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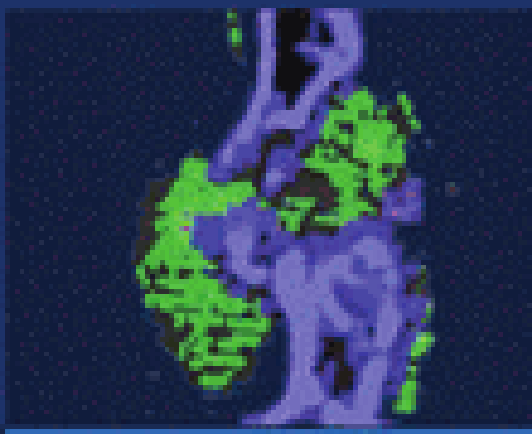

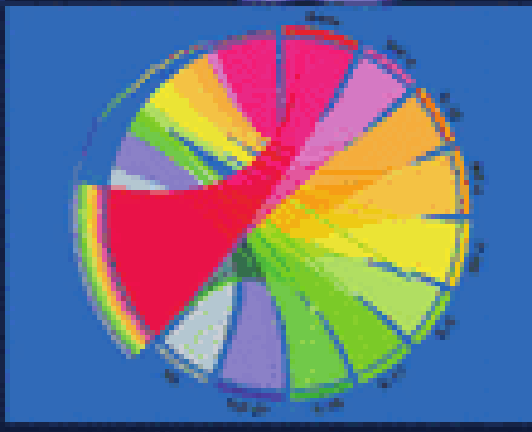

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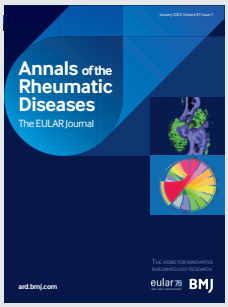

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Editorials

Review

Viewpoint

Recommendations

Rheumatoid arthritis

Psoriatic arthritis

MORE CONTENTS ►

Osteoarthritis

Systemic lupus erythematosus

Systemic sclerosis

Vasculitis

Epidemiology

Letters

Electronic pages

Greetings from the editor 2022

Josef S Smolen

When a year closes and gives way to its successor, it is good to pause for breath to reflect on the achievements of the past and contemplate the prospects for the incoming year.

So when looking back at the year just past and the tension that always exists between expectations and realisation, your editor must admit to being overwhelmed and humbled by the fantastic activities of our authors who have continued to submit the output of their excellent research work to the *Annals of the Rheumatic Diseases*; but also by the work of the Associate Editors, the Editorial Board and all Reviewers who supported the journal in an amazingly efficient, knowledgeable, thoughtful and balanced way. Most may think that this should be self-evident for the leading scientific journal in rheumatology, however, in this day and age, when COVID-19 continues to dominate, this is anything but a matter of course. I would therefore, like to place on record from the outset, my sincere gratitude to the referees, the Associate Editors and Editorial Board members and all authors—thank you!!

PANDEMIC AND ARD

Against our hopes, COVID-19 is far from over. Vaccinations have not been taken up to an extent that will permit herd immunity across Europe, let alone across the world. Part of this reflects supply issues and rate of feasible uptake across distinct healthcare economies. Sadly, large parts of the population are deceived by misleading information, not for the first time in this context,[1] and as such the greatest weapon to combat pandemics and prevent the ailments induced by infectious diseases, namely vaccination, is rendered less effective. For many decades, indeed, for more than a century, it is firmly established that 'vaccination is the most effective medical intervention ever introduced and, together with clean water and sanitation, it has eliminated a large part of the infectious diseases that once killed millions of people'[2]; 'Arguably the single most life-saving innovation in the history of medicine'.[3 4] Today, we remain convinced, maybe even more so. Many European politicians act with populist intention and an eye to the polls, and apparently seek to prevent short-term economic losses, rather than fighting robustly, and with moral determination to prevent deaths and gain long-term economic stability. This journal reminds the political class that the right to life is the foremost human right.[5] Are human rights enforceable by law? When I sent you my *Greetings* last year, I mentioned that the global death toll caused by the pandemic amounted to 1.5 million people.[6] By now, this toll has exceeded 5 million deaths,[7] and more will follow to our common dismay, many of which should be preventable if political decision makers would put in place appropriate rules, regulations or laws—*morituri te salutant*!

COVID-19 and the effects of vaccination in patients with rheumatic and musculoskeletal diseases (RMDs) was, unsurprisingly, a major research focus for many rheumatologists throughout the past year, and this was also reflected in ARD, just as for many other journals. Indeed, while ARD published accepted papers consistently across the year, the October 2020 issue in particular, presented several papers related to SARS-CoV-2 vaccination and immune responses in the general rheumatic and musculoskeletal disease population and in those receiving immunomodulatory therapy,[8–14] accompanied by a fine editorial that examined the broad scope of this issue.[15] Of note, EULAR is currently preparing a document concerning SARS-CoV-2 vaccination which soon may be presented in ARD after appropriate review.

NEW INSIGHTS INTO RHEUMATIC AND MUSCULOSKELETAL DISEASES

Obviously, while all rheumatologists were confronted with the worries of their patients regarding the pandemic around both disease risks and those of vaccines, and many had to take care of patients with RMD who developed COVID-19, rheumatology cannot primarily focus on the pandemic, nor can ARD. Consequently, many papers provided novel insights across the range of RMDs, from osteoarthritis[16–18] to systemic sclerosis[19] and from spondyloarthritis[20 21] to gout,[22 23] to name a few topics and cite randomly selected papers, focussing at translational and clinical aspects including novel or re-purposed therapies.

Last year several EULAR task forces provided ARD readers with the results of their work after approval by the EULAR Council, starting in January with a EULAR definition of difficult to treat rheumatoid arthritis (RA) and management of checkpoint inhibitor-induced RMDs[24 25] and ending with recommendations regarding self-management strategies and points to consider in patients at risk of RA, that is, pre-RA.[26 27] Needless to say the numerous correspondences to various articles and the recently introduced rapid responses are a living embodiment of the interest of our readership in the papers published in the journal.

INCOMING 2022: A EULAR ANNIVERSARY

In 2022 ARD will continue its path, striving to present breakthrough research activities across basic/translational and clinical/outcomes sciences, already exemplified in this January issue, in which we learn about several studies on the potential prevention of the evolution of psoriatic arthritis in patients with psoriasis undergoing effective treatment,[28–30] accompanied by an editorial that summarises these findings and puts them into perspective.[31] But this edition harbours yet another publication, which for the first time appears simultaneously in all five EULAR and ACR journals, accompanied by a commentary from all five editors, making authors aware that all these journals will require adherence to ACR, EULAR and joint recommendations and criteria more strongly in future papers than done before.[32–36] This will also be reflected in the respective instructions to authors.

Of particular note, ARD has engaged in a collaborative effort among many journals to publish an editorial on the emergency of combatting climate change[37] and is conscious of environmental issues, and such efforts were also initiated some time ago in EULAR and EULAR is committed to continuing them. Much to our dismay, the EULAR congresses in 2020 and 2021 were only virtual and personal contacts between researchers and clinicians, have not been possible. This even puts more emphasis on the EULAR Journal to be an open—peer-reviewed—forum for research. Hopefully, the physical interactions between participants can be resumed in June in Copenhagen.

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Speaking of EULAR: while 3 years ago ARD, the oldest journal devoted to rheumatology, celebrated its 90th anniversary and 2 years ago the 20th anniversary of becoming 'The EULAR Journal',[38] yet another anniversary must be honoured in 2022, namely the founding of EULAR itself in 1947. EULAR's 75th anniversary will obviously be celebrated at the EULAR congress, but with the turn of the year, ARD hereby conveys sincere congratulations to its parent organisation on this occasion with many thanks for the terrific collaboration over the decades!

Let me please close where I started, thanking wholeheartedly the reviewers, authors, editorial board and especially you, the readers! As always, I will be happy to receive your feedback on the scope and quality of the papers and on topics that you may wish to see covered in the future so that we can consider further important steps into the coming years.

And let me please use this opportunity to wish you and your families a happy, successful, safe and healthy New Year!

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Re-examining remission definitions in rheumatoid arthritis: considering the 28-Joint Disease Activity Score, C-reactive protein level and patient global assessment

David Felson ,[1,2] Diane Lacaille,[3,4] Michael P LaValley,[5] Daniel Aletaha [6]

Editors' note

The Editors of the 5 journals of the American College of Rheumatology and European Alliance of Associations for Rheumatology have been reminded by this editorial that ACR and EULAR have jointly agreed on various classification criteria, definitions, recommendations, or points to consider, which do not always find reflection in manuscripts submitted to the journals. Consequently, in the future, the Editors will enforce the use of the products obtained in the course of joint ACR/EULAR or EULAR/ACR activities in all respective papers. For rheumatoid arthritis this would mean use of the ACR/EULAR or EULAR/ACR classification criteria, remission definitions, recommendations on what to report in clinical trials, and others, as pertinent. The same applies to other diseases. There are valid and important reasons that these activities have been undertaken by ACR and EULAR, and therefore, the conclusions of the various task forces, which have been endorsed by ACR and EULAR, should be respected by investigators and study administrators. This does not mean other methods could not be used in a study, but at the least, the reports should address the methods agreed on by the two organisations. Maintaining uniformity across major publications regarding rheumatoid arthritis remission or other definitions not only allows for more appropriate comparison across analyses, but also enhances readers' ability to interpret results. Author instructions across the five journals will more strongly reflect this requirement.

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Over the last 30 years, treatment for rheumatoid arthritis (RA) has improved dramatically. By the early 2000s, disease remission had become a realistic goal, although definitions of remission varied widely, making it difficult to compare treatment strategies and gauge how often remission occurred. In 2009, the American College of Rheumatology (ACR) and the European Alliance of Associations for Rheumatology (EULAR) created a joint committee whose charge was to recommend a definition of remission. Members of the committee suggested a large number of candidate definitions and, using a data-driven consensus process, statisticians and programmers tested these candidates in a bank of RA trial data to see which definitions performed best in predicting long-term good function and lack of radiographic progression. The committee endorsed a stringent definition using measures from the validated core set of outcome measures.

After reviewing analysis results, the committee selected 2 definitions of remission that were approved by the ACR and EULAR.[1 2] The first was a Boolean version in which, to be classified as having attained remission, a patient had to have tender and swollen joint counts of ≤1, a C-reactive protein (CRP) level of ≤1 mg/dL, and a patient global assessment of arthritis activity of ≤1 (on a 0–10 scale). The second recommended definition was a score of ≤3 on the Simplified Disease Activity Index (SDAI),[3] a scoring system that is based on the same core set outcome measures. While designed and validated in trials, these definitions could help assess treatment 'success' in clinical practice as well as in trials and, in practice, could serve as a 'treat-to-target' goal for some patients.

Like all developed criteria, the ACR/EULAR 2011 RA remission criteria were labelled as provisionally approved and awaited validation in an independent sample for final approval. A revised validated version of the remission criteria is pending full approval by ACR/EULAR. Many concerns have arisen since the publication of the provisional remission criteria. Among them is the continuing use in trials of 28-joint Disease Activity Score (DAS28) thresholds[4] to define remission, questions about the use of CRP as an element of remission definitions, and questions about the appropriateness of including patient global assessment in defining RA remission. This editorial will address each of these issues.

USING THE DAS28: WHEN 'REMISSION' IS OFTEN NOT REMISSION

The DAS28 is a widely used measure of disease activity. An ACR committee that critically evaluated RA disease activity measures for use in clinical settings found that the DAS28 met predefined criteria, including providing a score that stratified patients into at least three disease activity states, being measurable in the clinical setting, and having adequate psychometric properties. The DAS28 was one of 4 recommended RA disease activity measures[5]

The committee on RA remission considered several DAS28 thresholds as candidate definitions of remission, including the popular threshold of a DAS28 using the CRP level (DAS28-CRP) of <2.6 and an even lower threshold of <2.0. The DAS28 formula weights swollen joint count half as much as tender joint count and also underweights it relative to CRP (or erythrocyte sedimentation rate (ESR)). Therefore, a patient can achieve a low DAS28 score but still have a substantial number of swollen joints. The committee's analyses showed that 10% of patients with a DAS28 of <2.6 had≥4 swollen joints, and one patient had >20 swollen joints. When a lower DAS28 threshold of <2.0 was used, swollen joint counts of two or three were common and scores of up to six possible. In fact, if the tender joint count is 0, values for the other components of the DAS28 become irrelevant (figure 1). Values of up to 60 (of 100) for patient global assessment are consistent with remission according to the DAS28. Even if the tender joint count is one, the DAS28 score can be in the remission range when other core set measures show active disease. DAS28-CRP thresholds differ substantially from those obtained with the DAS28 using the ESR (DAS28-ESR)[6], and with the DAS28-ESR, RA would be even more likely to be classified as being in remission when disease is in fact active.

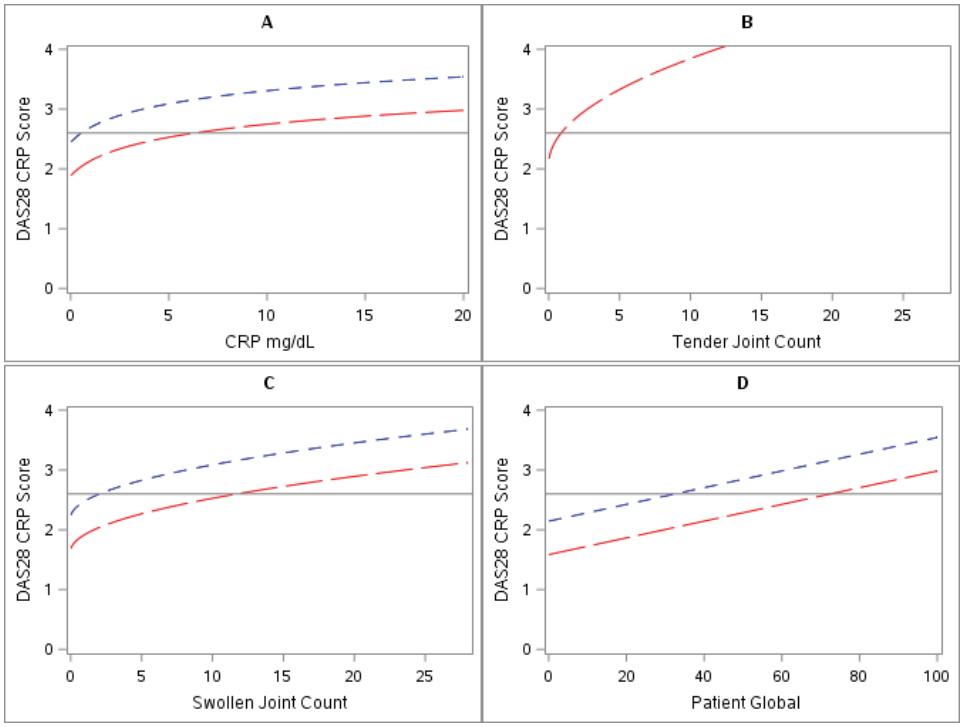

Figure 1 The contribution of each component of the 28-joint disease activity score using the C-reactive protein level (DAS28-CRP) to remission (score <2.6 (solid horizontal line)) when other components are in the range of remission. Red dotted line represents TJC = 0 and blue dotted line represents TJC = 1. The DAS28-CRP is composed of 4 components: CRP level (A), tender joint count (TJC) (B), swollen joint count (SJC) (C), and patient global assessment of arthritis activity (D). in each graph, it is assumed that the three components other than the one depicted met the threshold for remission (CRP 0.5, TJC 0 (red dashed lines) or 1 (blue dashed lines), SJC 0, patient global assessment 1). Note that when the TJC is 0, most values of CRP and patient global assessment yield a DAS28 of <2.6 ('remission'), and SJC <10 yields DAS28 'remission'.

One other major criterion was that patients whose disease was in remission at 6 months or 12 months in a 2 year trial should be likely to have both good and stable functional and radiographic outcomes later in the same trial. Patients in whom DAS28 remission was achieved had worse radiographic outcomes than those achieving remission according to other definitions (no change in the Sharp score[7] or the Sharp/van der Heijde score).[8] Ultimately, the committee rejected DAS28 candidates as definitions of remission because swollen joint counts were too high to be consistent with clinical remission and because DAS28 'remission,' even with the use of stricter thresholds, did not predict good combined functional and radiographic outcomes as well as the predictive ability that was observed using the remission definitions selected by the committee.

Other studies carried out since the publication of ACR/EULAR remission criteria provided additional evidence that the DAS28 should not be used to define remission. Saleem *et al*[9] demonstrated that among patients whose RA was in remission according to the DAS28, power Doppler ultrasound showed considerable disease activity unless disease was also in remission according to the SDAI. Lee *et al*[10] reported that joint pain was present and persisted in patients whose disease was in remission according to the DAS28 but was absent if remission was classified according to the Boolean definition. Analyses from the AGREE trial of abatacept vs placebo[11] confirmed that patients in whom remission was achieved according to the DAS28 subsequently had worse mean scores on the Health Assessment Questionnaire (HAQ)[12] than those in whom remission was attained according to the SDAI. Schoels *et al* reported, from an analysis of 3 large multicentre RA trials, that among patients with a DAS28 of <1.9, those whose disease was not in remission according to the ACR/EULAR criteria still had an average of 2–3 swollen joints.[13]

Given the problems with use of the DAS28 to define remission, why is it so widely used? First, the DAS28 is a commonly used disease activity measure and it is easy to apply a threshold in data already being acquired, although the requisite elements of the ACR/EULAR definitions of remission are also acquired. Another potential reason relates to industry-sponsored RA trials. A definition based on a DAS28 of <2.6 yields remission rates far higher than definitions endorsed by the ACR/EULAR, and treatments therefore appear more efficacious with use of the DAS28. Further, use of a definition that yields a higher remission rate improves statistical power. The same absolute difference in remission rates between two drugs is more likely to reach statistical significance when remission rates are higher. Finally, DAS28 use is mandated by some regulatory agencies. Many reports do not even include data on other measures of remission.

WHEN REMISSION DEFINITIONS FAVOUR SOME TREATMENTS OVER OTHERS

Reliance on the CRP level to define RA remission is an emerging concern.[14] CRP is the second most heavily weighted variable in the DAS28 formula. The armamentarium for treatment of RA includes effective biologic agents that have different effects on CRP; interleukin-6 and JAK inhibitors both directly reduce CRP, whereas abatacept and rituximab do not. If the DAS28-CRP is used in a trial comparing the efficacy of abatacept and JAK inhibitors, even if effects on joint counts and patient-reported outcomes are the same, JAK inhibitors would score better, as seen in one recent trial.[15] In

Table 1 Proportion of patients with good outcomes (both radiographic and functional) in three multicentre rheumatoid arthritis trials†

	Candidate remission definition	
Patients with good outcomes*	TJC, SJC, and CRP level all ≤1	TJC, SJC, CRP level, and patient global assessment all ≤1
In remission, %	46	66
Not in remission, %	17	17
Positive likelihood ratio (95% CI)	3.1 (1.9 to 5.3)	7.2 (3.5 to 14.8)

*Based on remission status at 6 months after baseline. Good radiographic outcome was defined as a change of 0 in the Sharp/van der Heijde score between 12 months and 24 months after baseline. Good functional outcome was defined as a change of 0 in the.Health Assessment Questionnaire between 12 and 24 months after baseline and a score of ≤0.5 at both 12 and 24 months.
†Excluding patient global assessment compromises the ability to predict good outcomes (from ref. 1). TJC=tender joint count; SJC=swollen joint count;.
CRP, C reactive protein.;

another trial comparing biologic agents, the authors acknowledged avoiding use of the DAS28-CRP because of this bias.[16] The ACR/EULAR provisional criteria allow for remission definitions that exclude acute-phase reactants, using a 3-variable version of the Boolean definition and the Clinical Disease Activity Index[17] instead of the SDAI. Further, while the full ACR/EULAR remission definitions include acute-phase reactants, they are not weighted as heavily as in the DAS28-CRP (or the DAS28-ESR).

CONCERNS ABOUT INCLUSION OF THE PATIENT GLOBAL ASSESSMENT

Yet another concern about the provisional definitions of remission has been championed by Ferreira *et al*.[18] They point out that a patient's global assessment of their arthritis activity often is based on considerations unrelated to current disease activity, such as pain from joint damage, and that this measure should not be included in definitions of remission. The factors that most influence the patient global activity measure are pain and fatigue. Ferreira *et al* analyses suggest that removing the patient global assessment would not compromise the ability to predict later radiographic outcomes in RA, although they acknowledge that patient global assessment is a powerful predictor of function (as measured by the HAQ). High patient global assessment scores not only correlate with poor concurrent physical function, but they identify patients whose physical function is worsening.[19 20] If patient global assessment is removed, remission criteria no longer predict future patient function well.

In addition to its being the only patient-reported outcome measure included in remission definitions and the importance of including the patient perspective, there are other critical reasons to include patient global assessment as a component of remission. First, the patient global assessment reflects components of disease activity that are otherwise not captured, including fatigue and pain, as well as inflammation in joints not included in a 28-joint count, such as the feet and ankles. This may be why high patient global assessment scores, even when 28-joint counts are low, identify patients at high risk of later functional loss. Second, the patient global assessment is among the most sensitive, if not the most sensitive, outcome measure in RA.[20] It improves much more with active RA treatment than with placebo, suggesting that it provides a window into disease activity related to systemic inflammation not detected by tender and swollen joint counts. Therefore, eliminating patient global assessments from RA trial outcomes would compromise the ability to distinguish the comparative efficacy of different treatments. This would occur at a time when, given the large armamentarium of treatments available, there is a particular need to maximise the ability to differentiate their efficacy. In addition, inclusion of patient global assessment markedly increases the likelihood that patients in whom remission is attained will have both good radiographic outcomes and good functional outcomes later (table 1), and ensures that the definition of remission captures non-radiographic outcomes that are important to patients.

CONCLUSIONS

With remission achievable in RA, making the definition of remission stringent will ensure that patients benefit from comprehensive control of their disease. The DAS28 should not be used to define remission because, even with the use of low thresholds, many patients whose disease is in 'remission' will still have a number of swollen joints and active disease. Also, given its dependence on the CRP value, use of the DAS28 makes it difficult to differentiate efficacious treatments with dissimilar effects on acute-phase reactant levels. Defining remission without asking patients to provide any information about their disease activity—not to mention failing to collect data on any patient-reported outcomes—risks losing valuable information on treatment efficacy.

