.002	–	–
Antibiotic therapy					
Delay from first symptoms to antibiotic initiation, days	20.8 (±32.8)	21.2 (±45.2)	0.588	1.01 (1.00 to 1.02)	0.224
Total duration, days	47.0 (±20.6)	44.5 (±38.6)	0.325	–	–
Intravenous duration, days	17.3 (±15.5)	15.8 (±14.2)	0.509	–	–
Oral route, days	29.1 (±27.9)	20.6 (±39.3)	0.204	–	–
Joint drainage					
Needle aspiration	61/312 (19.6)	2/19 (1.0)	0.396	–	–
Surgical management	161/332 (48.5)	10/22 (45.5)	0.955	–	–
Outcomes					
Duration of hospitalisation stay, days	22.5 (±16.5)	17.2 (±9.7)	0.171	–	–
One-year mortality	25/284 (8.8)	3/21 (14.3)	0.414	–	–
Adverse joint outcome within the year	116/261 (44.4)	9/17 (52.9)	0.667	–	–

Results are presented as mean±SD and number (%), unless indicated.
*Chemotherapy, csDMARDS, bDMARDS, immunosuppressive therapy, treatment for organ graft in the previous 6 months.
bDMARDs, biological disease-modifying anti-rheumatic drugs; csDMARDs, conventional disease-modifying anti-rheumatic drugs; NJSA, native joint septic arthritis; NSAIDS, non-steroidal anti-inflammatory drugs.

in the previous 3 months before admission was associated with mortality. This observation might reflect the frailty of patients who are more likely to contract multiples infectious diseases, and/or that antibiotics prescribed before microbiologic samples might complicate the diagnostic and therapeutic management of NJSA. Besides, antibiotics for NJSA started before microbiological samples could have been prescribed in frailty patients with more comorbidities.

Our study had important limitations, such as its retrospective nature. Outcomes, obtained from medical records by each participating centre, might lack of standardisation and precision. Nevertheless, it also has some strength such as its large sample size and representativeness of management of NJSA in rheumatology departments of both tertiary care centres and general hospitals. Since all patients were recruited in rheumatology departments, we cannot exclude a recruitment bias compared with surgical recruitment, notably older patients with more comorbidities. However, in France, management of NJSA relies on multidisciplinary approach formalised in many centres by the CRIOAc ('Centre de Référence des Infections Ostéo-articulaires Complexes' - reference center for complex joint and bone infections) healthcare network,[34] as observed here, with frequent involvement of infectious specialist (>75%) and orthopaedic surgeons (surgical management in ~50% of the patients).

In this study, NJSA has serious consequences with 9.2% mortality and 45.0% of adverse joint outcome within the year, increasing in older patients with comorbidities. This study emphasises that no antibiotic should be given before microbiological diagnosis. Management of NJSA was very heterogeneous across the different centres. Since then, new recent guidelines of the French society of rheumatology on management of NJSA have been published[9] and might have improved harmonisation

of NJSA management. Finally, whether antibiotic duration might be shortened or not, should be analysed in prospective randomised controlled trials.

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Fever, rhinosinusitis and glomerulonephritis with systemic inflammation and antimyeloperoxidase antibody

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A woman in her 80s presented with a month-long fever and nasal discharge, which did not subside by clarithromycin and levofloxacin. She did not have a history of allergic diseases including bronchial asthma. Physical examination showed no significant findings. However, laboratory tests revealed a high level of C-reactive protein (129 mg/L, reference range <1.4), positive antimyeloperoxidase antibody and active urine sediments including haematuria, proteinuria and cellular casts. Whole-body CT demonstrated no remarkable findings except abnormal soft tissue filling left maxillary sinus (figure 1A). The patient was referred to our department under the suspected diagnosis of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis with nasal manifestation and glomerulonephritis. However, CT reassessment revealed slight speckled calcification without bone destruction in the left maxillary sinus (figure 1B). MR fat-saturated-T2-weighted imaging also demonstrated low-intensity areas in the left maxillary sinus without dural thickening (figure 1C). In the nasopharyngoscopy procedure, the septum was found to be normal, but the lateral wall of nasal cavity was retracted with purulent discharges (figure 1D). (1–3)-β-D-glucan, serum fungal antigen and blood culture test results were negative. We suspected left fungal sinusitis and performed surgical treatment. Evaluation of the maxillary sinus indicated acute-angle branching, septate, filamentous fungi, suggesting *Aspergillus* spp (figure 1E). The mucosal biopsy of maxillary sinus exhibited inflammatory cells around vessels without fungal invasion (figure 1F).

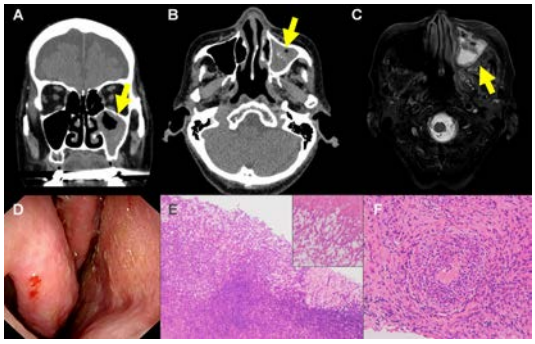

Figure 1 (A) Abnormal soft tissue density and fluid collection with air density in the left maxillary sinus (arrow). (B) Spotty calcification in the soft tissue filling left maxillary sinus (arrow). (C) Fluid collection and soft tissue congestion shown on the fat-saturated T2-weighted magnetic imaging (arrow). Air and calcification demonstrated as signal voids and low signal intensities, respectively. (D) The nasopharyngoscopy demonstrated the retraction of nasal cavity lateral wall with purulent discharges. (E) The contents of the left maxillary sinus included acute-angle blanching, septate, filamentous fungi. Right upper panel shows high-magnified image of the fungi. H&E staining. (F) Vasculitis without fungal invasion in the mucosa of the left maxillary sinus. H&E staining.

The fever immediately resolved after surgery. We eventually diagnosed the patient with non-invasive chronic fungal sinusitis, which immunologically triggered ANCA-associated vasculitis with glomerulonephritis. Previous reports demonstrated that cases of ANCA-associated vasculitis are frequently subjected to invasive mycoses in which *Aspergillus* spp are the leading strain.[1 2] Sporadic cases of systemic vasculitis due to invasive aspergillosis were noticed even in immunocompetent individuals.[3] Physicians should remember that non-invasive aspergillosis would induce autoimmunity of ANCA-associated vasculitis as in our case. Three months after the surgical removal of maxillary fungal balls, systemic inflammation, ANCA and active urine sediments had completely diminished without immunosuppressive therapy (see online supplemental table 1 for the follow-up results of laboratory findings).

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Rational use of tocilizumab in COVID-19

With 12 million cases and half a million deaths (as of 10 July 2020), the corona virus disease (COVID-19) has paralysed healthcare systems the world over. The observations by Potere *et al*[1] and mechanistic insights by Capecchi *et al*[2] merit a discussion of important considerations that should be borne in mind before using tocilizumab in severe COVID-19. The available evidence concerning its use, although promising, is preliminary and probably underpowered to detect important safety/efficacy signals.[3 4]

Patients with COVID-19 are not free from the risk of a bacterial infection, which could be secondary (nosocomial) or co-primary (community acquired). Concomitant bacterial infections have been reported in 10%–20% of COVID-19 cases and ~50% of COVID-19-related deaths, consistent with, and reminiscent of bacterial infections being the the most common cause of mortality in previous influenza pandemics.[5 6] This risk is higher in old age, presence of comorbidities and those requiring invasive mechanical ventilation (all probable candidates for use of tocilizumab). A hesitancy towards the use of CT and fiberoptic bronchoscopy at many centres to reduce COVID-19 transmission makes differentiation of COVID-19 acute respiratory distress syndrome (ARDS) from bacterial pneumonia challenging. Suppression of C reactive protein, leucocytosis and fever by tocilizumab render these parameters unusable for diagnosing an underlying bacterial infection. Inadvertent use of tocilizumab in the latter setting may be catastrophic.

To complicate things further, an increased risk of serious infections with tocilizumab (initially deemed unlikely with short-term use) has recently been demonstrated in COVID-19.[7 8] A 13% higher risk of new infections was seen with tocilizumab when added to standard-of-care, in the largest cohort study available until date.[7] Two deaths due to septic shock were seen in another cohort of 100 patients treated with tocilizumab.[8] Candidaemia with candidal endophthalmitis and endocarditis was reported in 3 of the 43 severe COVID-19 patients treated with tocilizumab in Italy.[9]

Gastrointestinal involvement in COVID-19 is fairly common (pooled prevalence=15%), especially in patients with severe disease.[10] Considering the compromised mucosal integrity in these patients because of intestinal hypoperfusion, and extrapolating the experience from rheumatic diseases, there is a realistic possibility of bowel perforation with the use of tocilizumab in COVID-19. Unsurprisingly, two such cases have already been reported.[8 11]

Most safety data for tocilizumab is derived from autoimmune rheumatic diseases, where routine screening, especially for viral hepatitis, dyslipidaemia, diverticular disease and latent infections including tuberculosis (TB), precedes its use. Whether short-term emergent use, as in COVID-19, warrants a similar or abbreviated screening is still unknown. The narrow window of intervention in severe COVID-19 necessitates prompt administration, often without screening, which could be potentially lethal. Mortality due to herpes simplex virus-1 reactivation-related liver failure was recently reported with the use of tocilizumab in COVID-19, along with hepatitis B reactivation.[7] There have also been reports of acute severe hypertriglyceridaemia (with subsequent pancreatitis) with tocilizumab, although confounded by concomitant use of lopinavir–ritonavir.[12]

Although data from randomised controlled trials (RCTs) and registries in non-endemic countries suggests a low risk for reactivation of TB with tocilizumab, data from high TB-endemic settings is unclear. Should empirical isoniazid prophylaxis be given to COVID-19 patients with a history of TB or those living in high-endemic countries? Should latent TB testing be done before administration using the interferon gamma release assay (considering tubercular skin testing will take 48–72 hours)? Or, considering short-duration use (one to three doses), are these concerns misplaced? There is no data to back any of these statements yet.

Finally, the cytokine release syndrome (CRS) of severe COVID-19 has many key players apart from interleukin 6 (IL-6), including tumour necrosis factor- alpha, granulocyte colony stimulating factor, interferon gamma and IL-1 beta.[13] In such a multipronged pathogenetic model, should tocilizumab be considered only in those patients with elevated serum IL-6 levels, as suggested by the Chinese guidelines? Since measuring IL-6 is difficult in routine clinical practice, could measurement of serum CRP be a reliable, cost-effective surrogate? A better understanding of COVID-CRS pathogenesis is required.

The use of tocilizumab in COVID-19 outside of an RCT setting thus needs to be rationalised. Its short-term (one to three doses) use is not free from serious adverse events, and a vigilant monitoring is mandatory. The efficacy data, although promising, are preliminary. Results of ongoing RCTs are eagerly awaited.

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Targeting IL-6 in COVID-19. Response to: 'Rational use of tocilizumab in COVID-19' by Jain and Sharma

Dear Editor,

Siddharth and Sharma[1] suggest caution in using interleukin-6 (IL-6) receptor blocking agents, namely tocilizumab, in the treatment of patients with COVID-19 infection, particularly those requiring invasive mechanical ventilation because of the increased risk of infections and bowel perforation, possibly also masked by the anti-inflammatory activity of the agent.[1] Generally speaking, this is a fully acceptable principle to be observed as caution when using drugs is a common rule in the clinical setting. Thus, no doubt that the use of tocilizumab should be carefully evaluated in individual cases. Nevertheless, the same authors state that 'the efficacy data are promising (although preliminary)'.

Specifically regarding safety, Siddharth and Sharma quote that a 13% higher risk of new infections was seen with tocilizumab when added to standard of care.[2] Indeed, in the study by Guaraldi *et al*,[2] 24 (13%) of 179 patients treated with tocilizumab were diagnosed with new infections, versus 14 (4%) of 365 patients treated with standard of care alone (9% higher risk), and the overall conclusions of the authors were that 'tocilizumab might reduce the risk of invasive mechanical ventilation or death in patients with severe COVID-19 pneumonia'. Additionally, it should be noted that 13% is in the range of the reported concomitant bacterial infections (10%–20%) of COVID-19 cases.[3] Also, Toniati *et al*[4] conclude their report on an uncontrolled series of COVID-19 pneumonia patients with adult respiratory distress syndrome including two deaths due to septic shock that the response to tocilizumab was rapid, sustained and associated with significant clinical improvement. Finally, when considering cases of bowel perforation during tocilizumab treatment, the possible role of the concomitant treatment with steroids should also be considered.[4 5] Nevertheless, we acknowledge that data on the efficacy of tocilizumab are still preliminary and sometimes conflicting, particularly in terms of rate of adverse events.[6–8]

One should also consider the putative role of IL-6 blockade in reducing the occurrence of COVID-19-associated cardiovascular events, particularly arrhythmias,[9] which are increasingly reported in the literature.[10] In this regard, although the COVID-19-associated long QT syndrome as a real risk factor for arrhythmic cardiac death is under strong consideration,[11 12] studies regarding the possible protective effects of IL-6 blocking agents are still in progress and when concluded they might provide further support to the use of tocilizumab (figure 1).

In conclusion, we agree with Siddharth and Sharma that data on IL-6 antagonists in the treatment of COVID-19 infection are still preliminary and in some way unconclusive as yet and that controlled studies with a larger number of subjects are needed before treatment with these agents may achieve any level of recommendation. Nevertheless, we still remain convinced that the rationale for targeting IL-6 in COVID-19 infection is strong and to some extent supported by preliminary evidence.