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Intercepting psoriatic arthritis in patients with psoriasis: buy one get one free?

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Psoriatic arthritis (PsA) mostly develops in patients with an established diagnosis of psoriasis (PsO).[1] Following the onset of PsA, structural articular damage and loss of function often occur, leading to impairment in quality of life above and beyond that seen in PsO alone.[2] PsO registry studies show a progression to PsA in around 1.5%–3% per year in PsO subjects, although figures may be even higher when PsO associates with other factors (eg, arthralgia).[3] Reducing this rate of PsA development and identifying PsO subjects at higher risk for PsA progression is of paramount importance, especially given that many PsO therapies have been independently verified as being efficacious for established PsA. Therefore, by extension, these therapies might also be expected to work at the earliest stages of PsO-associated inflammatory arthritis, where better therapeutic effectiveness is generally expected.[4]

The ability to characterise the preclinical phases of autoimmune diseases, as initially in type 1 diabetes (T1DM),[5] was followed by other diseases, including rheumatoid arthritis (RA)[6] or autoimmune connective tissue diseases.[7] They provide the unique window of opportunity for therapeutic interventions in the preclinical stage of disease. Interventions applied at this point, as the hypothesis goes, would minimise disease burden and subsequent irreversible joint damage leading to functional impairment and long-term disability, eventually to reducing the complications and socioeconomic impact of disease. Historically, the prevention of diseases, such as T1DM, for example, with cyclosporine, was marred by incomplete responses and drug toxicity.[8] Nonetheless, proof of concept for disease prevention was established. In this editorial, we discuss emerging and conflicting evidence about early stage therapy for PsA. Specifically, we will explore the concept of prescription of disease-modifying antirheumatic drugs (DMARDs), including biological DMARDs (bDMARDs), in subjects with moderate-to-severe PsO, at no extra cost to the health payers and no additional risk for patients, and the related impact on the interception of the evolution of PsO to PsA (figure 1A,B). We dubbed this approach 'buy one, get one for free', as beneficial effects on one manifestation of psoriatic disease would result from interventions prescribed to treat other, different signs or symptoms.

Predictive markers for inflammatory disease development usually focus on laboratory biomarkers, like anticitrullinated protein antibodies used in RA (positive in 70% of cases). However, rather than laboratory tests in PsA the most relevant biomarker seems to be the clinical presentation of PsO itself, as present in 70% of subjects who will subsequently develop PsA.[2 9] This aspect of PsA disease interception is unique compared with other immune-mediated disorders prevention, where

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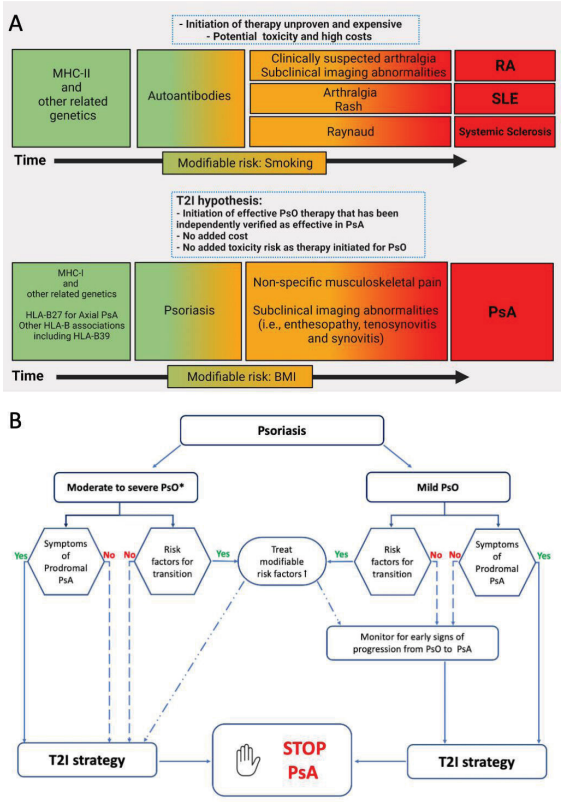

Figure 1 (A) Comparing autoimmune disease evolution to psoriatic arthritis (PSA) evolution. Unlike humoral immune-mediated autoimmune diseases where the autoantibodies that predate disease are a risk factor for disease that cannot be therapeutically manipulated at present, the psoriasis (PsO) biomarker is both a predictor of PsA and a target for therapy itself. This is a unique feature and means that the initiation of therapy does not increase risk of toxicity or costs providing the PsO is extensive enough to merit therapy that could intercept arthritis evolution.[30–33] (B) The concept of treat to intercept (T2I) algorithm for interception of PsA in PsO patients. *Moderate to severe PSO is defined as either extensive (body surface area involvement >10%), or as important to the patient: more limited PsO leading to significant impact on quality of life (eg, face/hand/feet/genital involvement). When patients have mild PSO but risk factors for PSA then systemic therapy for PSO would not ordinarily be initiated. However, risk factors for imminent PSA could make this group a potential target for therapy or T2I. BMI, body mass index; MHC, major histocompatibility complex; RA, rheumatoid arthritis.

interventions would be prescribed to otherwise healthy (at risk) subjects (figure 1). Different, interventions for preventing PsA in the presence of clinically active PsO would, if effective, mitigate the risks/benefits ratio considerably. Increasingly recognised shared immunepathological mechanisms between the skin and the enthesis—an early musculoskeletal key target lesion (figure 2) in PsA—are likely to provide a rationale for efficacy of PsO interventions beyond the skin level.

Therefore, several unique aspects around the potential for PsA prevention are distinguished from diseases like RA. First, several licensed systemic therapies for the treatment of PsO were independently verified as effective in established PsA.[10] While the therapy of PsO included conventional DMARDs (cDMARDs) initially, this evolved into the antitumour necrosis factor (TNF) agents about 15 years ago with these agents showing improved skin efficacy.[11] In recent years, the interleukin 23 (IL-23)/IL-17 axis cytokine blockade has been introduced to PsO where complete skin clearance has been reported in up to 50% of cases.[10] Such efficacy, an acceptable safety profile and the lack of clinically relevant neutralising antibodies, more often encountered with the use of the anti-TNF blockers, positions the IL-23/IL-17 blockers as therapies that are both liked, tolerated and show even better long-term retention. All factors that auger well for continuous use of therapies that could prevent manifestation of PsA in PsO patients. Some small preliminary studies have hinted that the use of both c-DMARDs and bDMARDs may be associated with a lower incidence of PsA development compared with topical or phototherapy.[12]

In ARD, the impact of systemic treatment on the development of PsA in PsO patients was evaluated in a retrospective cohort of PsO patients.[13–15] Gisondi *et al* and Acosta Felquer *et al* showed that PsO patients, without clinical evidence of PsA, treated with bDMARDs had a lower risk of PsA development compared with those treated with narrow-band ultraviolet light B (nb-UVB) phototherapy or those treated with topicals or without treatment.[13 14] Both studies found similar results in terms of incidence rates of PsA, (i.e., 1.2 and 1.6 cases per 100 patients/year, respectively) and nail involvement as predictor of later PsA development. The role of biologics as possible interceptor of PsA development in PsO was also described in a recent study, published in another journal.[16] In contrast, Meer *et al*, the third study on the topic published in this ARD issue, used an electronic health record database and found a higher incidence of PsA among PsO patients treated with bDMARDs than patients on oral or phototherapy.[15] These results appear inconsistent with clinical practice, in fact the authors stated these findings should not be interpreted causally, i.e, it is common experience that bDMARDs do not cause PsA. The key message of Meer *et al* is to use caution in interpreting results from retrospective studies. Several confounders and sources of bias should be taken in consideration, such as confounding by indication and the protopathic bias. Furthermore, results could be different depending on the cohort analysed (eg, dermatology clinic-based population or population based) and the way the data were analysed.

Hence, in the topic of transition from PsO to PsA, retrospective studies should be considered as hypotheses generating, but findings need to be validated in prospective studies and randomised controlled trials with an adequate follow-up depending on the selected PsO population.

Factors including the severity of PsO, nail involvement and family history of PsA are long-term predictors of PsA development, while the presence of arthralgia is a short-term predictor.[3 17] These factors for the transition from PsO to PsA may assist researchers in definition of target populations at risk and adequate timing of follow-up periods for prospective transition studies.

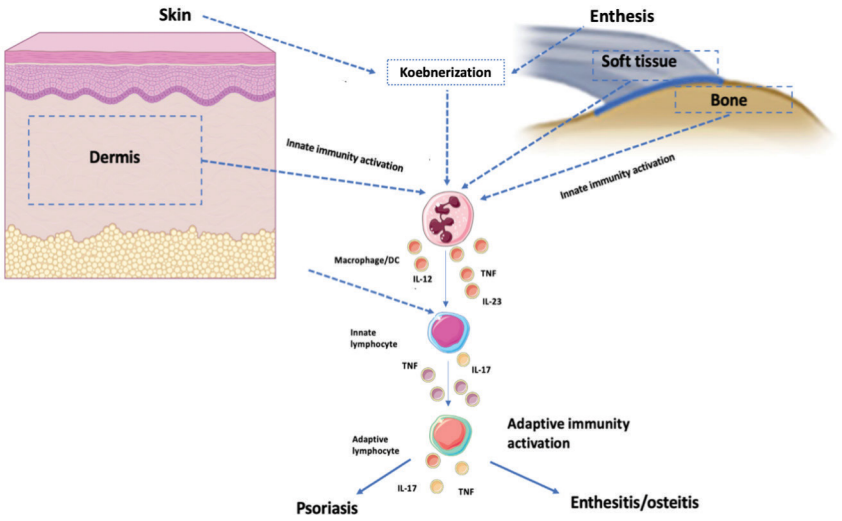

Figure 2 Emerging biological basis for the therapeutic prevention of PSA with drug use for PSO. When considering psoriasis (PsO) and PSA from the perspective of the enthesis, there is clear evidence for convergent paths from tissue microanatomy to immunological mechanisms. Both sites show microanatomical similarities including an avascular (epidermis and fibrocartilage zone respectively) and both are subject to Koebnerisation responses, whereby injury can trigger disease. Convergent immune homoeostasis mechanisms between both sites are increasingly recognised including resident myeloid cells capable of IL-23 production and the presence of both innate and adaptive T cells even in health including ILC3 and γδ T-cells at both the skin and enthesis. Conventional T cells including CD4 and CD8 T-cells including tissue resident memory (TRM) cells are present at both sites. Also some therapies show similar efficacy between skin and joints with respect to pathognomonic dactylitic lesion resolution. Collectively, these provide a strong basis for PSA prevention in PSO treated cases. DC, dentritic cell; IL-23, interleukin 23; PSA, psoriatic arthritis; TNF, tumour necrosis factor.

In the 'PsO to PsA march', there is good evidence that the earliest stage of PsA in experimental models and latterly in humans is linked to early enthesitis with subsequent inflammation spreading to the synovium.[18] In humans, PsO subjects without musculoskeletal complaints have a much greater burden of subclinical articular inflammation compared with healthy controls.[19 20] The evolution towards PsA is associated with the development of synovitis and tenosynovitis, which is again linked to the synovio-entheseal complex.[21] Ultrasound determined subclinical enthesopathy regresses under biological therapy in PsO subjects[22] and likewise subclinical MRI determined synovitis also regresses under biological therapy,[23] with both these studies providing mechanistic corroborative evidence that underpins the findings from the Gisondi *et al* and Acosta Felquer *et al* studies.[13 14]

POTENTIAL THERAPY FOR PSA INTERCEPTION

Noting the aforementioned arguments around the centrality of enthesitis it is noteworthy that methotrexate was thought not to work for enthesitis but is now endorsed in some quarters for that purpose.[24] This raises the possibility that c-DMARDs that are less effective for established enthesitis may nevertheless have a role in preventing it. The anti-TNF agents work less efficiently for skin disease compared with the emergent IL-23/IL-17 inhibitors and it will be interesting to see whether there are any emergent differences between biological classes for possible PsA interception.[25 26] Furthermore axial PsA evolution may overlap with ankylosing spondylitis (AS)—a disease where IL-23 pathway blockade failed.[27] However, in experimental models the pre-emptive use of IL-23 blockers was associated with the non-evolution of axial disease even though it could not treat established disease.[28] Overall, this supports the idea that there may be broad protection with different cDMARD and bDMARD classes for the interception of PsA.

BIOLOGICAL RATIONALE FOR PSA PREVENTION AND INTERCEPTION

It is increasingly clear that there is a close connection between the immunopathogenesis of skin and joint disease in PsO and PsA with both the normal skin and enthesis sharing IL-23/IL-17 axis immunogenetics and innate as well as adaptive IL-23/17 lineage immune cells in healthy tissue.[25 26] The emergent IL23/IL-17 axis blockers are associated with skin clearance in up to 50% of cases, however, responses in signs and symptoms of PsA (eg, American College of Rheumatology 20%/50%/70% response rates) are modest, which has been interpreted as a relative lack in depth of response in PsA. However, the clearance of dactylitis, the pathognomonic lesion of PsA, is reported up to 80%–90% of cases at 6 and 12 months.[29] This illustrates a closer therapeutic connection between skin and joint than hitherto appreciated as does the similar responses of PsA to IL-17A, IL23 and TNF inhibitor class drugs.

IMPLICATIONS

Many questions remain. Might the prevention of PsA, which is one systemic feature of PsO, also have implications for prevention of other complications of PsO as for example ischaemic heart disease that appears to be more frequent in PsA subjects ? What is the impact of different modes of action of bDMARDs on the metabolic syndrome and its clinical consequences? The majority of PsA patients present with mild skin involvement and usually do not need a systemic treatment or a dedicated dermatological follow-up. The crux for this group is how to prevent/intercept PsA in PsO patients if biological therapies for skin disease with lower PASI scores would be based on a higher PsA risk (figure 1). These milder PsO cases lead to the new hypothesis of 'Treat the skin To Intercept PsA' (figure 1B)—a fascinating challenge in the next years. Moreover, the consideration of reduction of PsA development as new clinical outcome/endpoint in PsO clinical trials may be of specific importance, particularly in the subset of PsO patients at high risk for transition.

To summarise, our dermatological colleagues may have already ushered in the era of PsA prevention without additional toxicity or cost implications. Validating and refining and understanding this across the full spectrum of PsO including mild disease represents a new challenge.

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Significance of structural changes in the sacroiliac joints of patients with axial spondyloarthritis detected by MRI related to patients symptoms and functioning

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ABSTRACT

Axial spondyloarthritis (axSpA) is a chronic inflammatory rheumatic disease that manifests primarily in the axial skeleton, initially mostly in the sacroiliac joints (SIJ), usually later spreading to the spine. The disease is characterised by inflammation and new bone formation which are mainly assessed by conventional radiography (CR) and magnetic resonance imaging (MRI). Tumour necrosis factor inhibitors (TNFi) and interleukin-17 antagonists have been shown to be efficacious and efficient in patients with axSpA. This treatment seems to also inhibit structural damage, for example, retard radiographic progression. Indeed, a reduction of new bone formation in the spine, as assessed by CR, has been reported to occur after at least 2 years of therapy with TNFi. Recently, a reduction of erosions and ankylosis in the SIJ has also been observed in axSpA patients treated with etanercept and filgotinib. In this narrative review, we discuss the limited significance of such findings.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory rheumatic disease[1 2] that covers both, radiographic axSpA (r-axSpA)—which is almost equivalent[3] to the classical ankylosing spondylitis (AS)—and non-raxSpA (nr-axSpA). This distinction, which is more important for classification than diagnosis,[4] is on the one hand based on the 2009 ASAS-classification and the 1984 New York (NY)-classification criteria for axSpA[5] and AS,[6] respectively. On the other hand, it is historically grown because the first biological diseas- modifying antirheumatic drugs (bDMARDs) had been approved for AS, now r-axSpA, first, and—to get an approval for the whole spectrum, a second approval for nr-axSpA had to be obtained. Only the fifth tumour necrosis factor inhibitors (TNFi) in line, certolizumab, tried to get both approvals at a time[7]—which has been difficult for various reasons but finally succeded.

AxSpA is characterised by inflammation of the sacroiliac joints (SIJ), the spine, peripheral joints and entheses as well as extramusculoskeletal pathology affecting eye, skin, gut and heart.[2] The main musculoskeletal findings are sacroiliitis, spondylitis, synovitis and enthesitis. Sacroiliitis is associated with inflammatory back pain localised to the buttocks that may alternate between both sides.[1]

The chronic inflammation in the axial skeleton may lead to erosions and new bone formation in the SIJ and the spine.[1 2] While inflammation—mostly in form of a bone marrow oedema —can only be detected by MRI, structural changes such as erosions and ankylosis can also be detected by conventional radiography (CR) and computed tomography (CT).[8] There is evidence that MRI can also detect structural changes including fat metaplasia that is not detected by any other imaging technique.[9 10] Assessment in SpondyloArthritis international Society (ASAS) recommendations for description and diagnosis of MRI changes in the SIJ have been recently updated.[11]

Neglecting the substantial genetic impact on the pathology of axSpA—which is not only due to HLA B27[12]—and concentrating on the established link between inflammation and new bone formation which has been defined several years ago[13 14] the hypothesis of the sequence of pathophysiologically relevant events is: inflammation[15 16] associated with osteoclastic activity[17]—repair and tissue transformation mechanisms such as fat metaplasia[9 18]— and new bone formation.[19] However, the significance of that sequence is limited to the presence of fat metaplasia in the joint space that has also been called 'backfill' in the SIJ, findings that may relate to subsequent ankylosis.[20 21] Indeed, SIJ ankylosis and fat metaplasia but not inflammatory lesions increased the propensity for spinal radiographic progression.[20]

BDMARDs such as the TNFi are clinically efficacious, recommended[22] and they can inhibit structural changes in the spine—with the main target syndesmophytes—of patients with r-axSpA when given over a period of several years.[23] In the last years, several studies have reported that the incidence of erosions in the SIJ can be reduced by treatment with TNFi—by both CR[24–29] and MRI,[21 30] as compared with a more natural course.[31–33]

The aim of this narrative review is to discuss the significance of such findings.

Detection of structural changes in the SIJ

Ever since the NY criteria (Rome, 1961) were first published in 1963,[34] structural changes in the SIJ have played a central role for identification of patients with AS. Even though it was tried to precisely define the SIJ changes seen on radiographs,[35] it was never easy to judge whether definite structural changes were present or not and even a special training did not lead to better results.[36] In studies aimed for an approval of bDMARDs for nr-axSpA, disagreement on the degree of SIJ structural changes occurred frequently.[37]

In an early German spondyloarthritis inception cohort (GESPIC), the first transition rate from no definite change to definite change in the SIJ or from nr-axSpA to r-axSpA was reported to be 12% within 2 years.[31] In the French early axSpA cohort "Devenir des Spondyloarthrites Indifférenciées Récentes (DESIR), the progression rate was lower: only 5% in 5 years.[32] One methodological problem with these studies is that the observed structural changes in the SIJ are not unidirectional[38] implying that there is also 'improvement', which is pathophysiologically unlikely to occur. Another problem is that many severe patients who develop structural changes early within the first 2 years of back pain can already be classified as r-axSpA before they are even diagnosed which has indeed been reported[39]—and it still takes an average of 5 years to be diagnosed with axSpA.[40]

There is increasing evidence that structural changes in the SIJ are also well detected by MRI.[9 41] The main pathologies observed are erosions and ankylosis. The diagnostic role of fat metaplasia in the bone or in the joint space (the latter also known as 'backfill') is less clear.[42] In all studies performed so far, the changes observed have been rather small. However, several studies have reported such small improvements. While it seems possible that erosions improve, there is so far no evidence that ankylosis can 'really' improve. Furthermore, the 'backfill' of an erosion has also to be considered as new bone formation which cannot really be considered as a relevant aim for therapeutic strategies. The methodological question whether spurious changes can be reliably differentiated from true changes in these MRI studies can only be answered no, we can't but we can come close to statistical truth by using clear definitions, several experienced readers, providing a good imaging quality and adequate statistical methods.

More recent data have even shown that the yield may be even better with MRI as compared with the historical routine standard CR.[43] A major argument backing this data is the fact that the latter is two-dimensional whereas MRI, similar to CT,[44] provides a three-dimensional image with much better anatomical insights. In addition, fat metaplasia cannot be detected by CR but may indicate a pathological change indicative of axSpA.[9] In summary, structural changes in the SIJ can also be detected by CR and MRI but, as shown in a recent direct comparison to MRI and CR, CT had the best accuracy for diagnosing axSpA.[45] Nevertheless, although CT can still be considered as the gold standard to detect structural changes in the SIJ, new methods seem to increasingly challenge this role.[46 47]

Detection of structural changes in the spine

The detection of structural changes in the spine by CR is usually quantified using the modified Stokes AS Spinal Score (mSASSS)—a widely used and established scoring system,[48] however, with some limitations: the most frequently affected thoracic spine is not included,[49] and only the anterior part of the spine is being assessed, while the posterior part and the facet joints are left out.

There is limited evidence on the significance of vertebral erosions[50 51] in axSpA. On the one hand, they are only infrequently found (about 1% of all vertebral bodies assessed) but, on the other hand, they seem to be somewhat predictive of syndesmophyte formation.[51] Since syndesmophytes are detected much more frequently in the spine than erosions, they are clearly in the centre of research and clinical care.[52] Low-dose CT seems to be a way to detect structural changes in the spine with better sensitivity and specificity.[53 54]

In summary, inflammation and new bone formation are the central pathological events in axSpA. Even though the exact sequence of events is not entirely clear, it seems likely that inflammation comes first, then erosions follow, mainly in the SIJ, while in the spine this is not often detected, and finally new bone formation comes in. All these events seem to possibly occur in parallel and fat metaplasia occurs rather between inflammation and bone formation but may also remain the only lesion.

Prediction of new bone formation in the axial skeleton

In GESPIC, the presence of syndesmophytes at baseline, elevated levels of acute-phase reactants, and cigarette smoking were all independently associated with spinal radiographic progression in patients with early axial SpA,[55] while there also seem to be differences between male and female patients with less damage in the latter.[56] Whether the degree of radiographic changes in the SIJ predicts syndesmophyte formation seems likely but has not been shown to date.