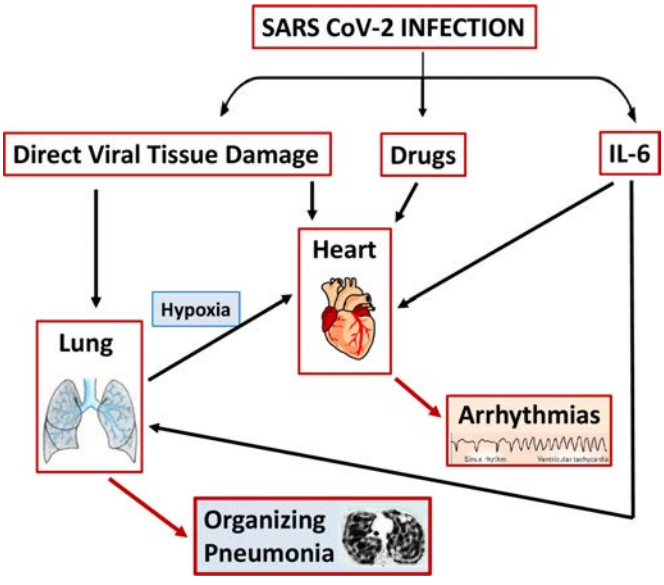

Figure 1 IL-6 in COVID-19. IL-6, interleukin-6.

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Response to: 'Rational use of tocilizumab in COVID-19' by Jain and Sharma

We thank Jain and Sharma[1] for their interest in our recent report on interleukin-6 (IL-6) receptor blockade with subcutaneous tocilizumab (324 mg, given in two concomitant 162 mg doses) in patients with severe COVID-19 pneumonia and hyperinflammation.[2] Jain and Sharma bring up an important point regarding the safety profile of subcutaneous tocilizumab in patients with COVID-19 at risk of concomitant bacterial infections.[1] As already concisely described, in our case–control series, only relatively young patients with severe COVID-19 pneumonia, requiring oxygen support through nasal cannulas or masks, hyperinflammation (C-reactive protein >20 mg/dL) and no contraindications to tocilizumab, including suspected concomitant bacterial infection, were included.[2] Well aware of the potential adverse effects of tocilizumab, our screening protocol to rule out concomitant bacterial infection was based on medical history, collected at the time of and during hospitalisation in advance of tocilizumab administration, absolute white blood cell, neutrophil and lymphocyte counts, serial procalcitonin (PCT) values, which were persistently <0.1 ng/mL in most patients, as well as imaging testing assessing for concomitant infection sites outside the lungs. Indeed, several patients were cautiously excluded due to suspicion of superimposed bacterial infection based on clinical input or PCT values above that threshold, and if immunodeficiency was clinically relevant or reasonably suspected. As reported, under these strict and cautious treatment criteria, the bacterial infection did not prove to revert the expected beneficial effects of tocilizumab administration.[2] The point raised by our colleagues is of utmost importance and may contribute to explain the remarkable difference in outcomes observed in some retrospective series reporting on the early beneficial administration of subcutaneous tocilizumab,[2–4] compared with other reports on intravenous tocilizumab in more severe and possibly intubated patients with COVID-19.[5 6] As a consequence, comments on the risks of late bacterial infections related to the treatment with tocilizumab, possibly causing an excess of bacterial adverse events, even severe, are welcome. Indeed, in future reports, the incidence of such events should be appraised as a composite endpoint including death, as patients meeting lethal outcomes cannot experience late infections. Therefore, although we agree that the available data on early subcutaneous treatment with tocilizumab are definitely preliminary, existing data suggest a survival benefit in selected patients with COVID-19, an evidence that may be considered for possible therapeutic decisions in the ongoing scenario of the present pandemic and that should be explored in adequately powered randomised controlled trials (RCTs). RCTs are indeed eagerly awaited even in the course of the present case escalation rates around the world,[7] provided an adequate setting to enrol such patients in an RCT. In the meantime, we agree once more that patients treated with subcutaneous tocilizumab for COVID-19 should be monitored for possible bacterial complications, as clearly stated in the prescribing information available to clinicians. Anyway, as our and other reports suggest, administration of subcutaneous tocilizumab early in the course of the disease, that is, before mechanical ventilation is required or resorted on, may result in greater clinical benefit compared with standard of care and infrequent occurrence of secondary infections, as mechanical ventilation is likely to represent a major risk factor for such complications in these prevalently young patients.[2–4]

As to the point of the possible need of screening for chronic underlying infections at risk of reactivation due to urgent tocilizumab exposure in patients with COVID-19, based on current evidence we feel that, under the strict exclusion/inclusion criteria recalled earlier, this possibility may be considered a minor issue, considering that patients with COVID-19 will be receiving one, or possibly two doses of subcutaneous tocilizumab, which is a therapeutic frame unlikely to induce enough immunosuppression and, in turn, cause reactivation of latent infection(s). As a consequence, empirical coprescription of isoniazid, even in countries with a high prevalence of active and latent tuberculosis, may be viewed at this stage as redundant, and possibly causing an excess of drug toxicity or drug–drug interaction issues in treated patients.

Finally, with regard to the possible IL-6-driven prescription of tocilizumab in patients with severe COVID-19 pneumonia, this may be interesting and possibly relevant, but at present beyond the purpose of our study.[2] Despite having the possibility of assaying IL-6 at our centre since 6 April 2020, IL-6 values assays were not mandatorily performed in our patients in advance of subcutaneous tocilizumab administration, as the inclusion criteria were once more only those described in our letter. We find that, for the time being, those treatment criteria may actually help timely and appropriate prescription of subcutaneous tocilizumab to patients with early and severe deterioration of respiratory function due to COVID-19 pneumonia in settings where, as in ours, IL-6 assays may be limiting.

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Antirheumatic drugs, B cell depletion and critical COVID-19: correspondence on 'Clinical course of coronavirus disease 2019 (COVID-19) in a series of 17 patients with systemic lupus erythematosus under long-term treatment with hydroxychloroquine' by Mathian *et al*

In a recent case series, Mathian *et al* reported on 17 patients suffering from systemic lupus erythematosus and COVID-19.[1] All of these patients received long-term hydroxychloroquine treatment and initial signs and symptoms of COVID-19 were similar to those previously described. However, as 50% of the patients remained hospitalised at the time of publication, the authors cannot comment on the duration and eventual outcome of COVID-19 in all of their patients. Furthermore, it is emerging that hydroxychloroquine does not alter COVID-19.[2–4] As such, we actually believe that other immunosuppressive, antirheumatic medications require more attention. Mathian *et al* rightfully pointed out that besides long-term hydroxychloroquine treatment, steroids and other baseline immunosuppressant drugs are often present in patients with rheumatic diseases. In this context, we found an altered immune response and noticeable prolongation of COVID-19 from the onset of symptoms to intensive care unit (ICU) admission in two patients pretreated with rituximab (RTX) (table 1).

Patient A (aged 40–60 years) suffered from rheumatoid arthritis, treated with daily doses of leflunomide and low-dose prednisolone. RTX was administered every 6 months. The patient was admitted to ICU 33 days after the onset of COVID-19 symptoms with severe acute respiratory distress syndrome and massively elevated interleukin-(IL-) 6 levels. A good response to low-dose circulatory support with norepinephrine and prone positioning improved the clinical situation. Extubation was successful on day 41 and the patient could be discharged from the ICU on day 44 in stable condition. Patient B (aged 40–60 years) presented himself to a regional hospital 19 days after the onset of fever and dyspnoea. COVID-19 was diagnosed and mechanical ventilation became necessary. Four months prior, the patient received an autologous haematopoietic stem cell transplantation due to a mantle cell lymphoma. Chemotherapy among others included RTX. Despite ARDS treatment and the use of IL-1 receptor antagonist anakinra to treat macrophage-activation-like syndrome, the clinical condition worsened. Transfer to tertiary care ICU was necessary on day 34. Massively elevated IL-6 and ferritin levels indicated hyperinflammation and treatment with tocilizumab was initiated. Moreover, the patient received hydrocortisone, convalescent plasma, granulocyte colony stimulating factor and immunoglobulins. After a complex clinical course, including acute renal failure, massive bilateral pulmonary embolism, episodes of ventricular tachycardia and a subarachnoid haemorrhage, the patient was successfully weaned from mechanical ventilation and finally discharged from ICU on day 58 without major respiratory or neurological residues.

Both patients had severe lymphocytopenia on ICU admission and B cell depletion persisted throughout the course of treatment, as repeatedly confirmed via flow cytometry. The patients were neither able to establish any anti-SARS-CoV-2-spike-receptor binding domaine (RBD) antibody titres, nor to eliminate the virus, as pharyngeal swabs and tracheal aspirates tested positive for SARS-CoV-2 until ICU discharge (figure 1).

Table 1 Clinical course

Demographics and ICU course	Patient A	Patient B
Age (years)	50–55	50–55
Body mass index (kg/m²)	29.4	25.7
Rituximab		
Indication	Rheumatoid arthritis	Mantle cell lymphoma
Last infusion (months prior to COVID-19 infection)	4	1
Dose (mg)	1000	828.75
B cell depletion	Complete	Complete
Gammaglobulin, baseline (mg/dL)	140	447
Further comorbidities	Hypertension	None
Tertiary care ICU admission		
Preceding length of COVID-19 symptoms (days)	33	34
Preceding hospital stay (days)	3	15
SARS-CoV-2 confirmation by RT-PCR	Yes	Yes
Mechanical ventilation	Yes	Yes
PaO_2/FiO_2 (mm Hg)	72	178
SOFA score	9	15
APACHE II score	31	39
Leucocytes (×1000/µL)	5.6	3.9
Lymphocytes (×1000/µL)	0.2	0.7
Thrombocytes (×1000/µL)	95	16
Erythrocytes (×1000/µL)	2.7	2.7
IL-6 (pg/mL)	641	518
Ferritin (µg/L)	6757	23 986
Tertiary care ICU course		
ICU stay (days)	9	25
Mechanical ventilation (days)	6	10
Renal replacement therapy	None	Intermittent
SARS-CoV-2 RNA, airway material	Cont. positive	Cont. positive
SARS-CoV-2 RNA, serum	Cont. positive	Cont. negative
Anti-SARS-CoV-2-Spike-RBD antibodies	Not detectable	Not detectable
Complications	Haemorrhage	Urinary tract infection
	PE	
	SAB	
	VT	
Survival on ICU discharge	Yes	Yes

APACHE, acute physiology and chronic health evaluation; cont., continuously; ICU, intensive care unit; IL-6, interleukin 6; PE, pulmonary embolism; RBD, receptor binding domaine; RT-PCR, reverse transcription PCR; SAB, subarachnoid haemorrhage; SOFA, sequential organ failure assessment; VT, ventricular tachycardia.

Previously a prolonged course of COVID-19[5] was attributed to the lack of B cell antigen presentation, which might concomitantly impair activation of immune cells and cytokine production.[6] We rather observed excessive immune activation and cytokine release with high proinflammatory markers. Hyperinflammation in the absence of B cells underlines the predominant role of the myeloid and T cell system in COVID-19 cytokine storm. The second interesting finding was that both patients could probably not completely clear the virus. This highlights the role of the B cell system and antibody production for virus elimination, as potentially neutralising anti-SARS-CoV-2-Spike-RBD antibodies were not detectable in both RTX patients.

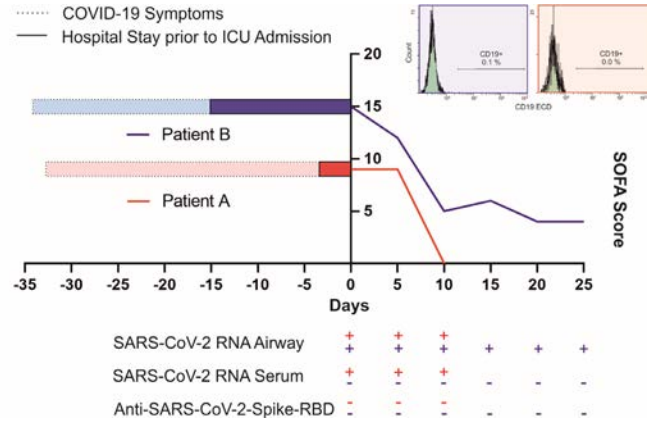

Figure 1 The prolonged clinical courses of the two patients are shown. Day 0 represents the admission on tertiary care intensive care unit (ICU). The onset of COVID-19 is marked with light red and blue bars; hospitalisation in a secondary care centre is highlighted in blue and red bars. The clinical course on ICU is represented by sequential organ failure assessment score with a line in corresponding colours. The line stops at the time of discharge from ICU. Patients had a continuous B cell depletion after rituximab treatment, as repeatedly confirmed via flow cytometry (representative inlays in corresponding colours, CD19 positivity indicated B cells). RNA from SARS-CoV-2 was positive (+) during the whole ICU stay. Anti-SARS-CoV-2-spike-receptor binding domaine (RBD) antibodies were negative (–) at all time.

Nonetheless, the clinical situation improved, indicating that deterioration was rather driven by the immune system and not by sheer presence of virus.

Overall, the case series by Mathian *et al* and our observations indicate that the presence of complex antirheumatic drug regimens further complicates COVID-19. However, the question if these patients are at risk of a more severe or just delayed course of COVID-19 remains unanswered. Larger and adequately powered studies are required, which concomitantly would provide evidence on how to handle immunosuppression in times of COVID-19.

COMPLIANCE WITH ETHICAL STANDARDS

The institutional ethic board of the University of Wuerzburg waived the need for a specific approval due to the context of sole retrospective chart review within standard care (63/20-kr 25.03.2020). Both patients have consented to the submission of this article to the journal. On behalf of all authors, the corresponding author states that there is no conflict of interest relating to the current work.