Therefore, the question arises whether structural MRI changes in the SIJ are important for the prediction of syndesmophytes, implying that a possible reduction of structural MRI changes in the SIJ may also be associated with the prevention of new bone formation in the SIJ and functionally more important, in the spine. This relates to the hypothesis that early anti-inflammatory interventions in axSpA—similar to RA—will prevent future radiographic damage and inhibit new bone formation.

Structural changes in the SIJ in axSpA patients on anti-TNF therapy

Four recent studies evaluated the significance of structural changes in the SIJ. In the first study, "Effect of Etanercept on Symptoms and Objective Inflammation in nr-axSpA" (EMBARK)[28], erosion and fat metaplasia of the SIJ were each scored from 0 to 8 per slice for five MRI slices (total score 0–40). Backfill and ankylosis were each scored from 0 to 4 per slice for five slices (total score 0–20). From baseline to 12 weeks, change in mean scores was significantly greater for etanercept than placebo for erosion (–0.57 vs –0.08, respectively) and backfill (0.36 vs 0.06) i.

In the second study on EMBARK data,[30] using CR, there was a slightly positive change (worsening) in the total SIJ score for the control group (DESIR) vs a slightly negative change (improvement) in the etanercept group after 104 weeks (least squares mean change: 0.08 (95% CI −0.04 to 0.20) vs −0.14 (95% CI −0.26 to −0.01)).

In the third study on EMBARK data[29], again MRI was used, with the same scoring as above. On etanercept, the erosion mean change was − 0.81 (95% CI − 1.09 to –0.53); for the control group (DESIR), erosion mean change was − 0.23 (95% CI − 0.64 to 0.18). The net percentage of patients with a decrease in erosion was significantly greater for etanercept vs controls: 23.9% (95% CI 15.7% to 32.2%) vs 5.3% (95% CI − 6.8% to 17.3%).

In the last one,[57] similar results were published for a targeted synthetic (ts) DMARD, the Janus kinase inhibitor (JAK) inhibitor filgotinib. Similar studies on other compounds such as the IL-17 inhibitor ixekizumab are on the way.

The pathophysiological general question whether improvement of erosions and ankylosis is possible at all—and if these are likely to occur at the same time—cannot definitely answered at present. As already mentioned, the observed changes are rather small but they point in the same direction in different studies. We do not think that these reports justify a claim of structural

disease modification. Thus, currently the main point is that the likelihood that these changes make a difference in terms of function is rather low.

Functional consequences of bone formation in the axial skeleton

There is no reasonable doubt that both, inflammation and new bone formation contribute to impaired function in patients with axSpA.[58] AxSpA patients participating in GESPIC had rather slow progression rates of structural damage in the spine.[59] Since these were patients in early disease stages, disease activity was a stronger determinant of function than radiographic damage, while this is different in later disease stages when bone formation is more important. Thus, only a minority of early patients suffers from rapid radiographic spinal progression which is different in later disease stages.[60]

When will structural damage become clinically relevant with functional impairment? In 10-year study on TNFi treated patients function, as assessed by the Bath AS functional index (BASFI) and spinal mobility, as assessed by the Bath AS mobility index (BASMI), remained stable over time - despite radiographic spinal progression, and no association between the change in mSASSS and BASFI was found, while there was some effect of mSASSS on BASMI changes over time. The data of that study also indicated that, over time, an increase of 20 and 12 mSASSS points would be responsible for an increase of one BASFI and one BASMI point, respectively.[61] However, that was a mixed population of patients with r-axSpA and nr-axSpA. Earlier studies on 'pure' AS populations have indicated that most of the functional decline occurred within the first 10 years.[62]

In another GESPIC paper[63] statistical analyses adjusted for structural damage in the spine (assessed by mSASSS), disease activity, as assessed by the Bath AS disease activity index (BASDAI), and gender, revealed an independent association of a sacroiliitis sum score with BASFI and BASMI. Change by one radiographic sacroiliitis grade in one joint was associated with BASFI/BASMI worsening by 0.10/0.12 points, respectively, independently of disease activity and structural damage in the spine. These data show that there is a minimal almost neglectable effect of structural SIJ changes on function and mobility. In comparison, structural changes in the spine are much more relevant for function and mobility. As recently discussed in much detail,[64] function is a major clinical outcome for patients with axSpA which needs to be put into perspective, also in comparison to treat-to-target strategies that concentrate more on disease activity.

CONCLUSION

In summary, there is some evidence that the clinical significance of very small changes in the structure of SIJ in patients with axSpA is rather limited. The low frequency of vertebral erosions makes it unlikely that this finding will ever be clinically relevant for spinal disease. Future research should focus on MRI changes in both, the SIJ and the spine with short intervals of examinations and long follow ups to shed more light on the fascinating pathology that can be observed in axSpA. The most important question whether the course of the disease can be substantially changed if anti-inflammatory interventions are set in place very early remains exiting. Predicting a more severe course of disease is one of the main challenges in that regard.

Finally, we like to clearly state that the rather small effect of b-DMARDS and ts-DMARDS on structural SIJ changes as assessed by MRI recently reported is not sufficient to make a claim for structural modification of any of the drugs tested. In our opinion, effects on new bone formation in axSpA need to be shown in the spine.

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From sequential to combination and personalised therapy in lupus nephritis: moving towards a paradigm shift?

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ABSTRACT

The current treatment paradigm in lupus nephritis consists of an initial phase aimed at inducing remission and a subsequent remission maintenance phase. With this so-called sequential treatment approach, complete renal response is achieved in a disappointing proportion of 20–30% of the patients within 6–12 months, and 5–20% develop end-stage kidney disease within 10 years. Treat-to-target approaches are detained owing to uncertainty as to whether the target should be determined based on clinical, histopathological, or immunopathological features. Until reliable non-invasive biomarkers exist, tissue-based evaluation remains the gold standard, necessitating repeat kidney biopsies for treatment evaluation and therapeutic decision-making. In this viewpoint, we discuss the pros and cons of voclosporin and belimumab as add-on agents to standard therapy, the first drugs to be licenced for lupus nephritis after recent successful randomised phase III clinical trials. We also discuss the prospect of obinutuzumab and anifrolumab, also on top of standard immunosuppression, currently tested in phase III trials after initial auspicious signals. Undoubtably, the treatment landscape in lupus nephritis is changing, with combination treatment regimens challenging the sequential concept. Meanwhile, the enrichment of the treatment armamentarium shifts the need from lack of therapies to the challenge of how to select the right treatment for the right patient. This has to be addressed in biomarker surveys along with tissue-level mapping of inflammatory phenotypes, which will ultimately lead to person-centred therapeutic approaches. After many years of trial failures, we may now anticipate a heartening future for patients with lupus nephritis.

INTRODUCTION

In different cohorts, 35%–60% of patients with systemic lupus erythematosus (SLE) develop lupus nephritis (LN) during their disease course.[1 2] Once established, LN should always be considered a severe condition. This is because each LN flare results in irreversible nephron loss corresponding to shortening of the kidney lifespan by decades. Persistently active LN increases the rate of nephron loss, leading to earlier onset of chronic kidney disease (CKD) and end-stage kidney disease (ESKD).[1] Therefore, flares and residual disease activity are essential predictors of long-term renal function impairment, and the management of LN resembles a race against nephron loss.

THE SEQUENTIAL TREATMENT PARADIGM

The current treatment paradigm is so-called 'sequential', comprising an initial phase aimed at inducing disease control and a subsequent phase aimed at maintaining the achieved response. First-line options for the initial or induction phase include oral mycophenolate mofetil (MMF; most commonly 2–3 g/day), or equivalent doses of mycophenolate sodium, and low-dose intravenous cyclophosphamide (CYC) 500 mg every second week for a total of six pulses; high-dose intravenous CYC (given monthly for 6 months, as per the NIH protocol) is still considered another option, however, with potentially more side effects, especially regarding fertility. Alongside, methylprednisolone at a total dose of 0.5–3 g is given in most cases, followed by oral prednisone at a dose of 0.3–0.5 mg/kg/day, tapered to ≤7.5 mg/day within 3–6 months. The subsequent or maintenance phase consists of either MMF or azathioprine (AZA). Calcineurin inhibitors (CNI) are used as an add-on option to optimise therapy, mainly in membranous LN, and are given at the lowest possible therapeutic dose, which necessitates measurement of drug concentrations. Hydroxychloroquine is recommended for all patients unless a contraindication exists.[3] In refractory cases, the treatment should be intensified, which may include therapy switch, addition of a CNI, or rituximab as an off-label option. Importantly, response to initial therapy should not lead to therapy discontinuation; it is recommended to continue for at least 3 years from the flare onset.[3]

With the current sequential treatment strategies, complete renal response (CRR) is achieved in a disappointing proportion of 20%–30% of the patients within 6–12 months from the onset of LN,[4 5] and 20%–35% of the patients who achieve adequate disease control show relapses within 3–5 years.[1 6] At least 20% of LN patients develop CKD and 5%–20% reach ESKD within 10 years from the LN onset,[7–9] not to mention the side effects of current immunosuppressants which contribute to increased morbidity and reduced quality of life.[1] On the whole, it is apparent that improvement of LN therapy and prognosis remains on the agenda. To address this need, the LN research community has focused on two main trajectories, that is, implementation of a treat-to-target approach and a paradigm shift from sequential to combination and personalised therapy.

TREAT-TO-TARGET APPROACH IN THE MANAGEMENT OF LN

With regard to the former trajectory, a key question is whether the target should be determined based on clinical, histopathological or immunopathological features. The Euro-Lupus[8] and MAINTAIN[10] Nephritis Trials provided robust evidence that early decrease in proteinuria predicts good long-term renal outcome, and data from three independent study populations indicated a cut-off of 0.7–0.8 g/day at 1 year to be the best predictor of creatinine values ≤1 mg/dL 7 years after the LN onset.[11–13] However, while the positive predictive value of this target was excellent, the negative predictive value was poor,[11 12] highlighting the need for predictors of poor long-term prognosis. In other words, the remaining challenge is to determine patients at high risk for renal function worsening irrespective of clinical response and might, therefore, benefit from early adjustments in therapy.

The concept of a histopathological target emerged from observations that clinical outcome based on proteinuria and/or urinalysis and histopathological outcome based on repeat kidney biopsies are discordant.[14–16] We recently showed that National Institutes of Health (NIH) activity and chronicity index scores >3 in per-protocol repeat kidney biopsies were associated with subsequent renal relapse and renal function impairment, respectively, while the baseline biopsy exhibited no predictive value.[16] Notably, active lesions in glomeruli mainly accounted for the association with renal relapses, whereas chronic damage in the tubulointerstitial compartment was an important contributor to the association with poor long-term renal function,[16] observations of particular importance in light of evidence that, although underemphasised in current LN classification,[17] the tubulointerstitial compartment is important in the inflammatory process in LN[18 19] and in renal prognosis.[20–22] Knowledge of these associations might help avert the poor renal prognosis in patients at risk by adjusting the treatment, addressed in the imminent multicentre randomised study entitled 'Per-protocol repeat kidney biopsy in incident cases of LN', abbreviated ReBioLup (http://rebiolup.com).[23] Systematic tissue-based survey within ReBioLup or similar endeavours may be foreseen to facilitate drug development through microarchitecture-level and single cell-level understanding of the role of the different compartments of the inflamed kidney towards more effective resolution of the inflammation and deceleration, or, desirably, reverse of fibrosis.

Lastly, recent implications that the degree of resorption of immune deposits in electron microscopy after induction therapy are associated with the clinical response to therapy[24] warrant further survey in relation to long-term prognosis, and support the notion that immunopathological targets may provide additive prognostic value to activity and chronicity scores.

FROM SEQUENTIAL TO COMBINATION THERAPY

Results of two recent randomised phase III clinical trials portend a new era in the management of LN, with combination regimens gradually substituting the sequential treatment paradigm. In fact, these trials led to labelling voclosporin and belimumab for LN.

Voclosporin

The potential of add-on CNIs emerged from multiple studies, particularly from Asia, examining the effect of tacrolimus as a monotherapy or combined with MMF.[25] The effect of a more modern CNI, that is, voclosporin, an analogue of ciclosporin with enhanced action against calcineurin, greater metabolic stability and a quicker elimination of metabolites minimising the need for monitoring, was recently studied in the phase II Aurinia Urinary Protein Reduction Active - Lupus With Voclosporin (AURA-LV) and phase III Aurinia Renal Response in Active Lupus With Voclosporin (AURORA 1) clinical trials of LN. AURA-LV comprised 265 class III–V LN patients and demonstrated higher proportions of CRR at week 48 among patients treated with low-dose (23.7 mg two times per day) or high-dose (39.5 mg two times per day) voclosporin (49% or 40%, respectively) versus placebo (24%) in combination with MMF (2 g/day) and glucocorticoids.[26] The difference at week 24 was statistically significant only for the low-dose voclosporin group (33% vs 19%). More serious adverse events were reported in the voclosporin groups, and a higher number of deaths in the low-dose group compared with the placebo and high-dose voclosporin groups (11%, 1% and 2%, respectively).[26] AURORA 1 comprised 357 patients with class III–V LN exposed to voclosporin (23.7 mg two times per day) or placebo (1:1) on top of MMF and low-dose oral glucocorticoids, commencing at a prednisone dose of 20–25 mg/day with a subsequent forced quick tapering schedule. This combination regimen was given for 52 weeks, with 41% of the patients in the voclosporin group achieving CRR (including urinary protein to creatinine ratio (uPCR)) ≤0.5 and estimated glomerular filtration rate (eGFR) >60 mL/min/1.73 m^2), compared with 23% in the placebo group. The separation was also significant at week 24 and remained significant after stratification into patients of Hispanic and non-Hispanic origin but did not reach significance in the subgroup of white patients (36% of the study population). Moreover, the separation between voclosporin and placebo was significant only in patients who were on MMF at baseline (55%), and not in patients who commenced at MMF at the study baseline (45%).[27] In AURORA 1, the adverse event profile was balanced between the voclosporin and placebo groups.[27]

Importantly, the voclosporin trials demonstrated that a low-dose glucocorticoid regimen is feasible, since the forced tapering schedule requiring that the prednisone dose was decreased to 2.5 mg/day by week 16 was achieved in ≥75% of the patients in each arm, in both trials.[26 27] Questions that remain to be answered include the long-term toxicity of voclosporin, its efficacy regarding prevention of renal flares and extrarenal activity, and the optimal time of withdrawal. As a matter of fact, some scepticism may be raised about proteinuria reduction at least partially being explained by the stabilisation of the podocyte cytoskeleton, thus overestimating the effect of voclosporin on inflammatory renal activity, and the potential tissue-level drug toxicity. Regarding the latter, repeat biopsy data would be desirable to appositely address this concern. Finally, while nephrologists have experience from CNI use and may therefore be expected to embrace a modern CNI with ease, the threshold for rheumatologists is anticipated to be higher.

Belimumab

B cell hyperactivity has a key role in SLE pathogenesis, and blockade of B cell activating factor with belimumab has proven successful in several phase III trials of SLE,[28] which however had excluded patients with severe active biopsy-proven LN. However, a post hoc analysis revealed significantly greater proteinuria reductions in belimumab-treated versus placebo-treated patients,[29] constituting the main rationale for the LN-specific phase III Belimumab International Study in Lupus Nephritis (BLISS-LN) trial.[30] BLISS-LN assigned 448 patients with

biopsy-proven class III–V LN at a ratio 1:1 to intravenous belimumab or placebo as add-on to oral glucocorticoids and either oral MMF or intravenous CYC followed by AZA for a total of 104 weeks. The primary endpoint was the so-called primary efficacy renal response (PERR) at week 104, a composite measure requiring uPCR ≤0.7 and eGFR no worse than 20% below the preflare value or ≥60 mL/min/1.73 m^2. PERR at week 104 was achieved in 43% of patients who received belimumab and 32% of patients who received placebo, yielding a statistically significant separation of 11%, that was also significant at week 52 (47% vs 35%). Consistent separations were seen regarding CRR, with a more stringent uPCR cut-off set at <0.5 (30% vs 20% at week 104), and no safety signals emerged.[30]

While these results may indeed be considered auspicious, worth noting observations from subgroup analyses include that a benefit from belimumab was documented for patients who received belimumab on top of MMF but not for patients who received it on top of CYC/AZA, and in non-black patients but not in the black patient population.[30] Nevertheless, patients in the CYC/AZA group had a more severe disease profile, evidenced by higher levels of proteinuria, lower eGFR, lower complement levels and longer disease duration at baseline. Moreover, African-Americans are known to have a more severe disease course.[1] Among the pros, BLISS-LN is the largest to date clinical trial of LN. Some scepticism is raised about the magnitude of response not exceeding 30% for CRR, along with some recently reported de novo LN cases during belimumab therapy.[31] This said, since belimumab already is an established biological for SLE, the threshold for clinicians to also use it in active LN may be expected to be low.

Other promising add-on agents: obinutuzumab and anifrolumab

B cell depletion is not a new concept for LN. Following encouraging observations from off-label use of the chimeric anti-CD20 monoclonal antibody rituximab, the Lupus Nephritis Assessment with Rituximab (LUNAR) trial tested its efficacy on top of MMF and high-dose glucocorticoids but failed to demonstrate superiority over placebo.[32] Despite the failure of LUNAR and no further investment from the industry, its off-label use in refractory cases is now even recommended.[3] Moreover, the combination of belimumab and rituximab is currently trialled in investigator-initiated settings (SYNBIoSe, NCT02284984[33]; SynBioSe-2, NCT03747159). Among more modern B cell depleting agents, obinutuzumab is a fully humanised anti-CD20 monoclonal antibody that has a partially dissimilar to rituximab binding specificity on CD20, resulting in greater and more sustained depletion. It was recently tested for LN in the phase II NOBILITY trial, that randomised 126 patients with class III/IV±V to receive two pulses of intravenous obinutuzumab or placebo on top of MMF and glucocorticoids, and two more pulses after 6 months. CRR requiring uPCR <0.5 yielded a separation of 12% between the obinutuzumab and placebo group at week 52 in favour of obinutuzumab (35% vs 23%) that became greater and statistically significant at week 104 (41% vs 23%).[34] While NOBILITY was a rather small phase II trial comprising a vast majority of Hispanic participants, the favourable results along with the absence of safety signals warranted the phase III REGENCY trial (NCT04221477) that is currently recruiting patients from a more diverse population.

Anifrolumab is a fully human monoclonal antibody against the type I interferon receptor that has been tested with encouraging results in two phase III clinical trials of non-renal SLE, the Treatment of Uncontrolled Lupus via the Interferon Pathway (TULIP)-1[35] and TULIP-2[36] trials. The phase II TULIP-LN trial randomised 145 patients with biopsy-proven LN to anifrolumab basic regimen (BR; 300 mg), anifrolumab intensified regimen (IR; 900 mg for three doses, 300 mg thereafter) or placebo every fourth week, on top of MMF and glucocorticoids.[37] The trial did not meet the primary endpoint, but IR anifrolumab was numerically superior to placebo across multiple clinical outcomes, including CRR (requiring uPCR ≤0.7) at week 52 (46% vs 31%) and steroid sparing effects. An important observation was that anifrolumab clearance was higher in patients with LN compared with previous experience from the non-renal TULIP trials owing to proteinuria, resulting in suboptimal serum concentrations in the BR group. Apart from the expected higher incidence of herpes zoster in anifrolumab-exposed patients, adverse events were non-serious and similar across groups. Collectively, despite the failure to meet the primary endpoint, the CRR frequency of 46% with the IR justifies the imminent phase III trial.

Lastly, several other pharmaceuticals are currently tested, for example, add-on interleukin-17A inhibition with secukinumab in the phase III SELUNE trial (NCT04181762).

HOW TO CHOOSE? TOWARDS PRECISION MEDICINE

Undoubtedly, the treatment landscape within LN is changing. Until recently, there was no drug approved for LN, with first-line therapy relying on off-label use of mainly MMF and CYC. We are currently witnessing a rapid enrichment of the therapeutic armamentarium with new-generation drugs, shifting the challenge from the need for new treatments to the need to determine how to use them wisely. Indeed, how will clinicians choose among add-on voclosporin, belimumab, obinutuzumab, anifrolumab and future agents?

Initially, drug availability, costs, pregnancy issues and the clinician's experience and gut feeling will steer the choice of therapy. Thus, patients with extrarenal manifestations such as arthritis or skin disease will likely be given a B cell targeting add-on agent rather than a CNI. Hopefully, biomarker mining will soon shed more light on which patient subgroups are expected to respond better to each one of these new regimens, based on tissue-based approaches,[38 39] urinary markers[40] and molecular/cellular signatures. Borrowing examples from rheumatoid arthritis, for example, the recently suggested RNA sequencing-based treatment selection,[41] application of such technologies in tissue-level investigations can be anticipated to entail a vertical take-off towards precision medicine in LN in the future. Moreover, associations between transcriptional signatures in renal tissue and urine samples may engender the prospect of the readily available and non-invasive 'liquid biopsy' in treatment selection and evaluation, as recently contemplated.[39]

CONCLUDING REMARKS

Although direct comparison across trials should be avoided to reckon definition dissimilarities, CRR attainment was ~30% in the placebo arms, illustrating that the current treatment paradigm does not meet the expectations. However, even in the active agent arms, CRR did not exceed—at best—46% which abates the enthusiasm and points to remaining need for improvement also after the introduction of those new therapeutic modalities. This probably addresses the concern about the risk for overtreatment in the abundance of new options. In fact, how many patients do we need to treat differently

to avoid one case of ESKD with current sequential and how many with future combination regimens? Importantly, however, even if the benefit from addition of the newly approved agents voclosporin and belimumab to standard therapy may be considered moderate, preserving renal function for an additional small number of patients is an achievement in the right direction.

Also, should add-on agents be part of the initial phase of treatment or be initiated after a first period of inadequate response? Frankly, are we able to address this question when treatment evaluation relies on an indirect outcome, that is, proteinuria, especially when agents with particular antiproteinuric potency come into play? Until reliable non-invasive biomarkers exist, tissue-level approaches and repeat biopsies will be essential towards treatment optimisation through precision medicine, with the ultimate goal being nephron loss deceleration and preservation of the renal function. For the moment, the research agenda is long, and includes biomarkers in 'liquid biopsy' reflecting kidney histopathology, exploration of the value of repeat kidney biopsies for treatment fine-tuning (for example, in ReBioLup) or withdrawal, and early markers of long-term prognosis. In light of the recent drug approvals, it also includes survey on biomarkers for person-centred treatment selection, optimal time for add-on agent and optimal treatment duration. It is important to bear in mind that trial settings never resemble reality; in fact, both AURORA 1 and BLISS-LN excluded patients with severely impaired renal function and failed to show efficacy of add-on voclosporin in MMF naïve patients or add-on belimumab in black patients in subgroup analyses. Moreover, long-term data on renal relapses are awaited. Nevertheless, the recent trial successes may be an omen of a paradigm shift from sequential to combination regimens for the treatment of LN. In the fullness of time, we foresee a heartening future for patients living with LN.

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EULAR points to consider for the management of difficult-to-treat rheumatoid arthritis

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ABSTRACT

Objective To develop evidence-based European Alliance of Associations for Rheumatology (EULAR) points to consider (PtCs) for the management of difficult-to-treat rheumatoid arthritis (D2T RA).

Methods An EULAR Task Force was established comprising 34 individuals: 26 rheumatologists, patient partners and rheumatology experienced health professionals. Two systematic literature reviews addressed clinical questions around diagnostic challenges, and pharmacological and non-pharmacological therapeutic strategies in D2T RA. PtCs were formulated based on the identified evidence and expert opinion. Strength of recommendations (SoR, scale A–D: A typically consistent level 1 studies and D level 5 evidence or inconsistent studies) and level of agreement (LoA, scale 0–10: 0 completely disagree and 10 completely agree) of the PtCs were determined by the Task Force members.

Results Two overarching principles and 11 PtCs were defined concerning diagnostic confirmation of RA, evaluation of inflammatory disease activity, pharmacological and non-pharmacological interventions, treatment adherence, functional disability, pain, fatigue, goal setting and self-efficacy and the impact of comorbidities. The SoR varied from level C to level D. The mean LoA with the overarching principles and PtCs was generally high (8.4–9.6).

Conclusions These PtCs for D2T RA can serve as a clinical roadmap to support healthcare professionals and patients to deliver holistic management and more personalised pharmacological and non-pharmacological therapeutic strategies. High-quality evidence was scarce. A research agenda was created to guide future research.

INTRODUCTION

Treatment options for rheumatoid arthritis (RA) have expanded with availability of biological and targeted synthetic disease-modifying anti-rheumatic drugs (b/tsDMARDs).[1] The updated European League Against Rheumatism (EULAR, from 2021, European Alliance of Associations for Rheumatology) recommendations for the management of RA[2] focusing on pharmacological therapy are similar to those developed by other international organisations.[3–5] Other recommendations and points to consider (PtCs) provide specific management support on cardiovascular disease (CVD) risk,[6] comorbidities,[7] imaging,[8] pain[9] and patient education.[10] Together with implementation of treat-to-target and tight control strategies,[2 11] specifically in the early phase of the disease, these have contributed to improved outcomes for the majority of patients with RA.

However, some patients with RA do not reach low disease activity or remission and/or remain symptomatic after several cycles of conventional synthetic (cs) DMARDs, bDMARDs and/or tsDMARDs.[12–14] Such patients may be referred to as having 'difficult-to-treat (D2T)' disease. Optimal management of these patients poses a significant challenge in clinical practice.[15] Hitherto, no specific guidance has been developed for the management of this complex patient population. Therefore, an EULAR Task Force was convened to develop PtCs for the management of D2T RA.

METHODS

Steering Committee and Task Force

The convenor (GN) and co-convenor (JMvL) formed the Steering Committee and Task Force that followed the EULAR standardised operating procedures (SOPs).[16] The Steering Committee included the (co-)convenors, a methodologist (DvdH), a co-methodologist (PMJW), a rheumatology post-doctoral fellow (Maria J H de Hair) and three fellows (NMTR, MK and AH). The Task Force comprised the Steering Committee members and another 18 rheumatologists (including 2 EMerging EUlar Network representatives), 3 patient partners, 1 rheumatology nurse, 1 rheumatology occupational therapist, 1 psychologist and 2 pharmacists. All rheumatologists were experienced in the treatment of RA, the majority with significant experience in clinical trials and some also in outcomes

Box 1 Definition of D2T RA[17]

All three criteria need to be present in D2T RA:

1. Treatment according to EULAR recommendations and failure of ≥two b/tsDMARDs (with different mechanisms of action)† after failing csDMARD therapy (unless contraindicated).†
2. Signs suggestive of active/progressive disease, defined as ≥one of:
   a. At least moderate disease activity (according to validated composite measures including joint counts, for example, DAS28-ESR >3.2 or CDAI >10).
   b. Signs (including acute phase reactants and imaging) and/or symptoms suggestive of active disease (joint related or other).
   c. Inability to taper glucocorticoid treatment (below 7.5 mg/day prednisone or equivalent).
   d. Rapid radiographic progression (with or without signs of active disease).‡
   e. Well-controlled disease according to above standards, but still having RA symptoms that are causing a reduction in quality of life.
3. The management of signs and/or symptoms is perceived as problematic by the rheumatologist and/or the patient.

b/tsDMARDs, biological and targeted synthetic disease-modifying antirheumatic drugs; CDAI, Clinical Disease Activity Index; csDMARD, conventional synthetic disease-modifying antirheumatic drug; D2T, difficult-to-treat; DAS28-ESR, Disease Activity Score assessing 28 joints using erythrocyte sedimentation rate; RA, rheumatoid arthritis.
†Unless restricted by access to treatment due to socioeconomic factors.
†If csDMARD treatment is contraindicated, failure of ≥two b/tsDMARDs with different mechanisms of action is sufficient.
‡Rapid radiographic progression: change in van der Heijde-Modified Sharp Score ≥5 points in 1 year[184] or a similar progression in another validated scoring method.

research and patient registries. All 34 Task Force members declared their potential conflicts of interest before the start of the project. Two of the Task Force members (Maria J H de Hair and Loriane Gutermann (pharmacist)) left the Task Force during the process, due to new positions, and did not attend the second and third Task Force meetings.

Target audience

In accordance with the EULAR SOP, the primary target audience of these PtCs is healthcare professionals (HCPs) and patients (and their carers).[16] In addition, these PtCs may serve to highlight unmet needs in D2T RA and, therefore, also target policy-makers, pharmaceutical and health insurance companies.

Definition

As an initial step, a definition and a uniform term for the patient population had to be established. The Steering Committee proposed terminology and created a first draft of a definition, guided by the results of the international survey and a scoping literature review.[15] These were discussed with the whole Task Force and amended during the first Task Force meeting (held in August 2018). The final terminology and definition were agreed by a voting process. All Task Force members agreed with 'D2T RA' as the term and the final definition (box 1).[17]

Clinical questions and systematic literature reviews

The Steering Committee formulated the clinical questions for the systematic literature reviews (SLRs). Clinical questions focused on techniques for the confirmation of the diagnosis of RA and/or a relevant differential diagnosis (either as alternative (ie, misdiagnosis) or coexisting disease mimics). Additional questions centred around the assessment of inflammatory activity in patients with RA in general and in those with specific comorbidities, which may influence this assessment, adherence, pharmacological and non-pharmacological therapeutic strategies for different aspects of D2T RA: patients with limited DMARD choices because of adverse events, comorbidities or other contraindications; patients in whom at least two b/tsDMARD with different mechanisms of action (MOA) failed; and patients with predominantly non-inflammatory complaints (not directly related to inflammation). In addition, the therapeutic role of lifestyle interventions, of goal setting between patients and HCPs and of self-management was assessed. All questions were discussed and finalised during the first Task Force meeting.

SLRs on these questions were performed by the fellows (NMTR, MK and AH) under supervision of the co-methodologist (PMJW) in accordance with the EULAR SOP.[16] As other ongoing EULAR projects were already focusing on adherence and lifestyle factors, it was decided not to perform separate SLRs on these topics, but to refer to the respective SLRs and PtCs.[18 19] For the other questions, PubMed, Embase and Cochrane bibliographic databases were searched for relevant papers until December 2019, as well as EULAR and American College of Rheumatology (ACR) conference abstracts from 2017 up to and including 2019. Relevant papers were selected and critically appraised. Results were summarised, including assessment of risk of bias (RoB).[16] Further details on the methodology and results of the SLRs are published separately.[20 21]

Consensus finding

Based on the results of the SLRs, draft of overarching principles and PtCs were proposed. The results of the SLRs as well as the proposed overarching principles and PtCs were considered, then presented by the Steering Group and discussed at three consecutive online meetings (the second Task Force meeting was split into three different online meetings) of the Task Force in September 2020 and October 2020. Twenty-five, 30 and 27 Task Force members, respectively, participated in these online meetings. Thereafter, overarching principles and PtCs were discussed and amended.

A voting process was applied per PtC. In round 1, a majority of at least 75% was required to accept the PtC. If this was not achieved, the PtC was discussed and amended and subjected to the second ballot. In round 2, a majority of at least 66% was required to accept the rephrased PtC. If this was not achieved, the PtC was discussed and amended again and subjected to the third ballot. In round 3, a majority of at least 50% was required to accept the rephrased PtC. If this was not achieved, the PtC was rejected.

After the meeting, the level of evidence (LoE) and strength of recommendations (SoR) according to the Oxford Centre for Evidence-Based Medicine system were determined.[22] The agreed overarching principles and PtCs were distributed among all Task Force members via email to assess their level of agreement (LoA) for each PtC. LoA was anonymously scored on a scale from 0 to 10 (0: completely disagree and 10: completely agree). LoA is shown as mean (SD) and as the proportion of Task

Force members with an LoA of at least 8. Additionally, a research agenda was created.

All Task Force members reviewed the draft of the manuscript. Thereafter, the manuscript was submitted to the EULAR Quality of Care Committee and the EULAR Council for review and approval. A third virtual meeting was held in April 2021 to discuss the comments by the EULAR Council, with 30 Task Force members in attendance. The manuscript was revised and the final version was submitted to EULAR and subsequently to the journal.

RESULTS

General aspects

Due to the scarcity of high-quality evidence (table 1), we prepared 'PtCs' for the management of D2T RA. Our PtCs complement current EULAR recommendations that also address elements of management of D2T RA.[2] The SLRs and the formulation of the PtCs predominantly focused on topics not addressed previously and refer to several published[2 6–10 23–25] and ongoing EULAR projects where appropriate.[19]

The discussion of the Task Force resulted in 2 overarching principles and 11 PtCs (table 1). The LoE ranged from 3 to 5 and the SoR ranged from C to D, predominantly, because high-quality evidence derived in the population of interest was scarce. The LoA was generally high and ranged from 8.4 to 9.6. The order of PtCs was presented in what was considered as logical sequence—in particular the first two PtCs, which serve as a basis for all subsequent items. The PtCs as presented can be used as a clinical roadmap (figure 1). Below, a point-by-point discussion is presented, explaining the reasoning behind the different topics and the supporting evidence.

Overarching principles

The Task Force formulated the following overarching principles.

(A) These PtCs pertain to patients who fulfil the definition of D2T RA and are underpinned by the EULAR recommendations for the management of RA including the overarching principles (LoA: 9.6 (1.0)).[2 17]

This principle emphasises the relationship between these PtCs and the EULAR definition of D2T RA.[17] All overarching principles and EULAR recommendations for the management of RA also apply to D2T RA.[2] Patients who fail at least two b/tsDMARDs with different MOA, and are, therefore, potentially classified as having D2T RA, fall in phase III of the management algorithm of the 2019 EULAR RA management recommendations. These D2T RA PtCs, therefore, provide further guidance on factors contributing to the D2T RA state. The Task Force unanimously agreed with this overarching principle (100% agreed, first round, n=27).

(B) The presence or absence of inflammation should be established to guide pharmacological and non-pharmacological interventions (LoA: 9.5 (1.3)).

The Task Force emphasised that confirming the presence of inflammatory RA disease activity is essential and should be done prior to adjustment of DMARD therapy. If the persistence of signs and/or symptoms is not caused by RA disease activity, DMARD therapy would in all probability be ineffective and may lead to apparent failure of multiple (b/ts)DMARDs. Concomitant fibromyalgia, osteoarthritis and/or psychological conditions, non-adherence, and comorbidities (eg, infections and malignancies) may contribute to the D2T state.[13 26] Moreover, when the presence of inflammatory activity has been ascertained, the coexistence and role of these factors should be considered. It was agreed that in the absence of inflammatory activity, DMARD therapy should not be escalated (figure 1), and careful tapering might be considered. This overarching principle was accepted in the second round of the voting process (78% agreed, second round, n=24).

Points to consider

(1) If a patient has a presumed D2T RA, the possibility of misdiagnosis and/or the presence of a coexistent mimicking disease should be considered as a first step (LoE: 5, SoR: D, LoA: 9.3 (1.2)).

An accurate RA diagnosis is the cornerstone of appropriate management. In the SLR, very few studies could be identified on this clinically relevant item.[20 27–31] Consequently, this PtC is based on expert opinion, reinforced by indirect evidence.

Misdiagnosis (ie, an alternative disease mimic) may be more common in seronegative disease,[32 33] but should be considered in all patients with D2T RA. Several diseases may mimic ongoing RA disease activity, such as: crystal arthropathies, polymyalgia rheumatica, psoriatic arthritis, spondyloarthritis, Still's disease, systemic lupus erythematosus, Rhupus (RA–lupus) syndrome, idiopathic inflammatory myopathies, vasculitis, remitting symmetric seronegative synovitis and pitting oedema, reactive arthritis (eg, parvo B19, rubella, Whipple's disease and hepatitis B virus (HBV) and hepatitis C virus (HCV) infections), paraneoplastic syndromes, osteoarthritis and fibromyalgia.[1 34] Furthermore, such other conditions may coexist and underlie signs and/or symptoms suggestive of active RA.

Current RA management approaches may also lead to misdiagnosis. Based on the 'window of opportunity',[35] EULAR and other international guidelines emphasise the importance of early diagnosis and immediate DMARD initiation to achieve optimal and sustained benefit.[2 3] However, this raises the possibility of misdiagnosis.[36] In this context, an RA treatment approach would inevitably lead to apparent inefficacy and unnecessary risk of toxicity.

The Task Force unanimously agreed with this PtC (100% agreed, first round, n=24).

(2) Where there is doubt on the presence of inflammatory activity based on clinical assessment and composite indices, ultrasonography (US) may be considered for this evaluation (LoE: 4, SoR: C, LoA: 9.2 (1.4)).

This PtC is linked closely to overarching principle B. In daily practice, composite indices (at patient level) and the clinical evaluation of a joint being swollen (at joint level) are most frequently used to assess the presence of inflammatory disease activity.[2] However, in patients with D2T RA in whom there is a doubt about the presence of inflammation[37] (see also PtC #1), these traditional measures may be difficult to interpret.

Limited (high-quality) evidence was found on diagnostics that can be used to assess the presence or absence of inflammatory disease activity in this patient group.[20] When traditional measures are challenging, US appears to be the most feasible measure to detect inflammatory activity both in patients with D2T RA in general and in those with conditions that might compound assessment, such as obesity or concomitant fibromyalgia. In the general population of RA (where composite indices can be considered reliable), moderate-to-strong correlations were reported between US sum scores and composite indices on a group level.[38–45] In a study in established patients with RA in whom there was explicit doubt about the presence of inflammation, only weak and non-statistically significant correlations between US sum scores and composite indices were found.[46]

Table 1 EULAR PtCs for the management of D2T RA

		LoE[22]	SoR[22]	LoA mean (SD)	≥8/10 (%)
	Overarching principles				
A	These PtCs pertain to patients who fulfil the definition of D2T RA and are underpinned by the EULAR recommendations for the management of RA, including the overarching principles.[2 17]	NA	NA	9.6 (1.0)	97
B	The presence or absence of inflammation should be established to guide pharmacological and non-pharmacological interventions.	NA	NA	9.5 (1.3)	91
	PtCs				
1	If a patient has a presumed D2T RA, the possibility of misdiagnosis and/or the presence of a coexistent mimicking disease* should be considered as a first step.	5	D	9.3 (1.2)	91
2	Where there is a doubt on the presence of inflammatory activity based on clinical assessment and composite indices, US may be considered for this evaluation.	4	C	9.2 (1.4)	91
3	Composite indices and clinical evaluation should be interpreted with caution in the presence of comorbidities‡ in particular obesity and fibromyalgia§ as these may directly heighten inflammatory activity and/or overestimate disease activity.	‡5 §4	‡D §C	9.2 (1.3)	88
4	Treatment adherence should be discussed and optimised within the process of shared decision-making.	5	D	9.5 (1.0)	97
5	After failure of a second or subsequent b/tsDMARD‡ and particularly after two TNFi failures§ treatment with a b/tsDMARD with a different target should be considered.	‡4 §3	‡C §C	9.2 (1.3)	94
6	If a third or subsequent b/tsDMARD is being considered, the maximum dose, as found effective and safe in appropriate testing, should be used.	3	C	8.4 (1.8)	75
7	Comorbidities† that impact quality of life either independently or by limiting RA treatment options should be carefully considered and managed.	5	D	9.3 (0.8)	97
8	In patients with concomitant HBV/HCV infection, b/tsDMARDs can be used‡ and concomitant antiviral prophylaxis or treatment should be considered in close collaboration with the hepatologist§.	‡4 §5	‡C §D	8.9 (1.4)	88
9	In addition to pharmacological treatment, non-pharmacological interventions (ie, exercise‡, psychological§, educational‡ and self-management interventions‡) should be considered to optimise management of functional disability, pain and fatigue.	‡3 §4	‡C §C	9.4 (1.2)	97
10	Appropriate education and support should be offered to patients to directly inform their choices of treatment goals and management.	4	C	9.4 (1.2)	97
11	Consider offering self-management programmes, relevant education and psychological interventions to optimise patient's ability to manage their disease confidently (ie, self-efficacy).	3	C	9.1 (1.7)	91

Continued

Table 1 Continued

	LoE[22]	SoR[22]	LoA mean (SD)	≥8/10 (%)

In case the LoE and SoR differed for different items within a PtC, differences in LoE and SoR are shown using the symbols‡ and §.
*Relevant mimicking diseases, for instance, crystal arthropathies, polymyalgia rheumatica, psoriatic arthritis, spondyloarthritis, Still's disease, SLE, Rhupus syndrome, vasculitis, idiopathic inflammatory myopathies, RS3PE, reactive arthritis (eg, parvo B19, Rubella, Whipple's disease, HBV and HCV infections), paraneoplastic syndromes, osteoarthritis and fibromyalgia.
†Relevant comorbidities: for instance, infections, malignancies, polymyalgia rheumatica and osteoarthritis, and consequences of longstanding destructive disease such as subluxations and joint dislocations.
b/tsDMARD, biological and targeted synthetic disease-modifying antirheumatic drugs; D2T, difficult-to-treat; EULAR, European Alliance of Associations for Rheumatology; HBV, hepatitis B virus; HCV, hepatitis C virus; LoA, levels of agreement; LoE, level of evidence (according to the standards of the Oxford Centre for Evidence-Based Medicine); NA, not applicable; PtCs, points to consider; RA, rheumatoid arthritis; RS3PE, remitting symmetric seronegative synovitis and pitting oedema; SLE, systemic lupus erythematosus; SoR, strengths of recommendations (according to the standards of the Oxford Centre for Evidence-Based Medicine); TNFi, tumour necrosis factor inhibitor; US, ultrasonography.

This suggests that US may be better related to 'true' inflammatory activity in these patients and may have additional value in patients with D2T RA in whom a doubt about the presence of inflammatory activity exists. However, the minimal number of joints that should be included in an US assessment remains unclear,[41] which hampers the use of a sum score to determine the overall level of disease activity in daily practice. Of note, no studies were found on tests in patients with comorbidities that may influence the assessment of disease activity.

The evidence for biomarkers (eg, miR-146, fibrinogen, resistin, matrix metallopeptidase 3, interleukin 6 and multibiomarker disease activity score) and other imaging measures (eg, MRI or optical spectral transmission measures) is currently less convincing.[20 40 47–61] The quality of this evidence was low to moderate and no evidence could be identified on their role in patients in whom there was explicit doubt about the presence of inflammatory activity resulting in indirectness. These limitations hamper the current use of these biomarkers and imaging modalities in daily practice.

The Task Force unanimously agreed with this PtC (100% agreed, first round, n=24).