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Response to: 'Antirheumatic drugs, B cell depletion and critical COVID-19: correspondence on 'Clinical course of coronavirus disease 2019 (COVID-19) in a series of 17 patients with systemic lupus erythematosus under long-term treatment with hydroxychloroquine by Mathian *et al*' by Notz *et al*

We thank Notz *et al* for their interest in our study reporting on the course of SARS-CoV-2 disease 2019 (COVID-19) in a case series of patients with systemic lupus erythematosus under long-term treatment with hydroxychloroquine.[1 2] Notz *et al* report on two patients who had been treated with the anti-CD20 monoclonal antibody rituximab (RTX), prior to SARS-CoV-2 infection, and who presented an exacerbated immune response, a noticeable prolongation of the COVID-19 course and a need for intensive care unit (ICU) admission and mechanical ventilation. Neither one of the patients was able to generate an anti-SARS-CoV-2 spike receptor-binding domain serum antibody response or to eliminate the virus prior to ICU discharge. Because our case series did not include patients receiving B cell depletion therapy, we can only make a general comment on the authors' very interesting observations.

At the start of the epidemic in Europe, it was already suggested that RTX may expose rheumatic disease patients to a significant increased risk of hospital admission.[3] In their study, Nuño *et al* reported that all seven patients under treatment with RTX in a cohort of 122 patients with rheumatic inflammatory disease infected with SARS-CoV-2 needed hospital admission and that one died.

This observation was corroborated by several other observations made in severe, sometimes fatal, COVID-19 in patients receiving RTX for the treatment of different pathologies, including rheumatoid arthritis,[4] granulomatosis with polyangiitis,[5 6] systemic sclerosis[7] and haematological malignancies.[8] Recently, Loarce-Martos *et al* confirmed that COVID-19 is not only common, but also particularly severe in patients with rheumatic disease who had been on treatment with RTX.[9] Indeed, in an observational study they reported that 13 out of 76 (17.1%) patients with rheumatic disease treated in their centre with RTX in the last 12 months prior to screening for the presence of SARS-CoV-2 had suspected or confirmed infection. A total of eight of these patients (61.5%) developed severe COVID-19 leading to hospitalisation, from which five (38.5%) fulfilled the acute respiratory distress syndrome criteria, whereas three (23.1%) eventually died. These findings underscore that while the innate immune system[10] and T cells[11] are paramount in the early antiviral response, B cells have also an important role to play in the anti-viral response. B cell depletion agents, while not improving the cytokine storm that causes severe morbidity, may dramatically inhibit the protective antibody immunity following infection and vaccination. This process is probably largely involved in cases of a prolonged and/or atypical course of COVID-19 characterised by a negative or delayed serological response against SARS-CoV-2 in B cell depleted patients.[12–15] It is of note however that many, non-serious, cases of COVID-19 in patients under treatment with RTX have been reported as well.[16 17]

Until further studies will help us to understand the risk with respect to COVID-19 severity, treatment with biological disease-modifying drugs, such as RTX, will have to be applied with particular caution in patients with rheumatic or autoimmune disease, especially if they suffer from other comorbidities which render them particularly at risk.

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Correspondence on 'Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): a multicentre cohort'

We read with interest the clinical study entitled 'Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): a multicentre cohort' by Pouletty *et al*.[1] In this series, the authors suggest that paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 (PMIS-TS) may represent a new inflammatory syndrome, different from classical Kawasaki disease (KD) as it occurs at an older age, and with a higher frequency of severe myocarditis.[1]

Likewise to this study, our Pediatric Tuscany Network (PTN)—16 paediatric units serving a region of 593 606 people aged less than 18 years—worked out the COVASAKI survey to detect the incidence of PMIS-TS cases and the eventual rise of KD in Tuscany during COVID-19 pandemic. Between 1 February 2000 and 30 June 2020, we tracked children with PMIS-TS and KD, aiming to compare the number of KD cases in the same 5 months of the previous 5 years and overall with the total number in the last 5 years.

No PMIS-TS cases were reported in our region. Ten KD children were diagnosed in 5 units (incidence two per month). Demographics, clinical and imaging findings, treatment and outcome of patients are reported in table 1. No specific intensive support was required. No coronary involvement was reported. Nasopharyngeal swabs (performed in 7/10) and serological test (available in 6/10) for SARS CoV-2 resulted negative.

From 1 January 2015 to 31 January 2020, 165 KD were diagnosed (incidence 2.7 per month): 59 were incomplete; 3 developed macrophage activation syndrome (MAS) and 1 KD shock syndrome (KDSS). Thirty-eight showed coronary involvement, with persistent ectasia/aneurisms in five. Eleven children

Table 1 Demographics, clinical findings, imaging findings, treatment and outcome of patients with Kawasaki disease

	Age weight comorbidities	Clinical presentation	Pharmacological treatment	Imaging results	Laboratory results	SARS CoV-2 tests	Hospital length of stay	Outcome
Patient 1 (female, Caucasian)	3 years, 15 kg no comorbidities	5 days fever (T>38°C), rash, palm-plantar oedema, conjunctivitis, cheilitis, lymphadenopathy, irritability, arthralgia	IVIG, aspirin and intravenous antibiotics	Normal abdominal US and echocardiography	WCC 14.38 10^9/L, ESR 53 mm/hour, CRP 12.5 mg/dL, ALT 238 UI/l	Nasopharyngeal swab: negative Serological test: negative	8 days	Complete recovery
Patient 2 (male, Caucasian)	4 years, 15 kg, no comorbidities	6 days fever (T>38°C), rash, conjunctivitis, cheilitis, lymphadenopathy, irritability, vomiting	IVIG, aspirin and intravenous antibiotics	Normal abdominal US and echocardiography	WCC 25.57 10^9/L PLT 528, ESR 120 mm/hour, CRP 16.4 mg/dL, fibrinogen 937 mg/dL	Not performed	7 days	Complete recovery
Patient 3 (male, Caucasian)	4 years, 17 kg, no comorbidities	9 days fever (T>38°C), rash, palm-plantar oedema cheilitis, lymphadenopathy, irritability, myalgia	IVIG, aspirin and intravenous antibiotics	Normal echocardiography	WCC 11.55 10^9/L, ESR 63 mm/hour, CRP 10 mg/dL, ferritin 116 ng/mL	Nasopharyngeal swab: negative Serological test: negative	8 days	Complete recovery
Patient 4 (female, Caucasian)	2 years, 11 kg, congenital hypothyroidism	5 days fever (T>38°C), rash, conjunctivitis, cheilitis, dyspnoea, irritability	IVIG, aspirin and intravenous antibiotics	Normal echocardiography, reactive lymphadenopathy at neck US, pneumonitis at chest US	WCC 3.47 10^9/L, L 0.7 10^9/L, ESR 4 mm/hour, CRP 2.1 mg/dL	Not performed	16 days	Complete recovery
Patient 5 (female, Caucasian)	2 years, 11 kg, no comorbidities	5 days fever (T>38°C), rash, conjunctivitis, cheilitis, lymphadenopathy, irritability	Methylprednisolone, IVIG, aspirin and intravenous antibiotics	Normal abdominal US and echocardiography, pneumonitis at chest XR	WCC 9.36 10^9/L, PLT 277 10^9/L Hb 8.3 g/dL, ESR 76 mm/hour, CRP 4.61 mg/dL, ferritin 866 ng/dL, triglycerides 419 mg/dL, albumin 1.98 g/dL	Nasopharyngeal swab: negative Serological test: negative	15 days	Complete recovery
Patient 6 (female, Asiatic)	2 years, 12 kg, no comorbidities	5 days fever (T>38°C), febrile seizures, rash, conjunctivitis, cheilitis, palm-plantar oedema lymphadenopathy, arthritis	IVIG (2 courses), aspirin and intravenous antibiotics	Normal echocardiography, pneumonitis at chest XR, hydrops of the gallbladder at abdominal US	WCC 12.60 10^9/L, ESR 59 mm/hour, CRP 26.02 mg/dL, ALT 485 UI/L, AST 536 UI/L, fibrinogen 924 mg/dL, ferritin 227 ng/mL	Nasopharyngeal swab: negative Serological test: negative	11 days	Complete recovery
Patient 7 (male, Caucasian)	1.5 years, 10.4 kg, no comorbidities	5 days fever (T>38°C), rash, conjunctivitis, cheilitis	IVIG, aspirin and intravenous antibiotics	Normal echocardiography	WCC 17.28 10^9/L, ESR 31 mm/hour, Hb 9.8 g/dL CRP 5.96 mg/dL	Not performed	10 days	Complete recovery
Patient 8 (female, Caucasian)	4 years, 15.5 kg, no comorbidities	5 days fever (T>38°C), rash, conjunctivitis, cheilitis lymphadenopathy	IVIG and aspirin	Normal echocardiography, hydrops of the gallbladder at abdominal US	WCC 19.90 10^9/L, ESR 120 mm/hour, CRP 25.72 mg/dL, fibrinogen 620 mg/dL, ferritin 183 ng/mL	Nasopharyngeal swab: negative Serological test: not performed	9 days	Complete recovery
Patient 9 (male, Hispanic)	4 years, 16 kg, no comorbidities	5 days fever (T>38°C), rash, conjunctivitis, cheilitis, palm-plantar oedema, lymphadenopathy	IVIG and aspirin	Normal echocardiography, hydrops of the gallbladder at abdominal US	WCC 21.36 10^9/L, ESR 120 mm/hour, CRP 12.00 mg/dL, fibrinogen 566 mg/dL, ALT 123 UI/L	Nasopharyngeal swab: negative Serological test: negative	16 days	Complete recovery
Patient 10 (female, Hispanic)	4 years, 15 kg, no comorbidities	5 days fever (T>38°C), rash, conjunctivitis, cheilitis lymphadenopathy	IVIG and aspirin	Normal echocardiography	WCC 21.29 10^9/L, ESR 89 mm/hour, CRP 9.16 mg/dL, fibrinogen 709 mg/dL, ALT 12 UI/L, AST 77 UI/L	Nasopharyngeal swab: negative Serological test: negative	13 days	Complete recovery

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; IVIG, intravenous immunoglobulins; L, lymphocytes; PLT, platelets; US, ultrasonography; WCC, white cell count; XR, radiography.

received steroid pulses and additional three biological therapy. No significant difference has been shown regarding the incidence/month (RR 1.21, 95% CI 0.60 to 2.20), neither limiting the analysis to the 56 children with KD diagnosed during the same corresponding 5 months of the last 5 years: 2.2 versus 2.0 incidence/month (RR 0.89, 95% CI 0.42 to 1.69).

The KD incidence rate adjusted for the 3801 children hospitalised in Tuscany in the 2020 index 5 months resulted 0.26%.

No significant differences were detected among the principal KD outcomes during the COVID-19 time and in the last 5 years: incomplete KD 59 versus 2, $\chi^2=1.03$; KDSS 1 versus 0, $\chi^2=0.06$; MAS: 3 versus 1, $\chi^2=2.81$; coronary involvement 38 versus 0, $\chi^2=2.92$. The same results have been observed limiting the analysis to the corresponding index 5 months of the last 5 years (p=n.s, Fisher's exact test).

From 1 February to 30 June, 8,637 nasopharyngeal swabs have been performed to the Tuscan children admitted to the hospitals: 157 resulted positive for SARS CoV-2. Serological tests have been performed in 2100 children: 127 were positive for antibodies.

Although the 10,500 COVID-19 Tuscany positive cases represent the fifth Italian highest number, our region reported a lower prevalence of infection compared with other high-prevalence areas in the North of Italy.[2] This epidemiological context may explain the lack of patients with PIMS-TS. However, our survey, as previously reported in other cohorts,[1 3–6] provides epidemiological evidence that the clinical spectrum of PMIS-TS differs from classical KD: median age of our patients with KD was lower (3.5 years), gastrointestinal symptoms were absent, any myocarditis was reported and all patients presented a benign disease course, responsive to a single dose of intravenous immunoglobulins in most of cases. Additionally, no significant increase of KD cases has been documented.

The PIMS-TS may in some cases mimic KD at onset, even if its typical clinical manifestations are characterised by a greater framework of systemic inflammation and haemodynamic involvement. At this regard, Whittaker *et al* and Cheung *et al* reported that only 28% (21/75) of PMIS-TS children met the American Heart Association criteria for KD diagnosis.[3 4]

These clinical differences also lead to pathogenetic implications. Most of patients with PIMS-TS presented a low viral load at diagnosis and/or showed positivity to serological tests. The high rate of SARS CoV-2 IgG positivity, usually mirror of a past infection, seems to suggest a reactive immunological response to a previous viral infection rather to an acute one, that is, instead, traditionally assumed as potential causative trigger of KD.

These considerations pose the clinical question whether different treatment approaches, that is, the immunomodulating agents, may be preferable to that strategies, such as intravenous immunoglobulins, that evidenced benefits in KD.

In conclusion, the epidemiology COVASAKI survey showed a KD incidence rate during COVID-19 pandemic identical to what previously reported in Tuscany along with clinical characteristics of typical KD picture.[7]

The well-structured collaboration of our PTN has ensured a prompt recognition of children with suspected KD, thus avoiding diagnostic and treatment delay. Keeping updated our register, a comparison between the COVASAKI survey and the worldwide results will better define the multifaceted nature of the paediatric COVID-19 disease and, if any, the potential relationship between PIMS-TS and KD.

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Response to: 'Correspondence on 'Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID19): a multicentre cohort" by Mastrolia *et al*

We thank Mastrolia *et al* for sharing the paediatric tuscany network (PTN) experience during the first wave of SARS-CoV-2 and their survey to detect the incidence of paediatric inflammatory multisystem syndrome temporally associated with SARS-COV-2 (PIMS-TS) and the rise of Kawasaki disease (KD).[1] Interestingly, PTN did not observe any increase in the number of cases of KD and no cases of PIMS-TS in their region between 1 February to 30 June 2020, unlike the many cases described across Europe, North America and Africa.[2–9] We believe that several points should be discussed to understand these findings.