(3) Composite indices and clinical evaluation should be interpreted with caution in the presence of comorbidities‡, in particular obesity and fibromyalgia§, as these may directly heighten

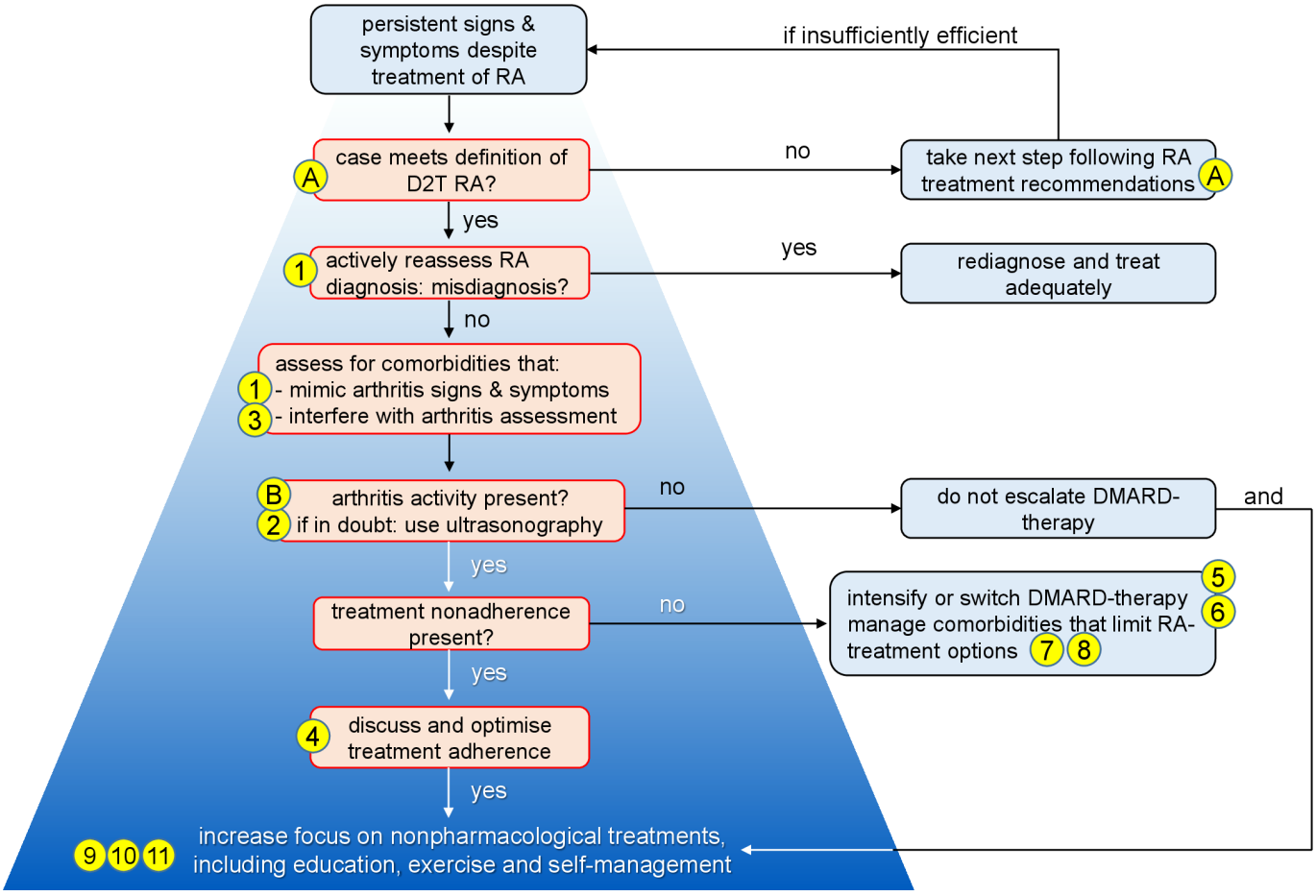

Figure 1 Algorithm based on the EULAR PtCs for the management of D2T RA. The pyramid background with increasing intensity of blue colour indicates non-pharmacological approaches and treatments, which are important throughout all phases of RA, but especially so if pharmacological treatment options are limited. The letters and numbers indicate the corresponding overarching principles and PtCs, respectively; see table 1. D2T, difficult-to-treat; DMARD, disease-modifying antirheumatic drug; EULAR, European Alliance of Associations for Rheumatology; PtCs, points to consider; RA, rheumatoid arthritis.

inflammatory activity and/or overestimate disease activity (‡LoE: 5, SoR: D; §LoE: 4, SoR: C; LoA: 9.2 (1.3)).

Although the Task Force was unanimous in its opinion that numerous comorbidities might influence the assessment of inflammatory disease activity, substantial evidence was only found for obesity and fibromyalgia.[20 62–65] These two conditions may also frequently coexist, further complicating the precise assessment of inflammatory disease activity. Other comorbidities (especially those increasing acute phase reactants: eg, infections, malignancies or polymyalgia rheumatica) may lead to misclassification of inflammatory RA activity, although no substantial evidence was identified to support this. In addition, no evidence was identified regarding the impact of osteoarthritis, subluxation or joint dislocations on clinical evaluation of joints.[20] It should be noted that the identification of synovitis and tenderness due to inflammation is generally more difficult in joints with destruction, since, for example, tenderness could be due to destruction rather than synovitis. The Task Force agreed that this PtC should refer to all potential comorbidities that may influence the evaluation of inflammatory disease activity. The Task Force unanimously agreed with this PtC (100% agreed, first round, n=24).

(4) Treatment adherence should be discussed and optimised within the process of shared decision-making (LoE: 5, SoR: D, LoA: 9.5 (1.0)).

In RA, drug non-adherence rates reportedly vary between 30% and 80%[18 66–68] and these rates are indicated to be substantially higher in patients with D2T RA compared with patients with non-D2T RA.[26] Suboptimal adherence is associated with higher disease activity levels, which may result in inappropriate treatment switches and reduced quality of life.[69–73] In a patient with D2T RA, this could exhaust all currently available (b/ts) DMARDs. Therefore, the Task Force unanimously agreed that adherence should be addressed as a standalone PtC. Another EULAR project has recently provided detailed PtCs for the detection, assessment and management of non-adherence in people with rheumatic and musculoskeletal diseases (RMDs). We, therefore, refer to their SLR and PtCs.[18 19]

The Task Force agreed to concur with WHO definitions[74] and especially considered ‘treatment adherence’ instead of ‘drug adherence’, as the PtC also applies to non-pharmacological strategies. There is no gold standard for identifying non-adherence. Questionnaires or serum and/or urine drug level measurements may be used.[18 75 76] If suboptimal adherence is present, this might be explained by various factors; both unintentional (eg, forgetting to take the prescribed drugs) and intentional non-adherence (driven by a decision not to take the prescribed drugs, eg, due to fear of side effects) are common in RA.[66 76 77] The patient’s evaluation of the risk–benefit ratio of the selected drug(s) is also of paramount importance. Therefore, discussions on adherence remain highly important. In addition to physicians, other HCPs, such as nurses experienced with patients with RA, psychologists and pharmacists, may also be involved in these discussions.

Shared decision-making is clearly vital to optimise adherence.[18 76] In this context, the quality of the relationship between the patient and the HCP is important.[78 79] As non-adherence is a vulnerable topic, the patient should be made to feel safe and supported to discuss all aspects. In addition, appropriate education, especially in case of intentional non-adherence, would be useful and could strengthen the process of shared decision-making (see also PtCs ‡9 and 10).[18 76] This PtC was accepted in the first round of the voting process (96% agreed, first round, n=28).

(5) After failure of a second or subsequent b/tsDMARD‡ and particularly after two tumour necrosis factor inhibitor (TNFi) failures§ treatment with a b/tsDMARD with a different target should be considered (‡LoE: 4, SoR: C; §LoE: 3, SoR: C; LoA: 9.2 (1.3)).

Increasing numbers of b/tsDMARDs (with different MOA) are available for the treatment of RA.[80] Switching within class as well as switching to a drug with a different MOA can be effective.[2 20 80] However, a considerable proportion of patients with RA fail at least two b/tsDMARDs with different MOA, which may result in reaching criteria for D2T RA.[12 13 81] In routine practice, a trial-and-error approach to DMARD cycling predominates when signs and/or symptoms suggestive active disease are present.[13] In the SLR, only limited evidence was identified on pharmacological therapeutic strategies in patients with RA in whom at least two b/tsDMARDs (specifically with different MOA) failed.[21] Several identified trials in patients with RA in whom multiple b/tsDMARDs failed did not clearly state reasons for previous DMARD failure (eg, toxicity, lack of efficacy or other factors). This resulted in the inclusion of heterogeneous patient populations, complicating interpretation of outcomes.

After failure of at least two b/tsDMARDs, some evidence was identified regarding the beneficial effect of treatment with a b/tsDMARD with a different target.[21] This evidence indicated that a third or fourth b/tsDMARD (ie, tocilizumab, tofacitinib, baricitinib, upadacitinib and filgotinib) is more effective than placebo.[82–87] However, no preference can be given to any of these DMARDs. In patients with failure of at least one prior bDMARD, TNFi, abatacept and rituximab were more effective than placebo,[80 88–92] although direct evidence was lacking about the efficacy as third and fourth bDMARD compared with placebo.[21] Where a higher number of prior bDMARDs had been ineffective, the extent of the beneficial effect of several b/tsDMARDs (TNFi and the lower doses of tocilizumab, tofacitinib and baricitinib) was less.[82 83 93–97] Furthermore, a tendency was identified for non-TNFis to be more efficacious than TNFis in patients in whom at least one bDMARD failed (predominantly if TNFi was failed).[88 89 95 98–115] Our current PtC proposes to switch to a b/tsDMARD of different MOA, after failure of a second or subsequent b/tsDMARD and, particularly, after failure of two TNFis. This PtC was accepted in the first round of the voting process (96% agreed, first round, n=24).

The Task Force emphasised that the current PtC is in line with the 2019 EULAR RA recommendation on b/tsDMARD switches. Our PtC adds the following: first, there is value in prescribing another b/tsDMARD after failure of a second or subsequent b/tsDMARD; and second, a b/tsDMARD with a different MOA is preferred after failure of a second or subsequent b/tsDMARD.[2] Concerning DMARD combination therapy, we refer to the 2019 RA EULAR recommendations, as no additional evidence was identified for D2T RA.[2]

(6) If a third or subsequent b/tsDMARD is being considered, the maximum dose, as found effective and safe in appropriate testing, should be used (LoE: 3, SoR: C, LoA: 8.4 (1.8)).

The extent of the beneficial effect of b/tsDMARDs was generally less in patients in whom a higher number of previous bDMARDs failed.[21] This tendency was not so apparent for upadacitinib and filgotinib, and for the higher doses of tocilizumab (intravenously administered, 8 mg/kg), baricitinib (4 mg once daily) and tofacitinib (10 mg two times per day, although tofacitinib is not licensed at higher doses than 5 mg two times per day because of safety concerns).[82 83 85 87 96 97] It should be noted, however, that baricitinib (4 mg once daily) should not be used in patients older than 75 years or those with reduced creatinine clearance (30–60 mL/min).

This suggests that the higher doses of intravenous tocilizumab, and tofacitinib and baricitinib may be preferred in patients in whom previously a higher number of bDMARDs failed.[82 83 96 97] The evidence supports the use of higher doses from the beginning, excepting patients in whom contraindications for this higher dose are present.

In addition, it was argued that this PtC might be more informative by including the names of the specific b/tsDMARD (baricitinib and tocilizumab, and not tofacitinib, as tofacitinib is not licensed at higher doses than 5 mg two times per day). The following wording was accepted (95% agreed, first round, n=22): 'If a second or subsequent b/tsDMARD has failed, and baricitinib or iv tocilizumab are being considered, the higher licensed dose should be used if appropriate'. However, it was also discussed that explicitly mentioning drug names (ie, baricitinib and tocilizumab) should be avoided in management PtCs as novel evidence may emerge for other drugs. Therefore, the Steering Committee initiated a new voting after the Task Force meeting regarding this PtC without explicit drug names. The Task Force members agreed to change the wording of the PtC and to exclude the drug names resulting in the current recommendation (94% agreed, second round, n=32).

(7) Comorbidities that impact quality of life either independently or by limiting RA treatment options, should be carefully considered and managed (LoE: 5, SoR: D, LoA: 9.3 (0.8)).

In clinical practice, comorbidities may significantly limit treatment options, potentially contributing to the D2T state.[7 13 15 116] The Task Force agreed to formulate a PtC on the importance of comorbidities (100% agreed, first round, n=28).

We sought evidence about safe and efficacious therapies in patients with such contraindications.[21] No studies were identified for patients with RA with HIV, gastrointestinal disease, latent tuberculosis and malignancies; only limited evidence was identified for patients with RA with extra-articular manifestations, hepatic disease, osteoporosis, psychological distress, pulmonary disease and renal disease. More than one study per intervention was identified only for patients with RA with HBV, HCV (see also PtC #8), CVD, before and during pregnancy and lactation, and obesity.

Concerning venous thromboembolisms (VTEs), higher frequencies of VTEs were reported in patients with RA using tsDMARDs at high doses, and in whom risk factors for VTE are present.[117] The Task Force unanimously agreed that in patients at risk for VTEs, tsDMARDs, specifically at high doses, should be used with caution and per drug label recommendations. As this item is covered in the 2019 EULAR RA management recommendations[2] and as the increased risk of VTEs is not specific for patients with D2T RA, the Task Force unanimously decided not to include this item as a standalone PtC (no formal voting). Nevertheless, the increased risk of VTEs should be considered as factor limiting treatment options, particularly for patients with D2T RA with VTE risk factors.

Recommendations about safe DMARDs use before and during pregnancy and lactation are published as 2016 EULAR PtCs and as a 2020 ACR guideline.[118 119] Few additional studies were identified, subsequently on these papers[21 120–122]; therefore, we refer to the existing guidance.[118 119]

Although obesity does not limit drug options per se, treatment efficacy might be different in obese patients.[13 123] Intravenously administered infliximab may be less effective in patients with a body mass index (BMI) above 30 kg/m^2 compared with those with a BMI below 30 kg/m^2.[124 125] The Task Force voted whether this issue should be a standalone PtC. The first vote did not clearly indicate the preference of the Task Force (formulate a separate PtC on this item 58%, n=24). Further discussion noted that evidence for several other comorbidities was lacking or very limited. Two studies of relevance had a high RoB.[124 125] The repeat vote indicated not to formulate a separate PtC on this item (formulate a separate PtC on this item: 12%, n=24).

Clinically meaningful contraindications of some therapies may result in limited treatment options, for example, tocilizumab in case of diverticulitis or janus kinase (JAK) inhibitors in case of repeated herpes zoster infections.[117] However, no substantial clinical evidence was identified about safe and/or efficacious therapies for patients with these conditions[21] and, therefore, no specific PtCs were formulated. A broad range of comorbidities and coexisting conditions were discussed at the Task Force meeting but are not explicitly part of the PtCs due to the lack of evidence.[21]

(8) In patients with concomitant HBV/HCV infection, b/tsDMARDs can be used‡ and concomitant antiviral prophylaxis or treatment should be considered in close collaboration with the hepatologist§ (‡LoE: 4, SoR: C, §LoA: 5; SoR: D, LoA: 8.9 (1.4)).

Substantial evidence was identified related to HBV and HCV infections prompting a standalone PtC.[21] TNFi, abatacept and tocilizumab may be considered in patients with HBV,[126–128] and TNFi in patients with HCV.[129 130] Furthermore, no evidence was identified regarding other b/tsDMARDs, but this does not indicate that these b/tsDMARDs are unsafe to use. Therefore, the Task Force voted not to include specific b/tsDMARDs in the PtC (83% agreed, n=24). Furthermore, the Task Force agreed that concomitant antiviral prophylaxis should be considered,[126] and that the treatment should be conducted in close collaboration with the hepatologist. The Task Force unanimously agreed with this PtC (100% agreed, first round, n=24). It should be noted that concomitant antiviral prophylaxis is appropriate for HBV infection in case of HCV infection, antiviral treatment is necessary.

(9) In addition to pharmacological treatment, non-pharmacological interventions (ie, exercise‡, psychological§, educational‡ and self-management interventions†) should be considered to optimise management of functional disability, pain and fatigue (‡LoE: 3, SoR: C; §LoE: 4, SoR: C; LoA: 9.4 (1.2)).

A wide spectrum of factors may contribute to the persistence of signs and/or symptoms, although these are not always directly related to inflammation (eg, functional disability, pain and fatigue).[13 26] Individually tailored non-pharmacological interventions are also important components of the management of D2T RA.[13 21 26] The SLR focused on non-DMARD interventions to improve non-inflammatory complaints in patients with RA who do not clearly have active inflammatory disease.[21] It is not always possible to disentangle inflammatory and non-inflammatory symptoms in clinical practice. Non-pharmacological interventions should also be considered in all patients with D2T RA[26] and not only in those patients without inflammatory RA activity.

Evidence emerged regarding the beneficial effect of exercise, education, psychological and self-management interventions to improve pain, fatigue and functional disability in RA, while substantial evidence regarding the role of non-pharmacological interventions to improve quality of life was lacking.[21] Benefit of exercise in RA is well established[131] and was specifically found to improve physical functioning. A wide range of physical activities might be advised in accordance with the patients' status, for example, aerobic exercises, water-based dynamic exercises, muscle strengthening or hand exercises.[132–144] Psychological interventions could be applied, specifically to reduce pain and fatigue, for example, cognitive behavioural therapy and interventions focusing on stress management.[142 145–149] Furthermore,

patient education can assist patients in learning about their disease and management options (see also PtCs #4, 9 and 10)[10] and was specifically found to improve physical functioning.[139] Education can be provided one on one, but also in group sessions promoting patients to learn from each other. Lastly, self-management programmes can be applied. These programmes are typically a combination of different non-pharmacological interventions (eg, exercise and education) and were found to optimise the management of pain, fatigue and functional disability (see also PtCs #9 and 10).[136 150–159]

Ideally, a package of care (ie, multimodal treatment) should be considered in accordance with the patient's needs and preferences. This individually tailored multimodal treatment can be provided by different members of the rheumatology team (eg, rheumatologists, rehabilitation physicians, nurses experienced with patients with RA, physiotherapists, occupational therapists, psychologists, pharmacists and podiatrists). The Task Force unanimously agreed with this PtC (100% agreed, first round, n=29).

(10) Appropriate education and support should be offered to patients to directly inform their choices of treatment goals and management (LoE: 4, SoR: C, LoA: 9.4 (1.2)).

Setting treatment goals is central in the management of RA. In the current EULAR RA management recommendations, clinical remission or at least low disease activity is the ideal target with adjustment of therapeutic strategies if there is no improvement at 3 months or if the treatment target is not achieved at 6 months (recommendation #3).[2] These treatment targets may be unrealistic to achieve for patients with D2T RA, considering their disease history, accrued joint damage and other factors that may contribute to the D2T RA state,[13] and lead to unnecessary DMARD switches. Accordingly, in D2T RA, treatment goals should be tailored to the individual patient.

Discordance in a given set target between the patient and HCP could negatively impact disease outcomes.[13] The SLR did not find a diagnostic method to identify a mismatch in treatment goals (between HCP and patient with RA).[21] Treatment goals should be discussed to be able to identify a mismatch in treatment goals and to optimise goal setting in shared decision-making.

Web-based education tools improve patients' knowledge and certainty in treatment decisions.[21 160–163] Such tools could be used in addition to providing information via usual discussions. As perceptions on treatment goals and management may change over time continuous education between patients and HCPs remains important. This PtC was accepted in the first round of the voting process (89% agreed, first round, n=28).

(11) Consider offering self-management programmes, relevant education and psychological interventions to optimise patient's ability to manage their disease confidently (ie, self-efficacy; LoE: 3, SoR: C, LoA: 9.1 (1.7)).

Self-efficacy refers to patients' ability to control or manage various aspects of their disease and has a major role in the well-being of patients.[164] Self-efficacy beliefs determine how individuals think, feel and act, and are an important aspect of self-management. People with low self-efficacy quickly give up their goals when faced with difficulties and are at higher risk of worse levels of pain, fatigue, depression, anxiety and stress.[164–166] All this may contribute to the D2T RA state.[13 26] In contrast, a strong sense of self-efficacy improves human performance and well-being in several ways, promotes the accomplishment of challenging goals and supports commitment to them.[164] Improved self-efficacy may not only improve disease outcomes such as mental well-being but may also improve many aspects of health behaviour, including treatment adherence and willingness to change lifestyle factors. Therefore, strengthening self-efficacy is specifically important in D2T RA.

The Arthritis Self-Efficacy Scale (ASES), a tool to measure perceived self-efficacy to cope with the disease,[167] was found as the most reliable measure of self-efficacy.[21 168] However, the ASES is perhaps too general to evaluate self-efficacy[168] and cut-offs for suboptimal self-management are not well-validated, so a standalone PtC regarding its application was not pursued (89% agreed, n=27). There was consensus that the ASES may be used as a screening instrument and to assess the change in self-efficacy over time. The Task Force considered it challenging to clearly define what constituted a suboptimal level of self-efficacy and agreed that offering interventions to improve self-efficacy could be beneficial for all patients with D2T RA.

The SLR identified self-management programmes, educational interventions and psychological interventions to have a beneficial effect on self-efficacy.[21] Some evidence suggested patients would like more education on disease processes.[21 169 170] Educational interventions, for example, individual education, a group education programme or education through a mobile app, specifically improved self-efficacy and RA knowledge.[154 155 171–175] Psychological interventions, for example, cognitive behavioural therapy or relaxation therapy, not only improve self-efficacy, but may also reduce symptoms related to anxiety and depression.[148 151 176] Self-management programmes (ie, typically a combination of different non-pharmacological interventions) were also found to be effective in improving self-efficacy.[136 143 151–153 155–158 177–181] In addition, mobile health applications may improve self-management.[182]

The Task Force thoroughly debated if these interventions should be offered to every patient (mandatory) or should be considered only (optional). The Task Force agreed that self-management programmes should be optional (agreed 82%, n=28). If a patient wishes to improve their self-efficacy, a shared decision-making that captures the patient's status and preferences should decide the type of intervention. This PtC was accepted in the first round of the voting process (96% agreed, first round, n=28).

Research agenda

The Task Force created a research agenda containing research questions that are considered most relevant to address (table 2).

DISCUSSION

The term 'D2T RA' has recently been defined to characterise a heterogeneous group of patients with RA with persistent signs and symptoms.[8 10 12 26] While the typical patient with D2T RA is characterised by longstanding disease and structural damage in whom (b/ts)DMARDs have been ineffective (multidrug resistant or 'true refractory' RA), this only represents a subgroup of this heterogeneous patient population. Identification of all factors potentially contributing to D2T RA warrants a holistic management approach and is essential in order to tailor management strategies to the individual patient. D2T RA constitutes an area of unmet need, which motivated our Task Force to develop a roadmap for clinical decision-making by HCPs and patients laid out in the current PtCs on diagnostic challenges and pharmacological and non-pharmacological therapeutic strategies (summarised in figure 1).

The PtCs promote individually tailored treatment interventions by addressing specific aspects of b/tsDMARD selection (including in patients with comorbidities and coexisting conditions) and non-pharmacological interventions to improve

Table 2 Research agenda

1	How can we optimally confirm a diagnosis of RA in patients with D2T RA?
2	Which reference standard should be used to assess the presence or absence of inflammation in patients with D2T RA, in whom there is a doubt after assessment by traditional measures?
3	What is the role of synovial biopsies in the assessment of the presence or absence of inflammation in D2T RA?
4	Could synovial tissue analyses be used to stratify b/tsDMARD treatment in D2T RA?
5	Could treatment history be used to stratify b/tsDMARD treatment in D2T RA?
6	Are any of the b/tsDMARDs superior to treat inflammatory disease activity in D2T RA?
7	Which DMARD is preferred in patients with D2T RA with specific adverse events, comorbidities (including extra-articular manifestations), other coexisting conditions and other contraindications that limit DMARD options?*
8	Could the development of the D2T RA state be prevented by adequate management of the potentially contributing factors in an earlier phase of RA?
9	Could the D2T RA state be ameliorated if potentially contributing factors are adequately addressed?
10	Does 'true' refractory RA (patients in whom (b/ts)DMARDs are truly ineffective) really exist?
11	Which immunological mechanisms and/or pathways underlie inefficacy to multiple b/tsDMARDs in D2T RA?
12	How does smoking impact D2T RA?
13	How does obesity impact D2T RA? And which treatment is preferred in patients with D2T RA with obesity?
14	What is the role of therapeutic drug monitoring to in the management of DT RA?

*For example, infections (HIV and TB); malignancies; lung disease (fibrosis, asthma and COPD); CVD (hypertension and cardiomyopathy); hyperlipidaemia; chronic kidney dysfunction; chronic liver dysfunction; liver enzyme elevation; osteoporosis; diabetes mellitus; thrombosis; depression and anxiety.
b/tsDMARDs, biological or targeted synthetic disease-modifying antirheumatic drugs; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; D2T, difficult-to-treat; RA, rheumatoid arthritis; TB, tuberculosis.

adherence, functional disability, pain, fatigue, goal setting and self-efficacy. Although some of these PtCs may seem self-evident, our purpose in offering this PtC is to promote the need to address each of them in D2T RA management strategies. This approach mitigates against both overtreatment as well as undertreatment.

Although the Task Force aimed to cover all potential aspects of D2T RA, not all relevant topics were addressed in the SLRs because of overlap with previous or ongoing EULAR projects (eg, treatment non-adherence, lifestyle factors, pain syndromes and osteoarthritis, see below). Joint replacement and reconstructive surgery, both of which may have relevance in D2T RA, were not included in the systemic literature search, as these were considered out of scope. There was no substantial evidence identified regarding non-steroidal-anti-inflammatory drugs and analgesics in the context of D2T RA.[21] For a few topics, the Task Force members considered a theme particularly relevant in the context of D2T RA as to merit highlighting herein. For instance, education is already addressed in separate EULAR recommendations[10] but is crucial in the management of D2T RA (§4 and 9–11). Additionally, treatment non-adherence is common in patients with RMDs and may also contribute to the D2T RA state[13 26 74 76]; therefore, it has also been addressed in the D2T RA PtCs (#4). Additional guidance on treatment non-adherence can be found in the recently published EULAR PtCs for the detection, assessment and management of non-adherence in people with RMDs.[19]

Furthermore, lifestyle factors, including diet, lack of exercise, smoking and alcohol consumption, might also be associated with D2T disease.[13 183] Therefore, the management of lifestyle factors in patients with D2T RA was raised as a clinically relevant issue at our first Task Force meeting and resulted in the formulation of a research question on this topic. However, an ongoing EULAR project is focusing on lifestyle behaviour PtCs to prevent progression of RMDs and will be published soon. The Task Force, therefore, decided to refer to these PtCs for the management of these factors, as evidence in patients with D2T RA specifically was expected to be lacking.

Concomitant fibromyalgia and other pain syndromes as well as osteoarthritis may coexist in patients with D2T RA and may (partly) explain the persistence of signs and/or symptoms suggestive of active disease.[13 26] Because previous EULAR projects focused on these conditions, it was decided to refer to their recommendations. Guidance on the management of these coexisting conditions can be found in the 'EULAR revised recommendations for the management of fibromyalgia',[23] 'EULAR recommendations for the health professional's approach to pain management in inflammatory arthritis and osteoarthritis',[9] '2018 update of the EULAR recommendations for the management of hand osteoarthritis'[24] and 'EULAR recommendations for the non-pharmacological core management of hip and knee osteoarthritis'.[25]

One of the main conclusions of the SLRs was the scarcity of high-quality direct evidence regarding D2T RA.[20 21] This is not surprising, considering the recent establishment of the EULAR definition of D2T RA.[17] However, indirect evidence (ie, in patients with RA in whom at least two b/tsDMARDs failed, especially with different MOA) was also scarce and the quality was generally low to moderate.[20 21] This lack of (high-quality) direct evidence can be seen as a limitation of these PtCs, but also as a stimulus for future studies to address patients with D2T RA specifically. Importantly, the heterogeneity of D2T RA should be considered when conducting such studies, as not all management strategies will be helpful in all patients with D2T RA. Selecting the appropriate patient population will, therefore, be crucial in order to obtain relevant results (see also table 2). As new evidence regarding D2T RA emerges, the PtCs on the management of D2T RA will need to be updated.

In summary, the evidence as identified in the SLRs together with expert opinion have resulted in a comprehensive set of overarching principles and PtCs for the management of D2T RA, promoting a holistic management approach and individually tailored pharmacological and non-pharmacological therapeutic strategies. Although high-quality evidence was scarce, these PtCs can be seen as a clinical roadmap and will provide assistance to HCPs and patients in the management of D2T RA. A research agenda was created to support future research in this emerging field.

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CLINICAL SCIENCE

2021 update of the EULAR points to consider on the use of immunomodulatory therapies in COVID-19

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ABSTRACT

Objectives To update the EULAR points to consider (PtCs) on the use of immunomodulatory therapies in COVID-19.

Methods According to the EULAR standardised operating procedures, a systematic literature review up to 14 July 2021 was conducted and followed by a consensus meeting of an international multidisciplinary task force. The new statements were consolidated by formal voting.

Results We updated 2 overarching principles and 12 PtC. Evidence was only available in moderate to severe and critical patients. Glucocorticoids alone or in combination with tocilizumab are beneficial in COVID-19 cases requiring oxygen therapy and in critical COVID-19. Use of Janus kinase inhibitors (baricitinib and tofacitinib) is promising in the same populations of severe and critical COVID-19. Anti-SARS-CoV-2 monoclonal antibodies and convalescent plasma may find application in early phases of the disease and in selected subgroups of immunosuppressed patients. There was insufficient robust evidence for the efficacy of other immunomodulators with further work being needed in relation to biomarker-based stratification for IL-1 therapy

Conclusions Growing evidence supports incremental efficacy of glucocorticoids alone or combined with tocilizumab/Janus kinase inhibitors in moderate to severe and critical COVID-19. Ongoing studies may unmask the potential application of other therapeutic approaches. Involvement of rheumatologists, as systemic inflammatory diseases experts, should be encouraged in clinical trials of immunomodulatory therapy in COVID-19.

Key messages

What is already known about this subject?

- Results from the previous systematic literature review highlighted that glucocorticoids, mainly dexamethasone, is the only drug with proven efficacy in reducing COVID-19 mortality in patients requiring oxygen therapy and in critically ill patients.
- Other immunomodulatory treatments used in rheumatology may be beneficial in selected subgroups of patients with COVID-19 and in specific phases of the disease.

What does this study add?

- We updated the existing EULAR points to consider (PtC) on immunomodulatory therapies in COVID-19 in light of the most recent literature available.
- Tocilizumab in combination with glucocorticoids is beneficial in COVID-19 cases requiring oxygen therapy and in critical COVID-19. Use of Janus kinase inhibitors (baricitinib and tofacitinib) is promising in the same populations.
- Anti-SARS-CoV-2 monoclonal antibodies and convalescent plasma may find application in early phases of the disease and in selected subgroups of immunosuppressed patients.
- Other immunomodulators failed to consistently demonstrate efficacy on mortality and other clinical outcomes at any disease stage or confirmatory evidence for biomarker-based stratification is currently lacking.

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INTRODUCTION

The use of immunomodulatory therapies in SARS-CoV-2 infection is a rapidly evolving field and it represents a challenge for the scientific community. New evidence informing best practice for clinical management of patients infected with SARS-CoV-2 and presenting COVID-19 are released on a weekly basis, leading to the continuous need for updated policies in the field. In this context, several scientific societies, including EULAR, have formulated guidance on treatment of COVID-19.[1–3] In order to propose the most up-to-date treatment strategies to physicians and patients, efforts to update these recommendations in a timely manner must be undertaken. The aim of this project was to update the EULAR points to Consider (PtC) on the use of immunomodulatory therapies in COVID-19 from the rheumatology perspective through a systematic literature review (SLR)-based approach.

Key messages

How might this impact on clinical practice or future developments?

- We propose for healthcare providers the most up-to-date treatment strategies of using immunomodulators in the treatment of moderate-to-severe and critical COVID-19.
- The updated PtCs open the way to a new paradigm: the treatment of severe and critical acute infections may benefit from immunomodulatory treatments usually reserved for autoimmune and inflammatory diseases.

METHODS

The multidisciplinary task force (TF) that developed the first version of the PtC guided by the 2014 updated EULAR standardised operating procedures.[4] reconvened in a virtual meeting on 30 June 2021. Two fellow clinicians (AA and AN), guided by the methodologist (PMM), performed an update of the SLR retrieving individual studies on the management of SARS-CoV-2 infection with immunomodulatory therapies published between 11 December 2020 and 30 June 2021 (subsequently updated up to 14 July 2021) (online supplemental text 1). In addition, a search to retrieve individual studies on the management of SARS-CoV-2 infection with anti-SARS-CoV-2 monoclonal antibodies was performed (online supplemental text 2). The SLR is published separately, however, it forms an integral part of the project. Grey literature, namely randomised controlled trials (RCTs) published as full online non-peer-reviewed preprints or in part as press releases, was also included for the sake of completeness but did not inform the PtC.

Statements updated by the steering group were presented to the TF, and discussed against the existing ones, based on the SLR results. The statements were accepted if more than 75% of the TF approved the wording in the first round (informal voting), 67% in the second voting round and more than 50% in the third round. The level of evidence (LoE) supporting each statement was assigned. Finally, TF members anonymously indicated their level of agreement with each PtC online (numerical rating scale ranging from 0='completely disagree' to 10='completely agree').

RESULTS

The updated PtCs are shown in table 1, and the modifications compared with the previous ones are shown in table 2.

The PtCs are intended to provide guidance on therapeutic aspects, and the target users are healthcare providers involved in the care of patients infected with SARS-CoV-2 infection, patients and policy-makers.

Overarching principles

The overarching principles remained unchanged compared with the 2020 version. More than a year after the start of the

Table 1 Overarching principles and points to consider on the use of immunomodulatory treatment in COVID-19, with levels of evidence (LoE) and levels of agreement (LoA)

	LoA mean (SD); % of votes ≥8/10
Overarching principles	
The phenotype of SARS-CoV-2 infection is heterogeneous ranging from asymptomatic to lethal disease due to multiorgan damage.	9.92 (0.3); 100
SARS-CoV-2 infection may need different treatment approaches, including antiviral, oxygen therapy, anticoagulation and/or immunomodulatory treatment at different stages of the disease.	9.92 (0.3); 100
Points to consider	
In non-hospitalised patients with SARS-CoV-2 infection there is currently no evidence to support the initiation of immunomodulatory therapy (LoE 2/3/4).	9.58 (1.0); 96
In hospitalised patients with SARS-CoV-2 infection that do not need oxygen therapy there is currently no evidence to support the initiation of immunomodulatory therapy to treat their COVID-19 (LoE 2/3/4).	9.04 (1.6); 88
Hydroxychloroquine should be avoided for treating any stage of SARS-CoV-2 infection since it does not provide any additional benefit to the standard of care, and could worsen the prognosis in more severe patients particularly if coprescribed with azithromycin (LoE 2).	9.92 (0.3) 100
In patients with COVID-19 requiring supplemental oxygen, non-invasive or mechanical ventilation, systemic glucocorticoids should be used since they can decrease mortality; most evidence concerns the use of dexamethasone (LoE 2/3).	9.75 (0.4) 100
In patients with COVID-19 requiring supplemental oxygen, non-invasive or mechanical ventilation combination of glucocorticoids and tocilizumab should be considered since it reduces disease progression and mortality (LoE 2). More data are needed to fully appreciate the effect of other IL-6R inhibitors (LoE 2/3).	9.17 (1.7) 87.5
In COVID-19 there is no robust evidence to support the use of anakinra or canakinumab at any disease stage (LoE 2).	9.16 (0.9) 96
In COVID-19 there is no robust evidence to support the use of low-dose colchicine at any disease stage (LoE 2)	9.5 (0.9) 96
In patients with COVID-19 requiring oxygen therapy, non-invasive ventilation or high-flow oxygen, the combination of glucocorticoids and baricitinib or tofacitinib could be considered since it might decrease disease progression and mortality (LoE 2).	8.92 (1.4) 87.5
An evolving RCT landscape cannot yet allow formal recommendation of the use of GM-CSF inhibitors (mavrilimumab, otilimab, lenzilumab) in COVID-19 (LoE 2)	9.13 (0.9) 92
In patients without hypogammaglobulinaemia and with symptom onset >5 days there is robust evidence against the use of convalescent plasma (LoE 2)	9.04 (1.9) 83.3
In patients at risk of severe COVID-19 course, symptom onset <5 days or still seronegative, monoclonal antibodies against SARS-CoV-2 spike protein should be considered (LoE 2)	9.29 (1.1) 92
In patients with COVID-19 there is currently insufficient evidence to recommend the use of other immunomodulatory drugs, including interferon alpha, interferon beta, interferon kappa, interferon lambda, leflunomide, non-SARS CoV-2 IVIg (LoE 2), eculizumab and cyclosporine (LoE 3)	9.79 (0.4) 100

GM-CSF, Granulocyte-Macrophage Colony-Stimulating Factor; IL-6R, Interleukin-6 receptor; RCT, randomised controlled trial.

Table 2 Comparison of the 2020 and 2021 points to consider on the use of immunomodulatory treatment in SARS-CoV-2 infection

2021 (current) version	Changes performed	2020 (previous) version
Overarching principles		
The phenotype of SARS-CoV-2 infection is heterogeneous ranging from asymptomatic to lethal disease due to multiorgan damage.	Unchanged	The phenotype of SARS-CoV-2 infection is heterogeneous ranging from asymptomatic to lethal disease due to multiorgan damage.
SARS-CoV-2 infection may need different treatment approaches, including antiviral, oxygen therapy, anticoagulation and/or immunomodulatory treatment at different stages of the disease.	Unchanged	SARS-CoV-2 infection may need different treatment approaches, including antiviral, oxygen therapy, anticoagulation and/or immunomodulatory treatment at different stages of the disease.
Points to consider		
In non-hospitalised patients with SARS-CoV-2 infection there is currently no evidence to support the initiation of immunomodulatory therapy (LoE 2/3/4).	Unchanged	In non-hospitalised patients with SARS-CoV-2 infection there is currently no evidence to support the initiation of immunomodulatory therapy (LoE 2/3/4).
In hospitalised patients with SARS-CoV-2 infection that do not need oxygen therapy there is currently no evidence to support the initiation of immunomodulatory therapy to treat their COVID-19 (LoE 2/3/4).	Unchanged	In hospitalised patients with SARS-CoV-2 infection that do not need oxygen therapy there is currently no evidence to support the initiation of immunomodulatory therapy to treat their COVID-19 (LoE 2/3/4).
Hydroxychloroquine should be avoided for treating any stage of SARS-CoV-2 infection since it does not provide any additional benefit to the standard of care, and could worsen the prognosis in more severe patients particularly if coprescribed with azithromycin (LoE 2).	Unchanged	Hydroxychloroquine should be avoided for treating any stage of SARS-CoV-2 infection since it does not provide any additional benefit to the standard of care, and could worsen the prognosis in more severe patients particularly if coprescribed with azithromycin (LoE 2).
In patients with COVID-19 requiring supplemental oxygen, non-invasive or mechanical ventilation, systemic glucocorticoids should be used since they can decrease mortality; most evidence concerns the use of dexamethasone (LoE 2/3).	Unchanged	In patients with COVID-19 requiring supplemental oxygen, non-invasive or mechanical ventilation, systemic glucocorticoids should be used since they can decrease mortality; most evidence concerns the use of dexamethasone (LoE 2/3).
In patients with COVID-19 requiring supplemental oxygen, non-invasive or mechanical ventilation combination of glucocorticoids and tocilizumab should be considered since it reduces disease progression and mortality (LoE 2). More data are needed to fully appreciate the effect of other IL-6R inhibitors (LoE 2/3).	Modified	An evolving RCT landscape cannot yet allow formal recommendation of the routine use of tocilizumab in patients with COVID-19 requiring oxygen therapy, non-invasive or invasive ventilation (LoE 2).
In COVID-19 there is no robust evidence to support the use of anakinra at any disease stage (LoE 2/4).	Modifies	In COVID-19 there is no robust evidence to support the use of anakinra or canakinumab at any disease stage (LoE 2).
In COVID-19 there is no robust evidence to support the use of low-dose colchicine at any disease stage (LoE 2)	New	Not applicable
In patients with COVID-19 requiring oxygen therapy, non-invasive ventilation or high-flow oxygen, the combination of glucocorticoids and baricitinib or tofacitinib could be considered since it might decrease disease progression and mortality (LoE 2).	Modified	In patients with COVID-19 requiring non-invasive ventilation or high-flow oxygen, the combination of remdesivir plus baricitinib could be considered since it can decrease time to recovery and accelerate improvement in clinical status (LoE 2).
An evolving RCT landscape cannot yet allow formal recommendation of the use of GM-CSF inhibitors (mavrilimumab, otilimab, lenzilumab) in COVID-19 (LoE 2)	New	Not applicable
In patients without hypogammaglobulinaemia and with symptom onset >5 days there is robust evidence against the use of convalescent plasma (LoE 2)	New	Not applicable
In patients at risk of severe COVID-19 course, symptom onset <5 days or still seronegative, monoclonal antibodies against antispike protein should be considered (LoE 2)	New	Not applicable
In patients with COVID-19 there is currently insufficient evidence to recommend the use of other immunomodulatory drugs, including interferon alpha, interferon beta, interferon kappa, interferon lambda, leflunomide, non-SARS CoV-2 IVIg (LoE 2), eculizumab and cyclosporine (LoE 3)	Modified	In COVID-19 there is currently insufficient evidence to recommend the use of other immunomodulators, including ruxolitinib, intravenous immunoglobin, convalescent plasma therapy except in Ig-deficient patients, interferon kappa, interferon beta, leflunomide, colchicine (LoE 2), sarilumab, lenzilumab, eculizumab, cyclosporine, interferon alpha (LoE 3), canakinumab (LoE 4).

GM-CSF, Granulocyte-Macrophage Colony-Stimulating Factor; IL-6R, Interleukin-6 receptor; LoE, lovel of evidence; ; RCT, randomised controlled trial.

pandemic, the heterogeneity of SARS-CoV-2 infection clinical picture, reflecting different pathogenic mechanisms, is widely recognised.[5] Patients infected by SARS-CoV-2 may experience a set of manifestations ranging from asymptomatic infection, mild disease to severe disease with acute respiratory distress syndrome, multiorgan failure and death. In this regard, response to immunomodulatory therapy varies according to disease stage, with the best efficacy of these compounds observed in severe but not critical disease (table 1).

Points to consider

Since the formulation of the original set of PtCs, over 300 articles with various LoE investigating immunomodulatory agents in SARS-CoV-2 infection were published.[6] Besides studies with drugs already mentioned in the previous PtCs, such as tocilizumab (TCZ) or anakinra, studies with new drugs including sarilumab, tofacitinib (TOFA), baricitinib (BARI) and colchicine, among others, were available, either as monotherapy or in combination treatment with glucocorticoids (GC). On this basis, the steering group agreed to keep PtC-1 and PtC-2 unchanged since they remain valid statements supported by current evidence and formulate new statements based on the recent evidence (or lack thereof) for individual classes of compounds, whenever possible or single drugs (tables 1 and 2).

PtC-1: In non-hospitalised patients with SARS-CoV-2 infection, there is currently no evidence to support the initiation of immunomodulatory therapy (LoE 2/3/4).

PtC-2: In hospitalised patients with SARS-CoV-2 infection that do not need oxygen therapy, there is currently no evidence to support the initiation of immunomodulatory therapy to treat their COVID-19 (LoE 2/3/4).

The group agreed to keep PtC-1 and PtC-2 unchanged since they remain valid statements supported by current evidence.

PtC-3: Hydroxychloroquine should be avoided for treating any stage of SARS-CoV-2 infection since it does not provide any additional benefit to the standard of care, and could worsen the prognosis in more severe patients particularly if coprescribed with azithromycin (LoE 2).

The group agreed to keep this PtC unchanged since further evidence against the use of hydroxychloroquine has emerged.[7–14]

PtC-4: In patients with COVID-19 requiring supplemental oxygen, non-invasive or mechanical ventilation, systemic GC should be used since they can decrease mortality; most evidence concerns the use of dexamethasone (DEXA) (LoE 2/3).

As PtC-1, the group agreed to keep this PtC unchanged but in this case on the basis of lack of new evidence. In fact, the three new RCTs gathered by the SLR update were underpowered, thereby providing unreliable results and therefore could not be used to formulate the PtC. One retrospective trial comparing the efficacy of methyprednisolone (MTP ≥1 mg/kg/days for ≥3 days) vs DEXA (DEXA ≥6 mg for ≥7 days) showed a reduction of mortality in the group of patients receiving MV treated with MTP (relative risk (RR) 0.48 (95% CI 0.23 to 0.96). However, the small number of patients, retrospective design and high risk of bias for this study did not allow definitive conclusions regarding superiority of any compound and could therefore not inform the PtCs.[15]

PtC-5: In patients with COVID-19 requiring supplemental oxygen, non-invasive or mechanical ventilation combination of GC and TCZ should be considered since it reduces disease progression and mortality (LoE 2). More data are needed to fully appreciate the effect of other IL-6R inhibitors (LoE 2/3).