First, as notified by our colleagues from PTN, Tuscany has been relatively preserved by the SARS-CoV-2 epidemic compared with the northern regions of Italy. Thus, on 30 June 2020 at the end of this survey, the Italian Ministry of Health[10] reported 93 901 cases of COVID-19 in Lombardy, where cases of PIMS-TS were described,[3] compared with 10 250 cases in Tuscany. An epidemiological study of PIMS-TS cases was conducted throughout France and 108 cases of PIMS-TS were reported.[8] According to the geographical distribution of PIMS-TS in France, many regions with lower incidences of SARS-CoV-2 epidemic were not affected with PIMS-TS case. The large majority occurred in the Great Paris region, one of the most affected COVID-19 areas. In this study, Belot *et al* estimated that the risk of PIMS-TS would be very low: less than 2 per 10 000 children. These data suggest that the epidemic might have been too weak in Tuscany to observe PIMS-TS cases, despite a highly efficient surveillance network.

Second, the hypothesis of a mutation of the virus during the epidemic could be discussed to explain the absence of PIMS-TS. However, the viral strain found in France seems to have remained the same as the one reported in Italy. The D614G spike protein mutation seems to have appeared mid-January 2020 in Europe, before the PIMS-TS epidemic, and currently seems to be the main strain worldwide.[11]

Third, different inborn errors of immunity may also predispose individuals for severe SARS-CoV-2 infection or PIMS-TS, according to the specific susceptibility or the inappropriate inflammatory activation they respectively convey. Indeed, Zhang *et al* raise the hypothesis of monogenic immunodeficiencies that may explain some severe COVID-19 cases, particularly in young patients with no comorbidities.[12] Several models of Mendelian susceptibility to viral infections have been indeed already described, such as mutations affecting the type I interferon pathway inducing herpes simplex virus encephalitis or severe influenza.[13 14] Moreover, monogenic autoinflammatory diseases are defined by Mendelian hyperactivation of several innate immune pathways (inflammasomopathies, type I interferonopathies).[15] Susceptible individuals with specific genetic background might be predisposed to such hyperinflammatory cytokine storm temporally associated to SARS-CoV-2 exposure.

Several studies have revealed that many patients affected with PIMS-TS were of Afro-Caribbean descent: 38%–62% in European cohorts.[2 5 7] Conversely, no cases have been described in China, the epicentre of the epidemic, even though the same phenomenon has been reported in Africa and the USA.[4 9] These observations remain to be confirmed by studies accounting for social environment factors, which may also play a role in these findings.[13] Nevertheless, if some populations are less predisposed to this specific condition, this may also explain why some regions did not report PIMS-TS cases.

Finally, these findings underline the need to further investigate epidemiological, immunological and genetic factors that may be associated with PIMS-TS. Deciphering its pathophysiology may help to better understand the discrepancy in the geographical distribution of this rare but severe condition.

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Correspondence on 'Lung involvement in macrophage activation syndrome and severe COVID-19: results from a cross-sectional study to assess clinical, laboratory and artificial intelligence–radiological differences' by Ruscitti *et al*

Our research team read Ruscitti *et al*'s article regarding lung involvement in coronavirus disease 2019 (COVID-19) and macrophage activation syndrome (MAS) with great interest.[1] This topic not only addresses the current pandemic, but also is of concern in our team's expertise—rheumatology. As we thoroughly examined the details of this research study, we noticed a few points we would like to address and discuss: the definition of patient selection criteria, the necessity of differentiating among COVID-19 CT patterns, the pathogenic mechanism differences between MAS and COVID-19, and the potential relationship between COVID-19 and vasculopathy.

First of all, this article states that age matching is not reliable. However, we contemplate that age is crucial in disease pathogenesis, such as immunosenescence, age-related inflammatory disease and periodontal disease, all of which could contribute to COVID-19 and MAS. Thus, "age" should still be an important factor to be considered and controlled as a confounding variable.[2 3] In addition, we doubt about the accuracy of diagnosis based on Yamaguchi criteria, as they simply serve as preliminary criteria for adult-onset Still's disease (AOSD),[4] which may led to misclassification. Furthermore, this article reports differences in H-scores between patients with MAS and COVID-19. If original diagnosis of MAS is preliminary, crosschecking with differential diagnoses may be preferable, especially through CT imaging.

Dai and Zhang reported cases from China in January 2020 that unenhanced CT scans showed (1) lateral basal ground-glass opacities (GGOs), (2) interlobular septal thickening with interlobar pleural thickening, and (3) patchy and partially consolidated lung tissues that gradually developed into reticular patterns and lesions.[5] Another study by Ye *et al* demonstrates chest CT manifestations of COVID-19 with disease progression. Initial degeneration of lung tissues originates with GGO in unilateral or bilateral lower lobes. As the hazing continuously develops, alveolar air starts being substituted by abnormal fluids, resulting in consolidation. Once consolidation could no longer be contained in a certain area of the lung, reticular patterns, namely, interlobular septa and intralobular lines, begin developing and are manifested by lesions. Consequently, thickening of such abnormality could demonstrate crazy paving patterns.[6] By close examination of CT scans from patients with MAS and COVID-19 from this paper and other previous studies, we could differentiate the two diseases prior to further comparison and contrast, thus improving current clinical practice.

Another area of our interest is the pathogenic mechanism differences between MAS and COVID-19, especially from the cytokine storm point of view. MAS inflammatory infiltrate was originally discovered to consist predominately of CD4 T cells and macrophages. Hence, scientists classified MAS as a secondary form of haemophagocytic lymphohistiocytosis.[7] Investigation into animal models of MAS reports that IL-6 overproduction results in autoinflammation or autoimmunity and suggests that interferon (IFN)-γ level is exceptionally elevated in experimental knockout mice. Since IFN-γ is a crucial cytokine that activates macrophages, we could infer that the primary cause of cytokine storm in MAS is IFN-γ.[7] On the other hand, for COVID-19, while IL-6 is continuously secreted by patients, high levels of other cytokines, such as IL-1β, TNF-α, CCL-2, CCL-3 and CCL-5, are also detected. However, the decisive difference between the two diseases is low levels of type I IFNs, an important factor for viral clearance, which may potentially be used as a differential diagnosis in the future.[8 9]

In continuation, our last point of comment is the correlation between COVID-19 and vasculopathy. Mondal *et al*'s research team stated that with an elevated level of IL-1β, cell pyroptosis, an inflammatory form of apoptosis, is indicated in the lymphocyte and macrophage cascade, leading to vasculopathy similarly to the previous studies of coronavirus family (severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus).[10] COVID-19 has an inhibiting effect on human haeme synthesis, which may trigger haemolysis, causing oxidative stress and leading to endothelial vasculature damage. With further evidence that shows a positive correlation between IL-6 and fibrinogen, this would raise the possibility of thrombosis in patients with COVID-19.[10] Another case series with regard to patients with COVID-19 originated from the intensive care unit (ICU) in Northern France drew our team's attention: 22 patients experienced pulmonary embolism (PE) among the first 107 confirmed COVID-19 cases, a statistically significant increase in comparison to other ICU patients. More shockingly, 20 of the 22 patients were already under prophylactic heparin at the time of PE diagnosis.[11] This wound lead to the fact that COVID-19 indeed has a positive correlation with vasculopathy.

In conclusion, the exact pathophysiology and mechanism of COVID-19 to lung and autoimmune injury are not well known yet. Ruscitti *et al* compare and contrast with MAS, opening up further discussions with various perspectives. We would like to contribute our share of knowledge to clinical science to improve patients' welfare.

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Response to: 'Correspondence on 'Lung involvement in macrophage activation syndrome and severe COVID-19: results from a cross-sectional study to assess clinical, laboratory and artificial intelligence–radiological differences' by Ruscitti *et al*' by Chen *et al*

Dear Editor,

We read with interest the correspondence by Chen *et al*[1] about our recent article on the comparison of clinical, laboratory and artificial intelligence–radiological findings in patients with lung involvement either from macrophage activation syndrome (MAS), a secondary form of haemophagocytic lymphohistiocytosis (HLH) or severe coronavirus disease 2019 (COVID-19).[2]

Age is one of the most common confounding factors in any observational study since it is associated with an increased risk of comorbidities, which may influence the outcome. In our study, the matching for age was not reliable because of higher prevalence of severe COVID-19 in elderly patients, who were admitted to intensive or subintensive care units of our hospital. These results mirror what was already observed in other observational studies,[3 4] in which the incidence and severity of COVID-19 are generally higher in elderly patients due to higher frequency of comorbidities, increased frailty and immunosenescence.[5] Conversely, MAS complicating adult-onset Still's disease (AOSD), as patients assessed in our study, usually affects young adults.[6] Considering the scientific debate behind our study,[2] about the possibility that severe COVID-19 could be considered or not part of HLH spectrum, the age of occurrence may further differentiate the clinical pictures between these diseases.

Furthermore, Chen *et al*[1] questioned the use of Yamaguchi criteria in classifying AOSD patients .[7] Although the classification criteria of AOSD may be considered an accessory part of our study, which evaluated patients with a fully developed MAS, we would like to point out that these criteria are widely used in the context of clinical research. Yamaguchi *et al* derived a set of major and minor criteria by a multicentre survey involving 90 AOSD patients and 267 controls.[7] Requiring five or more criteria, with at least two major ones, these provide 96.2% sensitivity and 92.1% specificity.[8] In addition, such criteria have been used as inclusion criteria in recent clinical trials on AOSD.[9–11]

Instrumental assessment by chest CT represents a key point for the staging and follow-up process in both COVID-19 pneumonia and MAS lung involvement. During the recent COVID-19 outbreak, CT examinations, due to the wide availability and rapid execution, also played a crucial role in the screening of many patients, along with the clinical laboratory data.[12] Several studies considered CT findings as a primary tool for the detection of COVID-19 in epidemic areas to optimise patient management, due to the high sensitivity and the important prognostic value.[13] In particular, the most typical findings in COVID-19 pneumonia, especially at the onset when most patients are examined, are represented by the presence of ground-glass opacities (GGOs) with bilateral peripheral localisation.[14] In our study, standard analysis of CT findings showed that peripheral, basal and bilateral GGOs were the most frequent findings in COVID-19 pneumonia when compared with MAS lung involvement. Nevertheless, CT findings of COVID-19 show overlapping features with other viral pneumonia (influenza and parainfluenza viruses, adenovirus and respiratory syncytial virus), as well as with other pulmonary conditions, such as pulmonary oedema, pulmonary haemorrhage, bronchiolitis obliterans and drug-induced lung disease.[15] Due to the cross-sectional design of our study,[2] we did not evaluate the evolution of radiological findings over time, but we assessed COVID-19 patients in the most severe phases, which led to the admission of the patients to intensive or subintensive care units, before the administration of any immunosuppressive therapy. In this context, a further analysis using dedicated artificial intelligence software could be an added value for a proper quantitative evaluation, crucial in disease staging, for quantitative correlation with clinical laboratory data at the time of diagnosis and during follow-up.[16]

As far as pathogenetic mechanisms of both diseases are concerned, it has been suggested that MAS and severe COVID-19 may share a similar cytokine profile.[17] As described in patients affected by MAS or primary HLH,[18] severe COVID-19 patients are burdened by a cytokine storm syndrome, a virally induced one, associated with a large release of proinflammatory cytokines.[5 17] According to Chen *et al*,[1] low levels of type I interferons (IFNs) could be a crucial pathogenic difference between MAS and severe COVID-19. This is an interesting topic deserving further investigations, probably able to differentiate these diseases, since a specific phenotype was observed in severe COVID-19 patients, consisting of no IFN-β and low IFN-α production and activity, associated with a persistent blood viral load and exacerbated proinflammatory response.[19]

Chen *et al*[1] also pointed out that COVID-19 may be associated with a relevant cardiovascular involvement, including myocardial injury, arrhythmias, acute coronary syndrome and thromboembolism.[20] Although we focused our interest on the differences concerning radiological findings and some laboratory biomarkers,[2] this is a relevant point, which should be addressed in the future. In any case, there are some important features differentiating the specific cardiovascular involvement in COVID-19 from what has been observed in MAS. In fact, during COVID-19, after having directly been infected by SARS-COV-2, the endothelial cells could attract proinflammatory cells, which lead to an endotheliitis and drive endothelial cell death.[20] Conversely, the cardiovascular involvement in MAS is usually associated with the multiple organ dysfunction syndrome, the leading cause of death of more severe patients.

In conclusion, despite overlapping clinical features, some differences could be recognised comparing patients with lung involvement either with MAS or severe COVID-19. Additional studies are needed to entirely elucidate these issues, furtherly investigating differences between these diseases, from a clinical as well as a pathogenic point of view.

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Correspondence on 'Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry' by Gianfrancesco *et al*. Disease activity, rather than glucocorticoid therapy, may be associated with COVID-19 severity in patients with rheumatic musculoskeletal diseases

The COVID-19 Global Rheumatology Alliance physician-reported registry has provided data on 600 individuals with rheumatic musculoskeletal disease (RMD) and COVID-19,[1] 277 (46%) of whom were hospitalised. The study was not powered to explore the association between disease activity and hospitalisation status; still, it was deemed to be non-significant (p=0.49). However, only 18% of patients had moderate and just 2% high disease activity. Therefore, the relationship between disease activity and COVID-19 severity deserves further investigation.

We collected clinical data of patients with RMD older than 18 years who reported a hospital admission for COVID-19 between 15 April and 15 June 2020. We retrieved 11 out of 1974 patients with RMD followed up in our rheumatology unit (0.55%) who tested positive for SARS-CoV-2 with real-time reverse transcription PCR analysis in the nasopharyngeal swab. We compared five patients with active versus six with remission disease status defined according to (1) persistency of signs or symptoms due to RMD for >50% of the time in the 3 months' prior hospital admission plus (2) laboratory or imaging abnormalities typical of disease activity or (3) escalation of treatment for the RMD (increase in the dose of immunosuppressive treatment, adding a drug or glucocorticoid for at least 30 days) (table 1).

All patients had established RMD including rheumatoid arthritis (n=3), rheumatoid arthritis and Sjogren syndrome (n=1), psoriatic arthritis (n=4), systemic sclerosis (n=1), spondyloarthritis (n=1) and microscopic polyangiitis (n=1). No patient in both groups was on prednisone>10 mg/daily (mean dose 2.5±2.5 vs 1.7±2.5 mg/daily), and 6/11 (40% vs 67%) patients were off-steroid; 2/5 (40%) were on biological disease-modifying antirheumatic drug (bDMARD) therapy in the active group vs 4/6 (67%) in the remission group. Chest radiographs showed more frequently interstitial infiltrates in the active RMD group, who were more frequently smokers; however, when chest CT was performed in 3/5, ground-glass opacities were found in all. The former group of patients had lower values at the admission of blood oxygen (pO_2 59±32 vs 74±28 mm Hg) than those in remission. ECG abnormalities were found more often in the active RMD group as well. The active RMD group had higher levels of C reactive protein (89±102 vs 44±32 mg/L), procalcitonin (1.9±2.0 vs 0.2±0.2 μg/L), ferritin (2019±1542 477±247 μg/L), D-dimer (1.7±1.7 vs 0.4±0.2 mg/L), lactate dehydrogenase (422±81 vs 265±66 IU/L), creatine kinase (188±185 vs 55±31 IU/L) and reduced estimated glomerular filtration rate (49±40 vs 78±26 mL/min/1.73 m^2). Modified Early Warning Score of ≥5 was found in 40% vs 16%. Similar rates between the two groups were found in terms of requirement of oxygen supplementation (100% vs 83%), mechanical or high-flow oxygenation (no patient in both groups), antibiotics, lopinavir/ritonavir and hydroxychloroquine use. One patient died in the active disease group, while the remaining had a comparable time to discharge (17±7 vs 15±5 days).

Table 1 Clinical characteristics of patients with rheumatic musculoskeletal disease according to their disease status

Clinical characteristics	Active disease (n=5)	Remission (n=6)
Age (years)	61±10	59±12
Female sex, n (%)	3 (60)	2 (33)
Body mass index (kg/m^2)	26±8	24±4
Ever used tobacco, n (%)	2 (40)	1 (16)
Disease duration (years)	10±11	13±10
Cardiovascular disease, n (%)	2 (40)	4 (67)
Biologic DMARD, n (%)	2 (40)	4 (67)
Conventional synthetic DMARD, n (%)	3 (60)	1 (16)
Glucocorticoid therapy, n (%)	3 (60)	2 (33)
Interstitial infiltrates, n (%)	4 (80)	2 (33)
Abnormal ECG, n (%)	4 (80)	2 (33)
Respiratory rate ≥22, n (%)	3 (60)	3 (50)
Heart rate ≥100 beat/min, n (%)	3 (60)	0 (0)
Temperature at admission (°C)	37.9 (0.9)	37.3 (1.0)

Continuous data are reported as mean±SDs.
DMARD, disease-modifying antirheumatic drug; ECG, electrocardiography.;à

In our series, patients with active RMDs on low-dose prednisone (< 10 mg/daily) and hospitalised for COVID-19 appeared to have a more severe systemic inflammatory response to SARS-CoV2 compared with those in remission. The key event in the infection of SARS-CoV-2 is the invasion of human tissues through the ACE 2 receptor expressed on the surface of alveolar epithelial cells and other target cells. Older individuals, especially those with hypertension and diabetes, have reduced ACE2 expression and upregulation of angiotensin II proinflammatory signalling.[2] Patients with RMD have reduced expression of ACE2 due to ageing, multiple comorbidities and autoantibodies.[3] Gianfrancesco and colleagues reported higher odds of hospitalisation with glucocorticoid therapy at prednisone-equivalent doses of ≥10 mg/day compared with no glucocorticoid therapy (OR=2.05, 95% CI 1.06 to 3.96; p=0.03). Noteworthy, systemic glucocorticoid therapy is not known to induce ACE2 receptor expression, but it can upregulate angiotensin II proinflammatory signalling and cause hypertension in a dose-dependent manner.[4] However, glucocorticoid therapy is also associated with active or poorly controlled RMD.[5 6] Although the authors confirmed the association between hospitalisation rates and glucocorticoid therapy after correction for disease activity (data not shown), the proportion of participants with moderate to high disease activity in the group taking prednisone-equivalent doses of ≥10 mg/day was not provided. Hence, one cannot exclude that patients with high levels of disease activity were treated with high doses of glucocorticoids for disease control, given the relatively small number of patients at prednisone-equivalent doses of ≥10 mg/day in each group (21/323 patients and 43/277 patients in the non-hospitalised and hospitalised groups, respectively).

In conclusion, we could not replicate the results by Gianfrancesco *et al*. Whether glucocorticoid therapy rather than disease activity is associated with COVID-19 severity in patients with RMD needs more robust data to be ascertained.

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Rheumatic disease activity, glucocorticoid use and COVID-19. Response to: 'Correspondence on 'Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry' by Gianfrancesco *et al*. Disease activity, rather than glucocorticoid therapy, may be associated with COVID-19 severity in patients with rheumatic musculoskeletal diseases' by Giollo *et al*

We thank Giollo and colleagues for their correspondence acknowledging the challenges of understanding those risk factors which contribute to severe outcomes in patients with underlying rheumatic musculoskeletal diseases (RMDs) who acquire the novel SARS-CoV-2 infection.[1] In their short case series, they highlight the balance and relative importance between disease activity and dose of glucocorticoid therapy, two factors which are highly linked within an individual. It is already known that both disease activity and glucocorticoid dose are independent risk factors for serious infection in patients with RMD, such as rheumatoid arthritis and systemic lupus erythematosus,[2–4] as are age, the underlying RMD diagnosis itself and the presence of other comorbidities. Disentangling the individual contribution of any of these individual risk factors will require robust analysis of large and diverse patient datasets. The Global Rheumatology Alliance (GRA)[5] aims to capture cases of COVID-19 among patients with pre-existing RMD and is reliant on the nature and diversity of the individual cases reported in order to provide a greater understanding of individual risk. By leveraging such a large dataset, we are able to conduct multivariable analyses that control for a number of different factors to estimate the contribution of individual risk factors. Future analyses of the GRA and other large patient registries will hopefully provide further insight into individual risk factors for severe outcomes in this patient group, such that we can offer the most relevant evidence to our colleagues and patients.

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Correspondence to 'Comparative effectiveness of first-line tumour necrosis factor inhibitor versus non-tumour necrosis factor inhibitor biologics and targeted synthetic agents in patients with rheumatoid arthritis: results from a large US registry study'

We read with great interest the article by Pappas *et al*[1] who evaluated the comparative effectiveness of a tumour necrosis factor inhibitor (TNFi) such as adalimumab, etanercept, certolizumab pegol, golimumab or infliximab versus a non-TNFi (abatacept, tocilizumab, rituximab, anakinra or tofacitinib) as the first-line treatment in patients with rheumatoid arthritis (RA). The authors concluded that there were no statistically significant differences observed between the TNFi and non-TNFi treatment groups for the outcomes assessed, including the Clinical Disease Activity Index (CDAI), the 28-Joint Modified Disease Activity Score, the Health Assessment Questionnaire Disability Index, EuroQol-5 Dimension score, morning stiffness and fatigue. In this observational study, data collected from 1 October 2001 to 31 January 2018 within a large US healthcare registry were evaluated, and 4816 patients who had a non-remission CDAI score at baseline were selected for further assessments. We would like to draw attention to some important points in this study.

First, the two key clinical outcomes in this study were 'achievement of low disease activity (CDAI≤10) among those with moderate or high baseline disease activity at baseline' and 'achievement of remission (CDAI≤2.8) among those with low, moderate (10<CDAI≤22) or high (CDAI>22) disease activity at baseline'. Although there was no statistically significant difference in the mean CDAI scores between the TNFi and non-TNFi treatment groups at baseline, it did not mean that the proportion of patients with moderate or high disease activity at baseline in the two groups was not significant. We think it could be a key effect modifier of the treatment outcome and should be adjusted in advance.

Second, the therapeutic effect of the different treatments used in patients with RA varies depending on whether conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) are used alone or in combination. The American College of Rheumatology (ACR) guidelines[2] for patients with RA recommends a treat-to-target approach that is guided by disease stage and treatment history. At baseline, there was no statistically significant difference in the proportion of patients receiving concomitant csDMARDs between the two groups. However, the proportions of patients receiving csDMARDs as monotherapy, dual therapy or triple therapy in the two groups during the 1-year follow-up visit were not specified in the study. Additionally, residual confounders, such as smoking status, baseline haemoglobin levels and dosage of the csDMARDs used, could exist.

Third, the number of patients on anakinra treatment in the non-TNFi group was only 14. The sample size of this subgroup was too small to extrapolate conclusions with statistical confidence in a study where the total number of patients was in the thousands. Considering its lower clinical efficacy than that of other biologics and the lack of long-term observational studies, most rheumatologists only prescribe anakinra for patients with RA who are intolerant to TNF-α inhibitors.[3 4] Anakinra was not included in the 2015 ACR guideline[2] for the treatment of RA because of insufficient data.

Finally, in the study design section, the sentence 'Patients who did have a non-remission CDAI score … were excluded from analysis' should be revised to 'Patients who did not have a non-remission CDAI …'. In figure 2, the term 'Non-TNFi' stated in the lower-left corner should be changed to 'Favours TNFi'.

Above all, we appreciate this real-world study that compared the effectiveness of individual TNFi and non-TNFi treatments in patients with RA. We recommend that in the proportion of patients with moderate or high disease activity and csDMARDs monotherapy, dual therapy or triple therapy, some residual confounders should be matched to ensure balanced baseline comparability.

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Response to 'Correspondence on 'Comparative effectiveness of first-line tumour necrosis factor inhibitor versus non-tumour necrosis factor inhibitor biologics and targeted synthetic agents in patients with rheumatoid arthritis: results from a large US registry study" by Zheng *et al*

We thank Zheng *et al*[1] for their interest in our study[2] and for providing constructive comments. They mention that even though the baseline mean Clinical Disease Activity Index (CDAI) was not statistically different, the proportion of patients with moderate or high disease at baseline was different and this could have been a key effect modifier.[1]

We used standardised differences to evaluate variables at baseline. Calculation of the standardised difference not only accounts for the means of the two cohorts, but also the SD. We noticed that continuous CDAI was balanced across the two therapy classes (as listed in the manuscript table 1, SD=−0.015)[2] and similarly observed for categorical CDAI (low/moderate/high; SD=0.0515; results not shown in the manuscript). Furthermore, in the postmatched population, continuous CDAI remained well balanced (manuscript table 1: SD=−0.0256)[2] as did the categorical CDAI measure (SD=0.0658; results not shown). Thus, we do not believe that baseline CDAI distribution could have influenced the observed results.

Zheng *et al* further point out that the proportions of patients receiving conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) as monotherapy, dual therapy or triple therapy in the two groups during the 1-year follow-up visit were not specified and that residual confounders, such as smoking status, baseline haemoglobin levels and dosage of csDMARDs used, could exist.[1]

In our study, coexisting csDMARD use was considered only at baseline, but not during follow-up.[2] Prior csDMARD use was also evaluated. Changes in concurrent DMARD therapy over the follow-up period were not evaluated. This is a limitation of the study, but the findings of a non-significant difference could be interpreted in the context that rheumatologists could modify concurrent therapy in real-life study settings. The study allows for changes in therapy and does not claim that final results were not statistically different with stable concurrent therapy. Smoking and baseline anaemia were considered as effect modifiers and were not found to be significantly associated with the outcome as described in the 'Determination of effect modifiers' section of the Methods.[2]

Although uncommon, some rheumatologists prescribe anakinra. We believe that the small number of patients on anakinra (14 patients among thousands in the 2 cohorts) did not affect the findings and would not alter the results if removed from the analysis.

Thank you for pointing out the mistakes and typos, we will work with the journal to proceed with a correction.

Dimitrios A Pappas ,[1,2] **Gregory St John,**[3] **Carol J Etzel,**[2] **Stefano Fiore,**[4] **Taylor Blachley,**[2] **Toshio Kimura,**[5] **Rajeshwari Punekar,**[6] **Kelechi Emeanuru,**[2] **Jeannie Choi,**[7] **Susan Boklage,**[3] **Joel M Kremer**[2]

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Correspondence on 'Effectiveness of secukinumab versus an alternative TNF inhibitor in patients with axial spondyloarthritis previously exposed to TNF inhibitors in the Swiss Clinical Quality Management cohort' by Micheroli *et al*

We read with great interest the article by Micheroli *et al*[1] for comparison of effectiveness between secukinumab (SEC) and tumour necrosis factor inhibitor (TNFi) in axial spondylitis (axSpA), with evidence conform to the current recommendation in a Swiss real-world setting.

According to current guidelines,[2 3 4]interleukin-17 inhibitor (IL-17i) or alternative TNFi are both recommended as the switch option of biological disease-modifying antirheumatic drug in patients with previous TNFi failure. Although head-to-head comparison clinical trials between IL-17 inhibition and TNF blockade have been conducted in psoriatic arthritis recently[5 6] and is ongoing in axSpA, we appreciated Micheroli *et al*[1] for their new information in this real-world indirect comparison study. However, some issues can be further discussed.

First, regarding the study design, it was not mentioned whether treatment adjustment was due to drug inefficacy or intolerability and the types of TNFi, receptor fusion protein or monoclonal antibody were unclear in the study. Since the administration time of SEC in the market was relatively short, subgroup analysis might be inconclusive due to underpowered sample size.

Second, there are huge difference in baseline clinical features between two groups. At baseline, patients in the SEC group had higher disease activity indexes including Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Ankylosing Spondylitis Disease Activity Score (ASDAS), poorer physician and patient global assessments, higher C-reactive protein (CRP) as well as worse Bath Ankylosing Spondylitis Functional Index (BASFI) and Bath Ankylosing Spondylitis Metrology Index (BASMI), and even higher health-related index of EQ-5D:Euro-Qol-5 Dimension (EQ-5D), compared with those in the TNFi group. Although authors had done propensity score matching and multiple regression models to adjust this baseline incomparability, residual confounders existed, for example, comorbidities should be adjusted or stratified. This confounding by indication may explain the high infection rate in the SEC group. Physicians may tend to choose SEC in high infection risk patients.