This PtC was modified encompassing not only TCZ but the entire class of IL-6R inhibitors. Four new RCTs pertained to TCZ[16–19] alongside the 90 days post hoc analysis of the CORIMUNO-19 TOCI trial.[20] Among these, RECOVERY, REMAP-CAP and the post hoc analysis of CORIMUNO-19 TOCI (the latter in the subgroup of patients with C reactive protein >15.0 mg/dL) showed reduction of death at day 21 (RR 0.27, 95% CI 0.12 to 0.72), day 28 (RR 0.82, 95% CI 0.75 to 0.90) and day 90 respectively (RR 0.79, 95% CI 0.63 to 0.97), respectively. In addition, a reduction of progression to invasive mechanical ventilation (IMV) or death at day 21[19] or day 90[20] or an increase in cardiovascular or respiratory support-free days[18] was observed. Of note, the proportion of patients receiving GC as part of the standard of care (SOC) was very heterogeneous among trials, with a difference observed between trials starting before and after the positive results of the GC arm of the RECOVERY trial. It is noteworthy that in contrast to two positive RCTs where a high percentage of patients were receiving concomitant GC (82%–93%),[18 19] only up to 50% of patients were receiving concomitant GC in the COVACTA trial, which failed to show efficacy in reducing death or improving clinical status.[16] In addition, a recent meta-analysis of RCTs published in JAMA confirmed the efficacy of TCZ on all-cause mortality (OR 0.83, 95% CI 0.72 to 0.94) and progression to IMV, extracorporeal membrane oxygenation or death (OR 0.74, 95% CI 0.66 to 0.82) at day 28.[21] It is important to mention that the survival benefit at 28 days was essentially observed only in patients also on GC. Furthermore, the statistically significant benefit in survival at 90 days is the most relevant finding. Of note, much of what drove the statistical significance for improved mortality were the non-blinded larger randomised trials.

The evidence regarding sarilumab (SARI) is scarcer although encouraging, with a small arm in REMAP-CAP trial (n=44 patients) showing a reduction in death and cardiovascular/respiratory organ-support free days[18] while another RCT comparing 200 mg or 400 mg of SARI and placebo showed no efficacy on death, progression to IMV or admission to intensive care unit.[22] Of interest, in a meta-analysis of IL-6R inhibitors, in the subgroup of patients receiving GC compared with those who did not, mortality at day 28 was significantly reduced only in the GC group for TCZ (ratio of OR (ROR) 0.69, 95% CI 0.52 to 0.91 p=0.008), with only a non-significant trend for SARI (ROR 0.77, 95% CI 0.64 to 1.31 p=0.34).

PtC-6) In COVID-19 there is no robust evidence to support the use of anakinra and canakinumab at any disease stage (LoE 2).

The only RCT available in the 2020 version of the PtC on anakinra used at a high dose of 400 mg/day for 3 to 6 days (CORIMUNO-19 ANA) was negative in patients with mild-to-moderate COVID-19 pneumonia requiring at least 3 L/min oxygen but not receiving non-invasive ventilation (NIV) or IMV at randomisation.[23] In addition, one RCT looking into a specific group of COVID-19 patients, namely those with elevated soluble urokinase plasminogen activator equal to or above 6 ng/mL which is considered as a predictor of unfavourable outcome. In this population, anakinra 100 mg subcutaneously for 7–10 days increased number of patients improving WHO CPS at day 28 (0.36 (95% CI 0.26 to 0.50) and decreased mortality at day 28: 3.2% vs 6.9% (HR=0.45, p=0.045).[24] Further studies are necessary to address the validity of this biomarker for predicting a possible effect of anakinra in this subgroup of patients. With regard to canakinumab, a 2020 press-release RCT indicated that it did not meet its primary and secondary endpoints.[25] Large trials recruiting severe cases of COVID-19 are warranted.

PtC-7: In COVID-19, there is no robust evidence to support the use of low-dose colchicine at any disease stage (LoE 2).

Compared to 2020, the new SLR updated gathered two additional RCTs, a large study enrolling almost 5000 non-hospitalised patients with mild disease[26] and a small study including 72 hospitalised patients, most of whom required oxygen therapy.[27] The results of both studies were not rated solid enough to recommend in favour of colchicine. Moreover, both studies used a rather low dose, hence the group deemed appropriate to specify this in the PtC since it was not possible to rule out whether higher doses might be beneficial. In addition, a press release reported that the colchicine arm of the RECOVERY trial, enrolling hospitalised patients with COVID-19, has closed due to lack of evidence that further recruitment will prove a reduction of mortality. The interim results have been published as preprint.[28]

PtC-8: In patients with COVID-19 requiring oxygen therapy, NIV or high-flow oxygen, the combination of GC and BARI or TOFA could be considered since it might decrease disease progression and mortality (LoE 2).

The only RCT available on BARI in SARS-CoV-2 infection included in the 2020 version[29] and compared remdesevir +BARI versus remdesevir +placebo. In addition, The Fourth iteration of the Adaptive COVID-19 Treatment Trial-4, although published in the grey literature and therefore not used to inform the PtCs; compared BARI+remdesivir+placebo versus remdesivir +DEXA+placebo and met predefined futility criteria in an interim analysis thereby closed enrollment in April 2021 according to a press release.[30] In a new study (COV-BARRIER trial), BARI in addition to SOC (80% participants receiving GC (92% DEXA)) showed no significant efficacy in reducing progression to the composite primary endpoint defined by the proportion who progressed to high-flow oxygen, NIV/IMV or death by day 28. However, the all-cause 28-day mortality in the

BARI group was decreased from 13% to 8% (HR=0.57 (95% CI 0.41 to 0.78); p=0.0018) and at day 60: 10% vs 15% (HR=0.62 (95% CI 0.47 to 0.83]; p=0.005).[31]

One new RCT[32] comparing TOFA+SOC (n=144) to placebo +SOC (n=144) reported a significant improvement of the composite outcome of respiratory failure or mortality at day 28 (RR 0.63, 95% CI 0.41 to 0.97) vs placebo +SOC in a population where 90% of patients were receiving GC as part of SOC. No new evidence other than the previously published negative RCT on ruxolitinib was retrieved.

PtC-9: An evolving RCT landscape cannot yet allow formal recommendation of the use of GM-CSF inhibitors (mavrilimumab, otilimab, lenzilumab) in COVID-19 (LoE 2)

The 2020 SLR gathered only a few studies with low LoE on GM-CSF inhibitors. Although the SLR update identified only one RCT on mavrilimumab, the group discussed the large proportion of ongoing RCTs, not only on mavrilimumab but also on other GM-CSF inhibitors (otilimab, lenzilumab), available in the grey literature (both as press releases and as preprints). On this basis, they deemed appropriate to formulate a PtC conveying the message that the current lack of evidence to recommend either in favour or against is accompanied by an evolving body of evidence that will soon be available in peer-reviewed journals.

PtC-10: In patients without hypogammaglobulinaemia and with symptom onset >5 days there is robust evidence against the use of convalescent plasma (CP) (LoE 2)

Among the RCTs published on CP (n=7), four were retrieved by the SLR update. Of interest, a distinction was drawn by the TF based on the timing of CP administration (ie, before or after day 5 of symptom onset). In fact, a large RCT including more than 5000 patients in each treatment arm (CP +SOC vs placebo +SOC), CP was not effective in reducing the composite outcome of progression to IMV or death at day 28 (RR 0.99, 95% CI 0.93 to 1.05 p=0.79) when administered after this time frame.[33] It is important to clarify that this PtC was informed by robust data against CP showing benefit while no evidence about CP being harmful was retrieved by SLR.

PtC-11: In patients at risk of severe COVID-19 course, with symptom onset <5 days or still seronegative, monoclonal antibodies against SARS-CoV-2 spike protein should be considered (LoE 2)

The new SLR conducted to gather studies on monoclonal antibodies against SARS-CoV-2 spike protein, retrieved four RCTs, three of which enrolled non-hospitalised patients with mild to moderate COVID-19[34–36] and one enrolling hospitalised patients with moderate-to-severe COVID-19.[37] The combination of bamlanivimab and etesevimab as well as of casirivimab and imdevimab administrated within the first week after symptom onset were able to significantly reduce viral load. However, casirivimab and imdevimab were effective only in patients seronegative at baseline.

Conversely, bamlanivimab monotherapy failed to significantly reduce viral load in non-hospitalised patients, and failed to provide any benefit on clinical outcomes (eg, 90 days mortality) in hospitalised patients.[37] It is important to mention that the specific monoclonal antibodies have different activities against variants, so in addition to the above-mentioned data, regional prevalence of variants must be taken into account when selecting a particular product.

PtC-12: In patients with COVID-19, there is currently insufficient evidence to recommend the use of other immunomodulatory drugs, including interferon alpha, interferon beta, interferon kappa, interferon lambda, leflunomide, non-SARS CoV-2 IVIg (LoE 2), eculizumab and cyclosporine (LoE 3).

Interferon lambda has been added since no RCT was available in the previous SLR and the two RCTs retrieved by the SLR update were not solid enough to formulate a new PtC. A change of LoE was done for interferon alpha since a small RCT was retrieved by the search update.[38] The group did not comment on drugs for which published literature was of LoE <3.

DISCUSSION

Since the release of the first EULAR-endorsed PtCs on immunomodulatory therapy of SARS-CoV-2 infection, new evidence has accumulated on the efficacy and safety of various compound with most evidence pertaining to moderate to severe/critical COVID-19. The aim of this update was to provide clinicians involved in the care of people with SARS-CoV-2 infection with an update on the use of immunomodulatory therapies in COVID-19, based on available literature and as seen from the rheumatology perspective.

All the statements are based on a thorough SLR and on conclusions of an international rheumatology/multidisciplinary team. All studies, although RCTs, were highly heterogeneous and at high or unclear risk of bias, hence the experts' opinion was instrumental to reach consensus on if and how to update the existing statements.

Until now, only three drugs have been recommended by WHO for COVID-19, DEXA and TCZ for patients requiring oxygen therapy and critical patients and the combination of casirivimab and imdevimab for early patients at risk of severe form and not vaccinated or having not responded to vaccination.[2]

Besides the three statements on HCQ, GCs and anakinra, the group developed several new PtCs and modified the existing ones since more evidence about numerous drugs has accrued (table 2). Moreover, the discontinuation of some RCTs for futility and the availability of interim data of some successful RCTs from the grey literature, clarified the role of some immunomodulatory compounds in the scenario of the pandemic although these could not be used to formulate recommendations in favour or against.

In particular, it was possible to formulate statements in favour of TCZ in combination with GCs and against CP, except in specific in subgroups of patients based on a consistent number of peer-reviewed RCTs. Based on the evidence on CP and monoclonal antibodies against SARS-CoV-2 spike protein, it is tempting to speculate that a polyclonal response may be better to activate effector functions than a monoclonal response.

Data on Janus kinase inhibitors are promising in some subgroups. Lastly, the use of colchicine and GM-CSF inhibitors is pending the release of more solid evidence.

In conclusion, the update of these EULAR PtCs provide relevant and updated guidance on immunomodulatory therapy utilisation from the rheumatology perspective and opens the way to a new paradigm: the treatment of immunopathology associated with severe and critical acute infections may benefit from immunomodulatory treatments usually given for autoimmune and inflammatory diseases.

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EPIDEMIOLOGICAL SCIENCE

Risk of herpes zoster (shingles) in patients with rheumatoid arthritis under biologic, targeted synthetic and conventional synthetic DMARD treatment: data from the German RABBIT register

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ABSTRACT

Objective To compare event and incidence rates of herpes zoster (HZ), also known as shingles, in patients with rheumatoid arthritis under treatment with conventional synthetic (cs), targeted synthetic (ts) or biologic (b) disease-modifying antirheumatic drugs (DMARDs).

Methods Patients were prospectively enrolled from 2007 until October 2020. Reported HZ events were assigned to ongoing treatments or those terminated within 1 month prior to the HZ event. Exposure-adjusted event rates (EAERs) of HZ were calculated per 1000 patient years (py) and adjusted HRs with 95% CIs computed. Inverse probability weights (IPW) were used to adjust for confounding by indication.

Results Data of 13 991 patients (62 958 py) were analysed, with 559 HZ events reported in 533 patients. The EAER of HZ was highest for tsDMARDs (21.5, 95% CI 16.4 to 27.9), followed by B cell targeted therapy (10.3, 95% CI 8.0 to 13.0), monoclonal antitumour necrosis factor (anti-TNF) antibodies (9.3, 95% CI 7.7 to 11.2), interleukin 6 inhibitors (8.8, 95% CI 6.9 to 11.0), soluble TNF receptor fusion protein (8.6, 95% CI 6.8 to 10.8), T cell costimulation modulator (8.4, 95% CI 5.9 to 11.8) and csDMARDs (7.1, 95% CI 6.0 to 8.3). Adjusted for age, sex and glucocorticoids and weighted with IPW, tsDMARDs (HR 3.66, 95% CI 2.38 to 5.63), monoclonal anti-TNF antibodies (HR 1.63, 95% CI 1.17 to 2.28) and B cell targeted therapy (HR 1.57, 95% CI 1.03 to 2.40) showed a significantly higher risk compared with csDMARDs.

Conclusion Our results provide evidence for a 3.6-fold increased risk of HZ associated with tsDMARDs and an increased risk of HZ under bDMARDs compared with csDMARDs.

Key messages

What is already known about this subject?
- Patients with rheumatoid arthritis have an increased risk of developing herpes zoster (HZ).
- Increased incidence rates have been reported under tumour necrosis factor and Janus kinase (JAK) inhibitors.

What does this study add?
- Comparative data on all disease-modifying antirheumatic drugs (DMARDs) provide evidence for an increased risk of HZ under JAK inhibitors.
- Treatment with biologic DMARDs showed a significantly higher risk compared with conventional synthetic DMARDs.
- Higher age and glucocorticoids were also associated with an increased risk of HZ.

How might this impact on clinical practice or future developments?
- An increased risk of HZ should be considered especially under JAK inhibitors, in elderly patients, and under glucocorticoid therapy.

INTRODUCTION

Herpes zoster (HZ), also known as shingles, remains a clinically relevant infectious event for patients with rheumatoid arthritis (RA). In addition to the generally increased risk with RA and older age,[1 2] current research focuses on the question to what extent the specific disease-modifying antirheumatic drug (DMARD) treatment of RA influences the risk of HZ. A comparison of all available biologic (b) DMARDs, including tumour necrosis factor (TNF) inhibitors, abatacept, rituximab and tocilizumab, in the US Medicare data of 2015 showed a similar risk of HZ across all biologic agents.[3] The newer targeted synthetic (ts) DMARDs, the Janus kinase (JAK) inhibitors, however, reportedly have an at least twofold risk of HZ, which was further increased by the addition of glucocorticoids.[4–7] Another meta-analysis using pooled data of 40 eligible randomised clinical trials and 19 observational studies until 2016 indicated an increased risk of HZ in immunocompromised patients receiving bDMARDs, especially of non-TNF blocking agents, mainly because studies comparing TNF inhibitors with controls were under-represented.[8] No European data on the risk of HZ under JAK inhibitors in real-world settings have been available to date.[9] The European Alliance of Associations for Rheumatology recommends considering HZ vaccination in high-risk patients with autoimmune rheumatic disease.[10] Data to date show that only a small percentage of patients have been vaccinated against HZ so far.[3 11]

To further investigate the risk of HZ when exposed to different antirheumatic therapies, we

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compared event and incidence rates of HZ in patients with RA under treatment with conventional synthetic (cs) DMARDs, tsDMARDs or bDMARDs and evaluated in addition the contribution of concomitant glucocorticoid therapy.

PATIENTS AND METHODS

Data source

The German Rheumatoid Arthritis: Observation of biologic therapy register RABBIT is a prospective longitudinally followed cohort of patients with RA that are included with a new start of a bDMARD/tsDMARD, or with a csDMARD treatment after at least one prior DMARD therapy. At the time of enrolment, at months 3 and 6, and then every 6 months during the time of observation, information is collected from rheumatologists and patients on demographics, clinical status including joint counts, treatment details (eg, start/stop dates of DMARDs, dosages of glucocorticoids), laboratory tests, patient-reported outcomes and adverse events. Rheumatologists are requested to classify reported events according to the International Conference on Harmonisation E2A guideline on serious and non-serious events and to provide additional information on serious adverse events, for example, providing hospital discharge letters if available. Patients are observed for up to 10 years, irrespective of treatment changes. For this analysis, patients enrolled from 2007 onwards with at least one follow-up were included.

Outcome and treatment exposure

All HZ events reported until 31 October 2020 were selected. Medical Dictionary for Regulatory Activities Terminology (MedDRA) terms used in this study included 'herpes zoster' (MedDRA code 10019974), 'herpes zoster infection neurological' (MedDRA code 10061208), 'herpes ophthalmic' (MedDRA code 10062004), 'herpes zoster oticus' (MedDRA code 10063491), 'herpes zoster disseminated' (MedDRA code 10065038), 'herpes zoster meningitis' (MedDRA code 10074259) and 'herpes zoster cutaneous disseminated' (MedDRA code 10074297).

Treatment with DMARDs was categorised into monoclonal anti-TNF antibodies (adalimumab, certolizumab, golimumab, infliximab), soluble TNF receptor fusion protein (etanercept), T cell costimulation modulator (abatacept), B cell targeted therapy (rituximab), interleukin (IL) 6 inhibitors (tocilizumab, sarilumab), JAK inhibitors (tofacitinib, baricitinib, upadacitinib) and csDMARDs as the reference group. Patients were considered receiving bDMARD/tsDMARD treatment at the time of an event if the respective treatment was ongoing or terminated within 1 month prior to the event. In a sensitivity analysis, this 1-month risk window was extended to 3 months.

Age, sex, disease duration, disease activity assessed by the Disease Activity Score of 28 joints (DAS28) using erythrocyte sedimentation rate and the Clinical Disease Activity Index (CDAI), physical capacity assessed by the Hannover Functional Status Questionnaire (FFbH, 0–100, with 100 representing full capacity), presence of rheumatoid factor and anticitrullinated protein antibodies (ACPA), level of C reactive protein (CRP), number of previous DMARDs, glucocorticoid therapy and doses, comorbidities (osteoporosis, hypertension, coronary heart disease, diabetes, chronic obstructive respiratory disease, chronic kidney disease, malignant neoplasia, lymphoma/leukaemia, mental illness/depression), and number of comorbidities (recorded by the rheumatologist as present or not) were used to characterise patients at baseline.

Statistical analysis

Differences at baseline between treatment groups were examined using descriptive statistics (mean, SD, median, IQR and percentage). Exposure-adjusted event rates of HZ (EAERs; defined as the number of HZ events divided by the total exposure time among patients in the respective treatment group) and exposure-adjusted incidence rates (EAIRs; defined as the number of HZ events divided by the total exposure time among patients in the respective treatment group and at risk of an initial occurrence of HZ) with 95% CIs were calculated per 1000 patient years (py).

The Andersen-Gill model, an extension of the standard Cox proportional hazard model used to include recurrent events by taking the complete follow-up time into account, was applied and adjusted HRs with 95% CIs were calculated to investigate risk factors for the development of HZ. Patient characteristics at baseline (age, sex) and characteristics varying with time during follow-up (treatment with bDMARDs/tsDMARDs and treatment with glucocorticoids) were considered as possible risk factors. The CIs of the HRs were calculated by means of robust sandwich estimates. Adjustment with inverse probability weights (IPW) was used to deal with confounding by indication. These weights were estimated by means of logistic regression with the covariates age, sex, disease duration, disease activity (DAS28), functional status (FFbH), previous treatment with bDMARDs/tsDMARDs and osteoporosis (yes/no). In a sensitivity analysis, IPW was calculated using CDAI instead of DAS28 as a measure of disease activity. Osteoporosis was used as an indirect indicator of long-standing disease activity in RA.

A secondary analysis was performed in a subsample of patients to evaluate the potential impact of unmeasured confounding. This subsample included only those patients who were (1) either enrolled with a certain bDMARD/tsDMARD treatment, had at least one interruption of this bDMARD/tsDMARD treatment during follow-up, and received only csDMARD treatment during this interruption time; or (2) patients who were enrolled with a csDMARD treatment and started a bDMARD/tsDMARD treatment during follow-up. Each patient in the subsample serves as her/his own control, observed under treatment exposure with a certain bDMARD/tsDMARD, but as well exposed to csDMARD alone, while carrying her/his own risk factors.

Missing values on DAS28 (CDAI) at the start of treatment were imputed by fitting regression models. Strongly skewed distributions, like the CRP, were logarithmised to prevent undesired effects and reverse-transformed for the calculation of regression coefficients.

Data analyses were performed with SAS V.9.4, and p values <0.05 were considered statistically significant.

RESULTS

Patient characteristics at baseline

A total of 13 991 patients (62 958 py of observation) were included. At baseline (ie, the time at enrolment in RABBIT with a new start of a DMARD therapy), 3242 patients started treatment with monoclonal anti-TNF antibodies, 2513 with soluble TNF receptor fusion protein, 817 with T cell costimulation modulator, 1431 with B cell targeted therapy, 1424 with IL-6 inhibitors, 713 with JAK inhibitors and 3851 with csDMARDs after at least one prior csDMARD therapy.