Third, for effectiveness, the primary outcome in this study was drug retention rate at year 1. The secondary outcome was to assess the proportion of patients reaching 50% reduction in the BASDAI (BASDAI50) at 1 year. We agreed that drug retention rate is a good endpoint for composite effectiveness and safety. But we suggest that ASAS20, ASAS40 and ASDAS-CRP would be more suitable endpoints to be presented to compare with existing literatures. Furthermore, previous studies showing that ASAS20 and ASAS40 were both higher in patients treated with ixekizumab (IXE) in COAST-V[7] study than in those in COAST-W[8] study, implicating the superiority of IXE in patients with TNFi naive over TNFi failure. We suggest that authors should discuss on this inconsistency.

Fourth, for the safety aspects, 18.4% to 27.4% of patients discontinued treatment due to the adverse events. Other adverse events including headache, generalised peripheral pain and acne conglobata were much higher in the TNFi group then in the SEC group. These non-specific adverse events might attribute to a wider and more potent proinflammatory effect of TNF than IL-17. Infection seemed to happen more frequently in patients treated with SEC. Among them, even one severe infection requiring hospitalisation occurred with unclear infection site and pathogen. Recurrence of breast cancer was identified in one patient treated with TNFi, which was considered possibly unrelated and inconclusive to the treatment from our point of view. For inflammatory bowel disease, conclusion was unfavourable and consistent with former randomised controlled trials (RCTs).[9–11] No uveitis developed in patients treated with SEC, which was inconsistent with former RCTs and required further investigation. Paradoxical psoriasis happened in two patients treated with SEC. It would be of great value to clarify the underlying mechanism for psoriasis pathogenesis despite IL-17i treatment.

Finally, for the reason of discontinuation, the percentage of ineffectiveness was comparable between TNFi and SEC (60.3% vs 58.1%), implying that over 50% of patients experienced unfavourable response to both drugs. We agree that there is still an unmet need for the treatment of the axSpA population. More therapeutic targets are required to improve the present status of treatment.

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Response to: 'Correspondence on 'Effectiveness of secukinumab versus an alternative TNF inhibitor in patients with axial spondyloarthritis previously exposed to TNF inhibitors in the Swiss Clinical Quality Management cohort' by Micheroli *et al*' by Huang *et al*

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We would like to thank Huang *et al*[1] for their interest in our study[2] and the opportunity to discuss some aspects in more detail.

The limited sample size precluded subgroup analyses with regard to either the type of tumour necrosis factor inhibitor (TNFi) agent or the reason for discontinuation of the previous biologic disease-modifying antirheumatic drug (bDMARD). Moreover, drug discontinuation might be due to a combination of reasons (eg, only partial effectiveness combined with otherwise acceptable adverse events (AE)) further impeding this type of analysis in a real-world setting and requiring the definition of a hierarchy of reasons for discontinuation as introduced in a previous study.[3]

We have used two different statistical methods to account for potential confounding by indication. A total of 16 covariates were incorporated in both propensity score-based analyses as well as covariate adjustment, with a particular focus in avoiding collinearity between variables. Given the observational nature of our study, we cannot entirely exclude residual confounding. However, the numerical higher rate of infection in patients treated with secukinumab (SEC) should not be interpreted as an indication of residual confounding, as the exact nature of AE leading to drug discontinuation was not known in 47% of patients.

The choice of our outcomes was informed by current international treatment recommendations in real-world settings.[4] We have preferred a reduction in the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) by 50% over the achievement of the 20% and 40% improvement criteria defined by the Assessment of Spondylo Arthritis international Society (ASAS20 and ASAS40, respectively), as it is more intuitive to assess response in day-to-day clinical practice. According to guidelines, continuing a bDMARD in axial spondyloarthritis (axSpA) should be considered if after at least 12 weeks of treatment a BASDAI improvement of at least 2 points or an improvement in the Ankylosing Spondylitis Disease Activity Score (ASDAS) of at least 1.1 points is achieved.[4] Given their clinical relevance, both outcomes were included in our study, as was the proportion of patients reaching an ASDAS <2.1. We do not recommend comparing endpoints between studies with different design, inclusion criteria and treatment in different calendar periods.

A superiority of interleukin-17 (IL-17) inhibitors in TNFi-naïve versus TNFi-experienced patients was indeed demonstrated for both SEC and ixekizumab.[5–7] There is no inconsistency with the results of our study, as the latter focused exclusively on TNFi-experienced patients.

Missing data precluded a formal comparison of specific AE rates between SEC and TNFi, as already mentioned. With regard to the two patients with paradoxical psoriasis during SEC treatment, this AE had already occurred during previous TNFi treatment in both patients and failed to improve during SEC. New onset of psoriasis induced by SEC in patients with axSpA has also been described and its pathogenesis remains largely unknown.[8 9]

A significant proportion of patients with axSpA did not respond adequately to both TNF and IL-17 inhibition and we agree with Huang *et al* that investigation of additional modes of treatment action is warranted.

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Correspondence on 'Systemic evaluation of the relationship between psoriasis, psoriatic arthritis and osteoporosis: observational and Mendelian randomisation study'

With great interest, I have read the article by Xia *et al*,[1] which evaluated the relationship between psoriasis, psoriatic arthritis (PsA) and osteoporosis. This Mendelian randomisation (MR) study suggests that the effect of PsA on osteoporosis is secondary, but not causal. However, it is important to discuss some methodological issues in MR. First, MR is a powerful tool for analysing causal relationships between exposures and outcomes because of minimisation of residual confounding.[2 3] However, MR is often vulnerable to bias resulting from pleiotropy.[2] Therefore, the use of a variety of robust methods working in diverse ways and relying on different assumptions has been recommended to derive valid inferences and assess the reliability of MR analyses.[4] In the MR-Egger test, the pleiotropic effects of single nucleotide polymorphisms (SNPs) on the outcome should be independent of the association between SNPs and exposure.[2] The weighted median method assumes that valid variants account for at least 50% of the total weight of the instrument.[5] The weighted median estimator has the benefit of preserving greater precision in the estimates, whereas the MR-Egger process results in loss of precision and power.[5] The authors should consider the weighted median method as a sensitivity analysis tool in this study. Second, patients with a low bone density at the calcaneus are at an increased risk of hip fracture. However, low bone density at the hip is a better predictor of hip fracture than bone density at other sites.[6] Similarly, bone density at the spine and hip measured using dual X-ray absorptiometry may be a more reliable predictor of spinal and hip fractures than bone density at other sites. In addition, the expected bone mineral density (BMD) is uncertain in most cases of osteoporosis diagnosed using ultrasonography.[7] Data on BMD measured using dual X-ray absorptiometry and fracture at the same site would give more accurate results. Third, it is necessary to determine the effect of disease-modifying antirheumatic drugs on bone metabolism as it is indicated in patients at a high risk of osteoporosis. The possible adverse effect of methotrexate (MTX), used for rheumatic diseases, on bone metabolism cannot be excluded in the study population despite controlling for factors such as concomitant therapies or disease type. However, the direct local effects of MTX on the bone must be weighed against the indirect anti-inflammatory effects of the drug.[8] Decreased systemic inflammation with MTX treatment in the study population appears to outweigh any direct local adverse effect that MTX may have on osteoblast and osteoclast function. Cohort and case–control studies have not demonstrated any substantial effects of MTX on BMD.[9] Many practitioners, including myself, do not believe that MTX treatment for rheumatic diseases has an adverse effect on bone density. It is imperative that the findings of this MR study be interpreted with caution considering the aforementioned methodological concerns.

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Response to: 'Correspondence on 'Systemic evaluation of the relationship between psoriasis, psoriatic arthritis and osteoporosis: observational and Mendelian randomisation study' by Lee

We appreciate Dr Young Ho Lee's interest in our study on the relationship between psoriatic arthritis (PsA) and osteoporosis,[1] and thank him for the comments brought in his letter.[2]

Over the past few years, several methods were developed to deal with the pleiotropic effect of instrumental single-nucleotide polymorphisms (SNPs) in Mendelian randomisation (MR) analysis, such as inverse variance-weighted (IVW), MR Egger, the weighted median,[3] weighted (simple) mode-based[4] and Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO).[5] However, the limitation of each method should be acknowledged.[6] The weighted median method estimated some SNPs as invalid instruments, but still kept at least half were valid instruments for the causal effect estimate to be unbiased. The advantage of this approach was the improved precision with reduced type 1 error compared with MR Egger, but less accuracy of IVW. In our study, we applied all the methods mentioned above, and the results complemented with each other that PsA had no causal effect on low bone mineral density (BMD).

However, in the large-scale observational study with UK biobank dataset, we found that PsA somehow associated with low BMD. Therefore, we started to think about how the inconsistent results between MR analysis and observational study could be explained. We speculated some secondary factors such as physical activity and medication treatments (methotrexate and ciclosporin) could rise this observational association, but this association was not genetically determined.[7] In the following conditional analysis and mediation analysis, we indeed observed that medication treatments might be the secondary factor causing the observational association. Therefore, we suggested that patients with PsA should be screened for BMD and proper management should be provided to reduce the fracture risk, especially for those who received treatment with methotrexate or ciclosporin. However, large-scale randomised controlled trial study was still needed to clarify the adverse effect of the methotrexate treatment. And we agreed that we should balance the treatment effect and the adverse effect of methotrexate.

In addition, we used quantitative ultrasound estimated BMD at heel as the outcome in our study. Although previous studies showed that quantitative ultrasound was also proven as a good predictor for the fracture risk,[8–11] BMD measured by dual-energy X-ray absorptiometry (DXA) was the golden standard in clinical practice. In UK biobank, only about ~5000 individuals had been measured by DXA; it is worth to check the association between PsA and BMD in the future if the DXA data are available for the ~500 000 individuals.

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Short duration antibiotic therapy for native joint arthritis caused by *Neisseria* infection?

We read with great interest the article "Two weeks versus four weeks of antibiotic therapy after surgical drainage for native joint bacterial arthritis: a prospective, randomised, non-inferiority trial" by Gjika *et al*.[1] As staff members of a French Regional Referral Centre for complex bone and joint infections, we want to share our experience with short-duration antibiotic treatment for native joint arthritis caused by *Neisseria gonorrhoeae* (Ng) and *Neisseria meningitidis* (Nm).

We conducted a retrospective study including all patients with arthritis caused by Gram-negative cocci treated in our institution from January 2018 to July 2020.

Ten patients were included (seven men, three women; median age 34 years; table 1). Most patients had monarthritis (n=6); knees were the most frequently affected joints (n=7). Fever was inconsistent (n=5). Blood culture analyses were performed for nine patients and were positive for three patients. All but two patients (because absence of joint fluid) underwent joint aspiration. Direct examination and bacterial culture were positive in five and six of eight patients, respectively. Diagnoses were made using PCR of synovial fluid in two patients (#7 and #8) because their bacterial culture were negative without explanation (in particular no prior antibiotic therapy), antibiotic sensitivity could not be assessed for them. Six patients were infected with Ng and four with Nm. Ng was always resistant to ciprofloxacin and exhibited intermediate sensitivity to penicillin G. Nm was always sensitive to amoxicillin with a minimal inhibitory

Table 1 Patients' characteristics

	Gender, age (years)	Infected joints	Microbiological diagnosis			Bacteria	Treatment			Clinical outcome at 2 months
			Blood culture	*Synovial fluid*	*Other*		*Antibiotic and duration*	*Surgery*	*Other*	
1	M, 47	Left thumb, ankles	Positive	ND	Oropharyngeal Ng positive PCR	*Neisseria gonorrhoeae*	Ceftriaxone IV, 2 g/day, 7 days	No	NSAIDs, 2 weeks	Complete resolution
2	F, 45	Right knee	ND	► Direct examination: positive (GNC) ► Culture: positive ► PCR: positive	Oropharyngeal, genital and anal Ng PCR positive	*Neisseria gonorrhoeae*	Ceftriaxone IV, 2 g/day, 7 days	No	No	Complete resolution
3	M, 54	Left knee	Negative	► Direct examination: positive (GNC) ► Culture: positive ► PCR: ND	No	*Neisseria gonorrhoeae*	Ceftriaxone IV, 2 g/day, 7 days	No	No	Complete resolution
4	M, 54	Left knee	Negative	► Direct examination: positive (GNC) ► Culture: positive ► PCR: ND	No	*Neisseria gonorrhoeae*	Ceftriaxone IV, 1 g/day, 7 days	Yes	Evacuation punctures before surgery and NSAIDs, 4 weeks	Complete resolution
5	F, 18	Left hip	Positive	► Direct examination: positive (GNC) ► Culture: positive ► PCR: positive	No	*Neisseria meningitidis* C	Cefotaxime IV 200 mg/kg/day, then amoxicillin IV, 200 mg/kg/day, 10 days	Yes	NSAIDs, 2 weeks	Complete resolution
6	M, 18	Knees, elbows, ankles, shoulders,	Positive	► Direct examination: ND ► Culture: positive ► PCR: positive	No	*Neisseria meningitidis* C	Ceftriaxone IV 4 g/day, then amoxicillin IV 200 mg/kg/day, 7 days	No	NSAIDs, 2 weeks	Complete resolution
7	M, 28	Right knee and ankle	Negative	► Direct examination: negative ► Culture: negative ► PCR: positive	No	*Neisseria meningitidis* C	Ceftriaxone IV 2 g/day, 7 days	Yes	NSAIDs, 2 weeks	Complete resolution
8	F, 24	Right knee	Negative	► Direct examination: negative ► Culture: negative ► PCR: positive	Positive oropharyngeal, genital and anal Ng PCR	*Neisseria gonorrhoeae*	Ceftriaxone IV 2 g/day, 7 days	No	NSAIDs, 2 weeks	Complete resolution
9	H, 39	Left knee, right foot, right ankle, right hand	Negative	ND	Positive oropharyngeal Ng PCR	*Neisseria gonorrhoeae*	Ceftriaxone IV 2 g/day, 10 days	No	NSAIDs, 2 weeks	In progress
10	H, 16	Left hip	Negative	► Direct examination: positive (GNC) ► Culture: positive ► PCR: positive	No	*Neisseria meningitidis* W	Ceftriaxone IV 2 g/day, then amoxicillin IV 200 mg/kg/day, 7 days	Yes	NSAIDs, 1 week	In progress

CRP, C-reactive protein; F, female; GNC, Gram negative cocci; IV, intravenous; M, male; MIC, minimum inhibitory concentration; ND, not done; Ng, *Neisseria gonorrhoeae*; Nm, *Neisseria meningitidis*; NSAID, non-steroidal anti-inflammatory drug.;

concentration (MIC) <0.125 mg/L. The most common treatment was ceftriaxone for 7 days (n=7). Two patients with Nm infections (#5 and #6) received first-line cefotaxime and ceftriaxone, respectively; after determination of the MICs, amoxicillin was used, thus yielding total treatment intervals of 7 and 10 days. Four patients required surgical drainage (#4, #5, #7 and #10). Concomitant non-steroidal anti-inflammatory drugs (NSAIDs) were used in eight patients (naproxen or ketoprofen), for reactive arthritis-like symptomatology, usually for 2 to 4 weeks. With a minimum of 2-month follow-up, outcomes were favourable for 8 out of 10 patients.

Most of native septic arthritis are caused by Gram-positive cocci;[2] Gram-negative cocci septic arthritis are rare and occur in 1% to 3% of affected patients.[3 4] While oligoarthritis or polyarthritis are reported as the most common clinical presentation in literature,[4 5] we observed a majority of patients with monarthritis, only one patient had oligoarthritis (#7) and three had polyarthritis (#1, #6 and #9). All patients exhibited arthritis in a large joint. The diagnosis of native arthritis can be made using blood cultures, direct examination and synovial fluid culture. Species-specific PCR can be performed to determine the presence of Ng in synovial fluid and specimens from other sites (eg, oropharyngeal, genital and rectal). Antibiotic susceptibility must be determined. Ng is typically resistant to penicillin and fluoroquinolones.[4] Nm is frequently sensitive to amoxicillin and third-generation cephalosporins.[6] The optimal therapy has not been established but our retrospective study supports the following hypothesis: ceftriaxone for Ng or amoxicillin for Nm (after determination of the MIC) for 7 days appears to be effective; surgical drainage is not required, except in patients for whom the infection cannot be controlled; meningococcal arthritis more frequently required surgery.

Overall, our study highlighted a completely different management of native joint arthritis due to Ng and Nm compared with others septic arthritis.[2] Since it is a rare condition with scarce literature, one can consider shorter antibiotics duration of 7 to 10 days (ceftriaxone 2 g/day for Ng and amoxicillin 100 to 200 mg/kg/day for Nm). Surgical drainage is not always mandatory. Finally, NSAIDs are often required and can be safely used in combination with antibiotics, if needed.

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Response to: 'Short duration antibiotic therapy for native joint arthritis cause by *Neisseria* infection?' by Durcours *et al*

We read with great interest the contribution of Ducours *et al*[1] to our article,[2] which randomised adult patients with native joint septic arthritis to either 2 or 4 weeks of systemic targeted antibiotic therapy after surgical drainage.[2] Ducours *et al* reveal a similar experience with a short duration of targeted systemic antibiotic therapy for adult native joint bacterial arthritis due to gonococci and meningococci.[1] There are substantial differences between our both studies: (1) Our arthritis episodes (majority hand arthritis) included all pyogenic bacteria, but not gonococci. In contrast, the Ducours group reports only *Neisseria* spp. (2) We randomised 154 cases, whereas Ducours *et al* resumed only 10 patients with mostly knee infections, although with a high proportion (30%) of bacteraemia. (3) Our minimal antibiotic treatment duration was 14 days (median 2 days of intravenous administration) compared with 7–10 days in the Ducours study (7 days of parenteral therapy).[2] (4) All our cases were surgically debrided, whereas most gonococcal cases were treated conservatively.

All these differences are explained by the nature of the pathogens: *Neisseria gonorrhoeae* or *N. meningitidis*. *Neisseria* spp classically require only a few days of targeted empirical therapy, when compared with other arthritis pathogens. This is a particularity of the pathogen, which is very (rapidly) susceptible to all appropriate antibiotic agents.[3] Indeed, native joint septic arthritis is a very heterogeneous group of clinical entities[4] with different epidemiological, microbiological and therapeutic aspects in humans.[5] Already in 2005, clinicians recommended a maximum therapy duration of 1 week with targeted antibiotics for disseminated gonococcal infection, including for arthritis.[6] The later US 2015 Sexually Transmitted Diseases guidelines reconfirmed this duration indicating the duration as 'for 7 days'.[7] Hence, the good results by Ducours *et al* are not surprising.

Nevertheless, although investigating the most easily treatable bacterial pathogen in septic arthritis,[4] Ducours *et al* underline the possibility of a short antibiotic treatment for septic arthritis,[5] even in bacteraemic cases.[1] This is important, because long parenteral antibiotic therapies (eg, 4 weeks) are still being reported for neisserial infections.[8] We therefore congratulate our colleagues for their study and encourage other researchers to perform prospective trials aiming at the optimisation of systemic antibiotic treatment for adult native joint septic arthritis.

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Correspondence on 'Changing the outcome measures, changing the results? The urgent need of a specific disease activity score to adult-onset Still's disease'

Our interest in correspondence of Ruscitti *et al* focusing on the analysis of the data of the multicentre double-blind randomised placebo-controlled trial assessing the efficacy and safety of canakinumab in patients with adult-onset Still's disease (AOSD) can be explained by the authors' astonishing assumption stating that the clinical trial by Kedor *et al* "… is a further example of how the absence of validated measures could impair the expected positive results, despite the strong scientific rationale."[1 2] Ruscitti *et al* seriously believe that changing the measurement units will change the results of the trial; therefore, a specific AOSD activity score is required immediately.[1] Kedor *et al* used the DAS28 to assess the disease activity, selecting patients with active joint involvement as previously performed by a number of researchers, including Ruscitti.[2–4] The primary endpoint was the proportion of patients with a clinically relevant reduction in disease activity at week 12 as determined by the change in disease activity score (ΔDAS28 >1.2). However, for some reason, Kedor *et al* did not compare this indicator with the current disease activity (DAS28) as recommended when evaluating the efficacy (good and moderate responses) of a treatment.[2 5] Remember that unlike the American College of Rheumatology improvement criteria, the EULAR response criteria include changes in disease activity as well as current disease activity.[5] High activity of disease was defined as a DAS28 >5.1. Low activity of disease was defined as a DAS28 <3.2. Good responders were patients with a significant change (ΔDAS28 >1.2) and low disease activity. Moderate responders were patients with a significant change and moderate/high disease activity or patients with a change <1.2 and >0.6 and low/moderate disease activity. Non-responders were the remaining patients.[5] If Kedor *et al* had taken this into consideration, the canakinumab efficacy in AOSD would have been different.[2] Furthermore, the following information is missing from the study by Kedor *et al*[2]:

1. The pattern of the clinical course of the disease:
   - The monocyclic (or self-limiting) pattern characterised by systemic symptoms occurring in a single episode of varying duration and subsequent complete remission.
   - The polycyclic or intermittent pattern characterised by two or more episodes of systemic symptoms, which are separated by clinical remission lasting at least 2 months.
   - The chronic articular pattern characterised by severe inflammation of joints, which can lead to joint destruction.
2. Systemic symptoms. The systemic score assigns 1 point to the following disease manifestations[6]: 1. Fever; 2. Rash; 3. Pleuritis; 4. Pneumonia; 5. Pericarditis; 6. Hepatomegaly or abnormal liver function tests; 7. Splenomegaly; 8. Lymphadenopathy; 9. Leucocytosis $\geq 15\times10^9$/L; 10. Sore throat; 11. Myalgias; 12. Abdominal pain.
3. Except for the name of the disease, the diagnosis does not specify
   - Clinical form: systemic (a monocyclic or polycyclic pattern) or a chronic articular pattern.
   - Activity based on the systemic score.
   - Refractoriness to administered medication therapy.
   - Radiology stage
   - Functional class (grades).
   - Complications.

In addition, the authors totally neglected the commonly accepted 'treat-to-target' recommendations that do not imply specific indicators for evaluation of the disease activity.

Apparently, everything listed earlier affected the results and "the study was terminated prematurely and the primary endpoint did not achieve statistical significance."[2] In the meantime, the published data support the treatment of patients with AOSD with canakinumab using 4 mg/kg body weight every 4 weeks.

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Response to: 'Correspondence on 'Changing the outcome measures, changing the results? The urgent need of a specific disease activity score to adult-onset Still's disease" by Muraviov and Muraviova

Dear Editor,

We read the correspondence by Muravion and Muravion[1] about our previous correspondence on a recent clinical trial investigating the efficacy of canakinumab on adult-onset Still's disease (AOSD)[2 3] with interest.

In this correspondence, Muravion and Muravion highlighted the role of disease activity score in 28 joints (DAS28) in assessing the disease activity in AOSD, also advocating American College of Rheumatology definitions of clinical response and treat-to-target recommendations.[1] This is relevant in the context of rheumatoid arthritis (RA). However, it is well recognised that AOSD is a different disease from RA, considering pathogenic mechanisms, clinical features and therapeutic strategies.[4 5]

Different from RA, AOSD-associated arthritis, usually an oligoarthritis, is present in two-thirds of these patients, migrating between joints at the very beginning and becoming stable within the course of the disease.[6] Although any joint might be affected, wrists, knees and ankles are frequently involved in AOSD arthritis. However, proximal interphalangeal and metacarpophalangeal joints of the hands and small joints of the feet, including the metatarsophalangeal joints, are scarcely affected in these patients.[6 7] Rarely, AOSD is characterised by symmetrical RA-like polyarthritis. This pattern of joint involvement does not fully justify the application of the DAS28 in AOSD. Furthermore, DAS28 does not entirely assess the systemic features of the disease. In fact, in previous studies, which are mentioned by Muravion and Muravion,[8 9] the clinical response has been defined combining DAS28 reduction and disappearance of fever in assessed patients.[8 9] In any case, the DAS28 is not validated for assessing AOSD disease activity so far; thus, it is not simply possible to translate its use in these patients based on evidence deriving from a different disease. The measures of outcome derived from RA do not fully evaluate the disease activity in AOSD, since these are characterised by the lack of comprehensiveness and responsiveness on these patients.

Furthermore, Muravion and Muravion suggested the use of systemic score as disease activity score.[1] The systemic score, proposed by Pouchot *et al*,[10] is designed as a severity score, and its sensitivity to change is not investigated so far. In a large cohort of patients with AOSD, one of the largest published in literature, the use of the systemic score has been validated as a prognostic tool, not as a disease activity score, identifying a subset of patients at higher risk of life-threatening complications and mortality.[11] In this context, some authors modified the systemic score to evaluate the activity of AOSD.[12] Despite its being closer to disease activity than other proposed measures, some variables, which are included in the score, are not clearly defined and could thus not be precisely measured.

As far as the strong rationale of inhibiting interleukin (IL)-1 in AOSD questioned by [1]Muravion and Muravion is concerned, multiple lines of evidence clearly reported the usefulness of biological disease-modifying antirheumatic drugs (DMARDs) targeting IL-1 in these patients.[13 14] The importance of inhibiting IL-1 is also confirmed by the possibility to change the natural history of the patients by an early administration during the first phases of the disease, as shown in the juvenile counterpart of AOSD.[15 16]

In conclusion, we still consider an urgent need the development of a validated disease activity score in AOSD. The lack of this clinical tool is also documented by the available clinical trials on AOSD, which developed their own criteria of response, consequently reducing the comparability and the reproducibility of obtained results.[3 17 18] Furthermore, the urgency of a validated disease activity score is suggested, since the therapeutic strategy in AOSD, including definition of refractory patients and choice of which class of biological DMARDs, is mainly related to the clinical judgement which combines scientific theory, but also personal clinical experience, patient perspectives and other insights.[19 20] However, with the rise of modern research methodology, the fallacious aspects of clinical judgement have been increasingly stressed, undertaking something like low-quality correlational statistics.[19 20] Thus, in the era of evidence-based and precision medicine, a validated score to accurately measure AOSD activity is of crucial importance to comprehensively investigate the disease, balancing appropriate therapy, minimising the exposure to iatrogenic harm and avoiding unnecessary expenditures. Conversely, the mere translation of evidence from another disease to AOSD might impair the management of these patients.

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Response to: 'Correspondence on 'Changing the outcome measures, changing the results? The urgent need of a specific disease activity score to adult-onset Still's disease" by Muraviov and Muraviova

Muraviov and Muraviova are asking for further[1] elucidation of the chosen endpoints and a more detailed characterisation of the included patients in our study of canakinumab for the treatment of patients with adult-onset Still's disease (AOSD) with articular involvement.[2]

As mentioned in our previous communication, at the time of Canakinumab for treatment of adult ONset StIll's Disease to achiEve Reduction of arthritic manifestation (CONSIDER) trial conception in 2012, there were no approved drugs and no validated scores available for AOSD. On the other hand, accumulating evidence suggested that inhibition of Interleukin-1 (IL1) could be beneficial for the patients.[2–5] Thus, our aim was to investigate the effects of canakinumab in a controlled setting to provide convincing data, which could even be useful for regulatory purposes. Since we decided to focus mainly on articular manifestation in AOSD, the chosen endpoint of Diasese Activity Score with a 28 joint count (DAS28) response was accepted.

In fact, the European Medicines Agency (EMA) granted approval of canakinumab for AOSD based on the concept of a disease continuum of systemic juvenile idiopathic arthritis (sJIA) and AOSD as well as on biomarker data from the CONSIDER trial already in 2016.[6] Recently, the results of our study were also evaluated by the US Food and Drug Administration (FDA) and canakinumab was approved for this indication also in the US in 2020.[7]

Of course, our study cannot answer all open questions. In response to some raised queries, we would like to refer to the published data including supplementary materials (results referring to current DAS28 status on figures 3 and 4; European League Against Rheumatism response criteria are presented in supplemental material in tableS3 and figure S1; table 1 describes systemic symptoms at baseline, functional class, etc).[2] Due to the design of a prospective study and the provided intervention by treatment, it was not possible to foresee the pattern of disease in patients with a short disease duration. However, in patients with a prolonged articular disease manifestation, a chronic course is typical. Radiographic examinations were not part of the study protocol, since a treatment period of 6 months is most likely to short for a differentiation of such an outcome especially without a validated radiographic staging system for AOSD. We were also not able to address treat-to-target recommendations, since there is no agreement about or definition for it in AOSD. We agree that further initiatives on an international basis are required to establish new outcome criteria for AOSD. We also hope that our CONSIDER study will stimulate discussions, further developments and investigations in this rare disease. Finally, we are very happy to have provided convincing data to the EMA and FDA for the granted approval of canakinumab in AOSD. This offers now the opportunity to patients with AOSD to receive an effective targeted therapy in many countries worldwide.

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Correspondence on 'Ultrasound shows rapid reduction of crystal depositions during a treat-to-target approach in gout patients: 12-month results from the NOR-Gout study'

With great interest, we read the article by Hammer *et al* reporting a treat-to-target (T2T) approach with urate-lowering treatment (ULT) resulted in significant reductions of crystal depositions via ultrasonic detection.[1] We agree with the authors that the use of new semiquantitative scoring system may increase the sensitivity to minor changes in lesions in contrast to a binary scoring system. We would like, however, to highlight some key points.

First and foremost, there is no control group in this study. Although patients with gout have rapid reduction of crystal depositions by a T2T approach, we cannot tell how much of this improvement is due to the effect of the treatment itself. Moreover, whether the operators and the participants are blind or not is unknown in the article. A double-blind, controlled study is essential for giving information on effectiveness. Next, there are medicines left out, and a subgroup analysis for different treatment is required. Allopurinol and febuxostat are the medication with ULT in the experiment. However, we caution that benzbromarone is another common medication when patients have intolerance for allopurinol and febuxostat. Previous researchers have taken benzbromarone in consideration during ULT research.[2] Also, the proportions and a subgroup analysis of patients using different medicines (allopurinol and febuxostat) are recommended for verifying effectiveness. Last but not the least, according to European League Against Rheumatism (EULAR), a lower target (under 5 mg/dL or 300 μmol/L) might be given if patients have severe gout with tophi, chronic arthropathy or very frequent attacks. In normal situation, the target of serum urate level is set up by 6 mg/dL. We note that the proportion of the patients whose target is under 5 mg/dL is unsaid, and that the results should be presented separately.[3]

There may be some information bias in the research. On the one hand, the locations for elementary lesions in Table 1 may be underestimated. Locations such as kidneys, tibialis posterior tendons,[2 4] peroneus, metacarpophalangeal joints (MCP)1, MCP3-5 and metatarsophalangeal joints (MTP)2-5 are not shown in your results[2] which may lead to inaccurate detection and wrong conclusions. On the other hand, erosions, one of the elementary lesions according to Outcome Measures in Rheumatology (OMERACT), have been missed out in Table 2. Since the sum scores of double contours, tophi and aggregates cannot represent all of the elementary lesions of patients with gout, we propose that erosions should be taken into account as an outcome during 12 months follow-up as in previous studies.[2 4 5] Another information bias is the unknowingness of the periods of recruitment, exposure and data collection. Besides, the medical institution where the experiment took place is yet to be clarified. We are looking forward to understanding further details.

Apart from the above, we are sincerely concerned about some selection bias and residual confounders in the situation. First, both patients being naïve to ULT or with previous or present ULT treatment were included in this study. However, the patients who have undergone ULT, or even by a T2T approach, before the enrolment may be a confounder. We suggest that a subgroup analysis for naïve, previous and present users is needed. Second, the rules of study patient enrolment may be inadequate. The inclusion and exclusion criteria in previous studies like age ≥18 years,[2 6] not to have recent corticosteroid[6] or glucocorticoid[2] injections before study entry, and no history of severe renal insufficiency, psoriasis, drug-induced gout and other secondary gout types[6] are not mentioned in the article. Finally, alcohol consumption,[2 6] smoking history,[2 6] comorbidities[2 7 8] and body mass index[6 8] may be some residual confounders unrevealed in the experiment. More investigation and stratification are expected to remove confounding bias.

To sum up, we are convinced that a double-blind, controlled study with different subgroups is necessary to enhance credibility. An outcome of erosions and some undetected locations should be included. Lastly, the enrolment criteria and residual confounders are requested to explication corresponding to your great work.

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Response to: 'Correspondence on 'Ultrasound shows rapid reduction of crystal depositions during a treat-to-target approach in gout patients: 12-month results from the NOR-Gout study" by Hung *et al*

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We appreciate the interest by Hung *et al*[1] in our article describing the rapid reduction of ultrasound-detected crystal depositions in gout during 12 months of treat-to-target follow-up on urate-lowering treatment (ULT).

Hung *et al* comment on the lack of control group in our study, and they suggest a double-blind, controlled study to explore the effectiveness of ULT, and they comment on the lack of information regarding blindness of operators and participants.[1] We certainly agree on a randomised controlled trial (RCT) being necessary to confirm our findings, which are based on an observational study without a control group, where neither sonographers nor patients were blind to the treatment. However, since in addition to our study, several smaller studies have shown the reduction of urate depositions during ULT,[2–6] and given the strong recommendations to reduce serum urate levels in gout to a target,[7 8] a long-lasting RCT with a control group not treated according to current guidelines, at least in Scandinavia, seems not ethically acceptable to patients.

In our study, we did not have focus on the different urate-lowering drugs applied but rather on whether patients achieved the treatment target of <6 mg/dL. In addition, all our patients were treated with either allopurinol or febuxostat, and none of the patients used probenecid or benzbromarone. The group of patients with tophi had a more ambitious treatment target of <5 mg/dL but accounted for less than 20% of patients, making meaningful conclusions in this cohort difficult.

There is no agreement on how many joints and tendons to examine by ultrasound when the load of depositions of crystals is assessed. We agree that additional joints and tendons could be explored with ultrasound, giving a more comprehensive examination of regions reported to be potential locations for depositions. We have, however, examined many joints, tendons and entheses, determined after careful literature research as well as discussions with experts in the field. A larger array of localisations would not be feasible in clinical practice as in our study but may be useful in other research settings.

The suggestion of including erosions as an outcome measure is relevant. Our study describes erosions of the medial part of the first metatarsal head to be present in about 60% of the patients. However, as could be expected, there was no change of the score of these erosions during the study. Thus, even if description of erosions is of interest to indicate the severity of the disease, it may not be appropriate as an outcome for treatment response.

Our study was performed at the Department of Rheumatology at Diakonhjemmet Hospital, Oslo, Norway, with inclusion between 2015 and 2018. The paper describes most of the inclusion criteria, and important exclusion criteria were severe comorbidity, including heart failure (New York Heart Association III–IV) or kidney failure (eGFR <45 mL/min, chronic kidney disease stage 3B). We thank for the interest in our study and will provide more clinical results from our study in future publications, including detailed information about treatment, clinical assessments and outcomes.

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Severe polymyalgia-like symptoms secondary to anti-PD1 therapy successfully managed without discontinuing checkpoint inhibitor

After reading the article from Braaten *et al*,[1] we reinforce our idea that suspending checkpoint inhibitors (CPI) due to musculoskeletal symptoms, even if severe, may not always be a good alternative, since in addition to losing an option in the treatment of cancer we do not know if the symptoms will actually resolve with treatment interruption. Here we present a patient who developed severe polymyalgia rheumatic-like (PMR) symptoms 7 months after initiation of nivolumab for metastatic melanoma. Since it was a grade 3 immune-related adverse event according to the guidelines published in the *Journal of Clinical Oncology*,[2] the oncologist initiated high-dose prednisone and opioids, considered interrupting the CPI, and requested rheumatology evaluation. After a shared discussion between the two specialties and the patient, considering his good life expectancy as well as the good tumour response to the drug, we decided to start methotrexate 15 mg weekly without stopping the anti-programmed cell death protein 1 (PD1) therapy. Weeks after, the patient evolved with a significant improvement in symptoms, successfully weaning off prednisone and stopping nivolumab 12 months later, without identifying any clinically significant drug interactions. Unfortunately, corroborating Braaten *et al*'s paper,[1] PMR-like symptoms persisted in the patient, still demanding methotrexate 2 years after stopping the CPI.

This case illustrates a different approach from what is usually recommended by recent guidelines.[2 3] Although several adverse rheumatological effects have already been described (eg, aggravation of degenerative conditions, drug-induced lupus, vasculitis and so on),[4] with regard to musculoskeletal manifestations, the guidelines suggest that for severe PMR-like symptoms (eg, grades 3–4) immunotherapy should be suspended.[2] Since we consider that this behaviour may not be the best for most patients with PMR-like or inflammatory arthritis, we wonder: should we stop the pain or stop the cancer[5]?

The authors believe that a good rheumatologist–oncologist relationship is essential before deciding to discontinue the CPI which has been fulfilling its role.[5] We suggest that, as a rule, non-life-threatening symptoms such as joint pain are not enough for the oncologist to suspend immunotherapy, and it is up to us to keep patients comfortable with the CPI, while ensuring them good quality of life. The rationale for this lies in the widely known safety of conventional disease-modifying antirheumatic drugs (DMARD) (eg, methotrexate, sulfasalazine and hydroxychloroquine) in the doses practised by the rheumatologist; in a faster weaning of prednisone, minimising its undesired effects; and that stopping CPI therapy due to articular pain will not necessarily cease the rheumatic symptoms, but may carry the risk of cancer advancing.[6]

Thus, the individualisation of treatment is crucial, and until there are more studies concomitant use of CPI therapy with conventional DMARDs should be chased. It is possible to stop pain and cancer,[7] and seeking this balance in real life is much more than following protocols and guidelines.

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Gender gap in rheumatology: speaker representation at annual conferences

Adami *et al*'s report of gender disparities in the first authorship of rheumatology guidelines was very interesting to read.[1] The authors highlight a fundamental issue: the existence of a gender gap—that is, proportionately more male than female physicians—within the authorship ranks in rheumatology. We had thought that it would be intriguing to investigate whether this difference permeates at the national conference level as well. Recent studies have quantified the gender gap among speakers at academic conferences in other specialties. The most extensive study of 181 medical conferences held in North America over the course of a decade (2007–2017) found an increase in the proportion of female speakers from 25% to 34% over time, and the under-representation of women to be more marked at surgical compared with medical conferences.[2]

Our goal was to describe the proportion of female representation among speakers and moderators at the American College of Rheumatology (ACR) meetings in 2017 and 2018. Using the ACR Session Tracker programme for these 2 years, we determined the proportion of women for each speaker or moderator slot. We further categorised by basic versus clinical science presentation and by type of session (premeeting, ACR general session, Association of Rheumatology Health Professionals (AHRP) general session, abstracts, workshop, study group or Meet the Professor).

Overall, the proportion of combined female speakers and moderators was 42.8% in 2017 and 47.0% in 2018. The representation of female speakers increased from 2017 to 2018 by 4.2%, which in a conference of approximately 1100 presenters (total presenters at the 2018 conference) amounts to 46.2 persons. There was a higher proportion of female speakers in the clinical than in the basic science presentations (mean 45.8% vs 40.5%). By session type, the AHRP sessions had the highest proportion of female representation (mean 65.3%) while Meet the Professor and workshops had the lowest (34.4% and 28.7%, respectively) (figure 1).

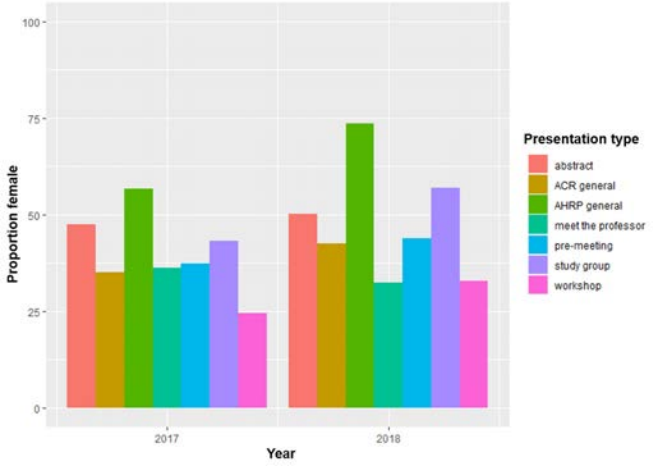

Figure 1 Proportion of female speakers at ACR, by year and presentation type. ACR, American College of Rheumatology; AHRP, Association of Rheumatology Health Professionals.

We found that the mean overall proportion of female speakers and moderators at ACR meetings in the past 2 years was 44.9%. The ACR had female representation above the mean compared with major North American medical conferences held in 2017. This proportion is also comparable to the estimated US adult rheumatology workforce data from 2015.[3] However, there remains a gender gap across most medical specialties in Canada and the USA despite the current gender parity in medical school. Limited numbers of role models, sponsors and mentors may cause and perpetuate the problem. Although the gender gap at recent ACR meetings was narrower as compared with other conferences, we must remain cognizant of its presence and continue to work towards equal representation.

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