The mean age of all patients was 57.7 years, whereby patients receiving monoclonal anti-TNF antibodies were younger (55.1 years) and those receiving JAK inhibitors were older (59.5 years) than patients in the other treatment groups. Patients under B cell

Table 1 Patient characteristics at baseline (N=13 991)

	Monoclonal anti-TNF antibodies n=3242 (23.17%)	Soluble TNF receptor fusion protein n=2513 (17.96%)	T cell costimulation modulator n=817 (5.84%)	B cell targeted therapies n=1431 (10.23%)	IL-6 inhibitors n=1424 (10.18%)	JAK inhibitors n=713 (5.10%)	csDMARDs n=3851 (27.52%)
Age, mean (SD), years	55.1 (12.9)	58.5 (12.8)	58.2 (12.9)	58.1 (11.8)	57.1 (12.7)	59.5 (11.9)	58.9 (12.6)
Women, n (%)	2439 (75.2)	1801 (71.7)	607 (74.3)	1102 (77.0)	1101 (77.3)	531 (74.5)	2837 (73.7)
RF positive, n (%)	2188 (69.0)	1664 (67.9)	599 (75.2)	1199 (84.3)	996 (74.0)	497 (71.2)	2173 (57.3)
ACPA positive, n (%)	1854 (68.6)	1486 (67.8)	482 (70.2)	679 (76.0)	844 (72.6)	468 (68.6)	1818 (54.5)
FFbH score, mean (SD)	67.7 (22.2)	65.9 (22.9)	59.5 (23.5)	56.0 (23.4)	63.3 (23.7)	63.4 (24.2)	71.3 (21.9)
Disease duration, median (IQR), years	7.0 (3.0–13.0)	7.0 (3.0–13.0)	10.0 (4.0–17.0)	12.0 (7.0–19.0)	9.0 (4.0–16.0)	8.0 (3.0–16.0)	3.0 (1.0–8.0)
DAS28, mean (SD)	4.8 (1.3)	4.9 (1.2)	5.2 (1.3)	5.3 (1.3)	5.1 (1.3)	4.7 (1.3)	4.4 (1.3)
CRP, median (IQR), mg/L	6.8 (2.9–15.9)	7.3 (3.0–18.0)	7.2 (3.0–19.3)	9.1 (3.3–22.9)	7.4 (2.6–20.0)	6.1 (2.3–14.1)	5.4 (2.3–12.8)
CDAI, median (IQR)	22.0 (16.0–31.0)	24.0 (17.0–32.0)	25.0 (18.0–34.0)	24.0 (15.0–34.0)	24.0 (17.0–31.0)	23.0 (16.0–31.0)	18.0 (12.0–26.0)
Previous csDMARD therapies, mean (SD)	2.2 (1.0)	2.2 (1.0)	2.5 (1.3)	2.7 (1.3)	2.3 (1.1)	2.0 (0.9)	1.3 (0.6)
Previous bDMARD/tsDMARD therapies, mean (SD)	0.3 (0.7)	0.2 (0.5)	1.3 (1.3)	1.7 (1.1)	1.1 (1.2)	1.0 (1.5)	0.0 (0.2)
Methotrexate, n (%)	1906 (58.8)	1277 (50.8)	556 (68.1)	804 (56.2)	513 (36.0)	263 (36.9)	2405 (62.5)
Glucocorticoids, n (%)	1934 (59.7)	1476 (58.8)	463 (56.8)	961 (68.0)	822 (57.8)	312 (43.9)	1823 (47.4)
Glucocorticoids, ≥10 mg, n (%)	490 (15.2)	377 (15.0)	128 (15.7)	332 (23.6)	269 (18.9)	64 (9.1)	291 (7.6)
Comorbidities, mean (SD)	2.1 (2.2)	2.5 (2.3)	3.1 (2.7)	2.8 (2.4)	2.4 (2.3)	2.8 (2.3)	1.9 (2.0)
Osteoporosis, n (%)	412 (12.7)	450 (17.9)	202 (24.7)	402 (28.1)	251 (17.6)	123 (17.3)	434 (11.3)
Hypertension, n (%)	1214 (37.4)	1124 (44.7)	398 (48.7)	600 (41.9)	590 (41.4)	340 (47.7)	1673 (43.4)
Coronary heart disease, n (%)	182 (5.6)	193 (7.7)	69 (8.4)	111 (7.8)	101 (7.1)	60 (8.4)	223 (5.8)
Diabetes, n (%)	349 (10.8)	312 (12.4)	102 (12.5)	161 (11.3)	175 (12.3)	96 (13.5)	429 (11.1)
Chronic obstructive respiratory disease, n (%)	119 (3.7)	145 (5.8)	59 (7.2)	83 (5.8)	64 (4.5)	41 (5.8)	173 (4.5)
Chronic kidney disease, n (%)	146 (4.5)	170 (6.8)	53 (6.5)	87 (6.1)	75 (5.3)	55 (7.7)	147 (3.8)
Malignant neoplasia, n (%)	90 (2.8)	95 (3.8)	36 (4.4)	142 (9.9)	48 (3.4)	26 (3.6)	157 (4.1)
Lymphoma/leukaemia, n (%)	3 (0.1)	4 (0.2)	3 (0.4)	39 (2.7)	5 (0.4)	3 (0.4)	20 (0.5)
Mental illness/depression, n (%)	246 (7.6)	199 (7.9)	70 (8.6)	85 (5.9)	127 (8.9)	84 (11.8)	250 (6.5)

ACPA, anticitrullinated protein antibodies; bDMARDs, biologic DMARDs; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; csDMARDs, conventional synthetic DMARDs; DAS28, Disease Activity Score of 28 joints using the erythrocyte sedimentation rate; DMARDs, disease-modifying antirheumatic drugs; FFbH, Hannover Functional Status Questionnaire; IL-6, interleukin 6; JAK, Janus kinase; RF, rheumatoid factor; TNF, tumour necrosis factor; tsDMARDs, targeted synthetic DMARDs.

targeted therapy were most often RF or ACPA positive (84% and 76%, respectively) and received most often glucocorticoid therapies (68%) compared with all other treatment groups. (The latter may be influenced by use of glucocorticoids as premedication of B cell targeted therapies to reduce possible infusion-related symptoms.) Meanwhile, patients under T cell costimulation modulator received most often concomitant methotrexate (68%). Patients in these two treatment groups tended to have the longest disease duration (12 and 10 years, respectively), the lowest functional capacity (FFbH score 56 and 59, respectively), the highest disease activity (DAS28 5.3 and 5.2, respectively), more often had osteoporosis (28% and 25%, respectively), and the highest number of previous therapies with csDMARDs (2.7 and 2.5, respectively) and bDMARDs/tsDMARDs (1.7 and 1.3, respectively). The presence of comorbidities other than osteoporosis was comparable between the treatment groups (table 1). All baseline characteristics are reported in table 1.

EAER of HZ

A total of 559 HZ cases in 533 patients were reported. The event rate per 1000 py was 8.9 (95% CI 8.2 to 9.6) over all treatments. The EAER of HZ was 9.3 (95% CI 7.7 to 11.2) for monoclonal anti-TNF antibodies, 8.6 (95% CI 6.8 to 10.7) for soluble TNF receptor fusion protein, 8.4 (95% CI 5.9 to 11.8) for T cell costimulation modulator, 10.3 (95% CI 8.0 to 13.0) for B cell targeted therapy, 8.8 (95% CI 6.9 to 11.0) for IL-6 inhibitors, 21.5 (95% CI 16.4 to 27.9) for JAK inhibitors and 7.1 (95% CI 6.0 to 8.3) for csDMARDs (figure 1). The event rate of HZ under JAK inhibitors with concomitant use of glucocorticoids was comparable with the event rate of HZ under JAK inhibitors without concomitant use of glucocorticoids (online supplemental figure S1). The EAER per 1000 py for each separate MedDRA term is presented in online supplemental table S1. Similar EAERs were observed when using a 3-month risk window (data not shown).

A total of 61 serious HZ events were reported in 61 patients. The event rate of serious HZ over all treatments per 1000 py was 1.0 (95% CI 0.7 to 1.2). The event rates between treatments did not differ considerably, except for higher event rates of serious HZ under B cell targeted therapy and JAK inhibitors (figure 2). The EAIRs of all HZ and of serious HZ are comparable with the respective EAERs and are provided in online supplemental figures S2 and S3, respectively.

Recurrent HZ events occurred in 22 patients. Patient characteristics at baseline and EAERs of HZ among patients with recurrent events are provided in online supplemental tables S2 and S3, respectively.

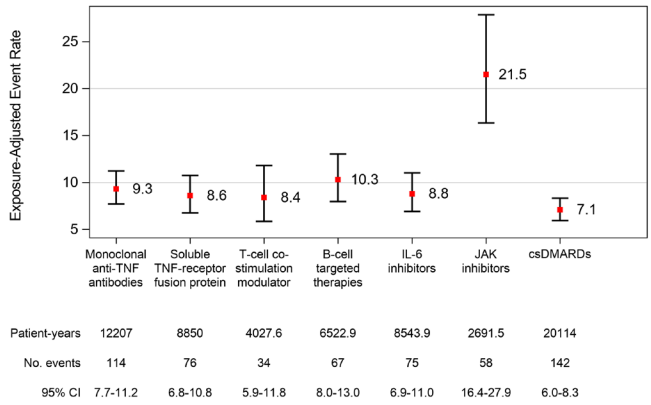

Figure 1 Exposure-adjusted event rates of all herpes zoster per 1000 patient years. csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; IL-6, interleukin 6; JAK, Janus kinase; TNF, tumour necrosis factor.

Risk of HZ

Adjusted for age, sex and glucocorticoid use, a significantly increased risk was observed for treatment with monoclonal anti-TNF antibodies (HR 1.73, 95% CI 1.34 to 2.24), soluble TNF receptor fusion protein (HR 1.45, 95% CI 1.09 to 1.94), B cell targeted therapy (HR 1.62, 95% CI 1.21 to 2.18), IL-6 inhibitors (HR 1.41, 95% CI 1.06 to 1.89) and JAK inhibitors (HR 3.23, 95% CI 2.32 to 4.48) compared with treatment with csDMARDs. Treatment with T cell costimulation modulator showed no significantly higher risk compared with csDMARDs. Furthermore, older age, female sex and glucocorticoid use in a dose-dependent manner were associated with an increased risk of HZ (table 2). Using IPW adjustment, a significantly higher risk of HZ remained under monoclonal anti-TNF antibodies, B cell targeted therapy and JAK inhibitors compared with treatment with csDMARDs (table 2). A sensitivity analysis, where IPW was estimated including CDAI instead of DAS28 as a measure of disease activity, showed similar results, except for B cell targeted therapy being no longer significant in comparison with csDMARDs (online supplemental table S4).

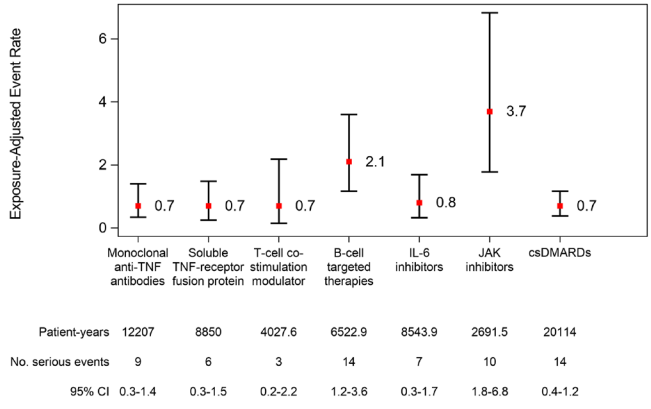

Figure 2 Exposure-adjusted event rates of serious herpes zoster per 1000 patient years. csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; IL-6, interleukin 6; JAK, Janus kinase; TNF, tumour necrosis factor.

Subsample analysis of patients with different treatment episodes

A total of 5974 patients were observed under treatment with a certain bDMARD/tsDMARD and with csDMARD alone, and thus included in the subsample analysis (a schematic representation of the design of the subsample is provided in online supplemental figure S4). These patients' first three treatment switches or (transient) interruptions are illustrated in online supplemental figure S5. It shows, for instance, that among the patients who started treatment with monoclonal anti-TNF antibodies at baseline, the majority experienced only (transient) interruption before receiving another bDMARD/tsDMARD, while 'treatment switch' in patients enrolled with csDMARDs mainly meant that they started TNF inhibitor treatment.

The mean duration of treatment with bDMARDs/tsDMARDs and csDMARDs is reported in table 3, together with the baseline characteristics of patients in the subsample, which were comparable with those in the entire study sample. A patient from this subsample who started treatment with monoclonal anti-TNF antibodies at baseline was treated, on average, for 12.9 months with this treatment and for 10.1 months with csDMARDs alone, whereas a patient who started treatment with JAK inhibitors at baseline was treated, on average, for 7.3 months with this treatment and for 6.7 months with csDMARDs alone (table 3).

Adjusted for age, sex and glucocorticoid use, a significantly increased risk in this subsample analysis was observed for treatment with monoclonal anti-TNF antibodies (HR 1.87, 95% CI 1.31 to 2.69), B cell targeted therapy (HR 1.71, 95% CI 1.22 to 2.39) and JAK inhibitors (HR 3.51, 95% CI 2.24 to 5.52) compared with treatment with csDMARDs, whereas treatment with soluble TNF receptor fusion protein, T cell costimulation modulator and IL-6 inhibitors showed no significantly higher risk compared with csDMARDs (table 4). Furthermore, older age, female sex and glucocorticoid use were associated with an increased risk of HZ (table 4).

DISCUSSION

This is the first analysis in a European prospective cohort study comparing the event and incidence rates and risk of HZ in patients with RA under treatment with six different DMARDs of variable modes of action with csDMARD treatment in one large national cohort. A significant association between HZ and treatment with tsDMARDs was found. The association between HZ and monoclonal anti-TNF antibodies as well as B cell targeted therapy was less influential and varied depending on the methodology used. A significantly higher risk of HZ was found for patients of older age and for treatment with glucocorticoids, with the latter being dose-dependent.

Previous data from the USA showed an increased risk of HZ in patients treated with tsDMARDs, but no clear association was found between HZ and use of bDMARDs.[2] Earlier data from the RABBIT register have already shown an increased risk of patients treated with a monoclonal anti-TNF antibody.[12] However, it remained unclear whether this was due to inflammatory activity of RA or due to the specific mode of action of the treatment administered, as patients treated with soluble TNF receptor fusion protein did not show an increased risk. In contrast, data from the British Society for Rheumatology Biologics Register provided evidence for an increased risk associated with all TNF inhibitors, while in other reports no increased incidence rates were found.[13–15] Furthermore, differences in the use of concomitant glucocorticoids and methotrexate were discussed as possible causes of these different results because, uniformly in all studies,

Table 2 Risk of herpes zoster: Andersen-Gill model with and without IPW

	Andersen-Gill model without IPW		Andersen-Gill model with IPW	
Characteristics	**Adjusted HR (95% CI)**	**P value**	**Adjusted HR (95% CI)**	**P value**
Female sex	1.36 (1.10 to 1.68)	**0.0051**	1.13 (0.85 to 1.50)	0.4122
Age per 10 years	1.21 (1.13 to 1.31)	**<0.0001**	1.25 (1.14 to 1.37)	**<0.0001**
Glucocorticoids, 5–10 vs 0 mg/day	1.42 (1.19 to 1.69)	**<0.0001**	1.47 (1.17 to 1.85)	**0.0008**
Glucocorticoids, >10 vs 0 mg/day	3.57 (2.36 to 5.39)	**<0.0001**	4.42 (2.50 to 7.83)	**<0.0001**
csDMARD treatment	*Reference*		*Reference*	
Monoclonal anti-TNF antibodies	1.73 (1.34 to 2.24)	**<0.0001**	1.63 (1.17 to 2.28)	**0.0042**
Soluble TNF receptor fusion protein	1.45 (1.09 to 1.94)	**0.0121**	1.28 (0.90 to 1.81)	0.1687
T cell costimulation modulator	1.25 (0.85 to 1.85)	0.2608	1.45 (0.86 to 2.46)	0.1652
B cell targeted therapy	1.62 (1.21 to 2.18)	**0.0013**	1.57 (1.03 to 2.40)	**0.0355**
IL-6 inhibitors	1.41 (1.06 to 1.89)	**0.0200**	1.44 (0.99 to 2.11)	0.0578
JAK inhibitors	3.23 (2.32 to 4.48)	**<0.0001**	3.66 (2.38 to 5.63)	**<0.0001**

P values <0.05 are shown in bold.
Weights were estimated using the variables age, sex, disease duration, DAS28, FFbH, previous treatment with bDMARDs/tsDMARDs and osteoporosis.
bDMARDs, biologic DMARDs; csDMARDs, conventional synthetic DMARDs; DAS28, Disease Activity Score of 28 joints using the erythrocyte sedimentation rate; DMARDs, disease-modifying antirheumatic drugs; FFbH, Hannover Functional Status Questionnaire; IL-6, interleukin 6; IPW, inverse probability weights; JAK, Janus kinase; TNF, tumour necrosis factor; tsDMARDs, targeted synthetic DMARDs.

glucocorticoids were associated with an increased risk of HZ. In a meta-analysis of 2014, a 61% significantly increased risk of HZ was calculated for patients receiving TNF inhibitors.[16]

The results of the analysis presented here comparing data on all DMARDs support our former findings of an increased incidence of HZ under bDMARDs compared with csDMARDs. Of note, this analysis was based on an independent data set with data collected between 2007 and end of October 2020, while the former analysis was performed on a data set of the same cohort with earlier data collected between 2001 and end of December 2006.[12] In agreement with our previous report, we showed a higher risk of HZ in patients receiving monoclonal anti-TNF antibodies but not in those receiving the soluble TNF receptor fusion protein. This difference may be explained by different mechanisms of action related to the induced cytotoxicity in TNF-expressing monocytes and T cells, yielding the expression of different leucocyte genes.[17 18] Due to the approval of additional therapies since the previous analysis, this time we were also able to investigate the risk of HZ for other mechanisms of action. This enabled us to investigate the risk of HZ also under B cell targeted therapy, T cell costimulation modulator, IL-6 inhibitors and tsDMARDs, of which the first and the latter were significantly associated with an increased risk of HZ compared with csDMARDs.

The possible mechanism leading to reactivation of the virus may differ with regard to the specific mechanisms of action. While monoclonal anti-TNF antibodies conjoin transmembrane TNF and induce apoptosis of T cells, the soluble TNF receptor

Table 3 Characteristics of patients who were either enrolled with bDMARD/tsDMARD treatment and had interruption(s) during follow-up in the course of which they received csDMARDs alone or who were enrolled with csDMARD treatment and started bDMARD/tsDMARD treatment during follow-up (n=5974)

	Treatment started at enrolment						
	Monoclonal anti-TNF antibodies	**Soluble TNF receptor fusion protein**	**T cell costimulation modulator**	**B cell targeted therapy**	**IL-6 inhibitors**	**JAK inhibitors**	**csDMARDs**
	n=1330 (22.26%)	**n=873 (14.61%)**	**n=395 (6.61%)**	**n=1296 (21.69%)**	**n=544 (9.12%)**	**n=132 (2.21%)**	**n=1404 (23.50%)**
Baseline							
Age, mean (SD), years	55.7 (12.8)	59.1 (13.2)	58.3 (13.3)	58.2 (11.7)	57.5 (12.7)	59.9 (12.4)	56.3 (11.8)
Women, n (%)	1019 (76.6)	653 (74.8)	300 (75.9)	999 (77.1)	418 (76.8)	98 (74.2)	1023 (72.9)
DAS28, mean (SD)	5.0 (1.3)	5.0 (1.3)	5.4 (1.3)	5.3 (1.3)	5.2 (1.3)	4.9 (1.3)	4.6 (1.3)
Glucocorticoids, n (%)	847 (63.7)	536 (61.5)	235 (59.6)	870 (68.0)	346 (63.7)	58 (43.9)	776 (55.3)
Glucocorticoids, ≥10 mg, n (%)	221 (16.7)	139 (16.0)	72 (18.3)	298 (23.4)	115 (21.2)	9 (6.9)	141 (10.0)
Follow-up							
Duration of treatment episodes, mean (SD), months							
Monoclonal anti-TNF antibodies	12.9 (15.5)	10.6 (13.0)	13.0 (15.9)	12.9 (14.0)	10.3 (14.0)	6.2 (6.5)	15.4 (18.5)
Soluble TNF receptor fusion protein	10.9 (13.0)	11.8 (14.5)	9.9 (11.5)	12.0 (14.8)	10.0 (11.7)	6.9 (6.4)	15.2 (17.2)
T cell costimulation modulator	12.4 (15.8)	11.0 (11.6)	12.0 (13.8)	14.4 (17.3)	12.6 (17.0)	6.6 (6.7)	14.2 (16.3)
B cell targeted therapy	8.6 (8.1)	8.0 (6.8)	8.4 (8.3)	8.2 (7.2)	8.5 (7.3)	8.5 (3.2)	9.3 (11.4)
IL-6 inhibitors	13.1 (15.6)	12.5 (15.9)	13.9 (17.2)	15.0 (18.5)	13.2 (15.9)	4.9 (3.3)	16.5 (18.5)
JAK inhibitors	9.5 (8.5)	8.5 (8.0)	8.5 (7.3)	9.9 (10.1)	10.7 (9.1)	7.3 (6.8)	9.7 (8.5)
csDMARDs	10.1 (14.1)	9.5 (12.2)	8.3 (10.9)	7.4 (10.2)	8.4 (12.0)	6.7 (6.7)	11.7 (14.9)

bDMARDs, biologic DMARDs; csDMARDs, conventional synthetic DMARDs; DAS28, Disease Activity Score of 28 joints using the erythrocyte sedimentation rate; DMARDs, disease-modifying antirheumatic drugs; IL-6, interleukin 6; JAK, Janus kinase; TNF, tumour necrosis factor; tsDMARDs, targeted synthetic DMARDs.

Table 4 Risk of herpes zoster for patients who were either enrolled with bDMARD/tsDMARD treatment and had interruption(s) during follow-up in the course of which they received csDMARDs alone or who were enrolled with csDMARD treatment and started bDMARD/tsDMARD treatment during follow-up

	Andersen-Gill model	
Characteristics	**Adjusted HR (95% CI)**	**P value**
Female sex	1.64 (1.20 to 2.24)	**0.0018**
Age per 10 years	1.31 (1.19 to 1.44)	**<0.0001**
Glucocorticoids, 5–10 vs 0 mg/day	1.24 (0.98 to 1.57)	0.0763
Glucocorticoids, >10 vs 0 mg/day	3.45 (2.14 to 5.55)	**<0.0001**
csDMARD treatment	*Reference*	
Monoclonal anti-TNF antibodies	1.87 (1.31 to 2.69)	**0.0006**
Soluble TNF receptor fusion protein	1.35 (0.87 to 2.10)	0.1819
T cell costimulation modulator	1.60 (0.98 to 2.59)	0.0581
B cell targeted therapy	1.71 (1.22 to 2.39)	**0.0019**
IL-6 inhibitors	1.48 (0.97 to 2.25)	0.0673
JAK inhibitors	3.51 (2.24 to 5.52)	**<0.0001**

P values <0.05 are shown in bold.
bDMARDs, biologic DMARDs; csDMARDs, conventional synthetic DMARDs; DMARDs, disease-modifying antirheumatic drugs; IL-6, interleukin 6; JAK, Janus kinase; TNF, tumour necrosis factor; tsDMARDs, targeted synthetic DMARDs.

does not. Etanercept lacks a specific domain for the docking of one of the complement components so that interaction proceeds differently.[19] From the reactivation of latent tuberculosis it is known that insufficient interferon-gamma (IFN-γ) production may also play a role, as TNF inhibitors but not B cell targeted therapy, T cell costimulation modulator and IL-6 inhibitors inhibit the IFN-γ production induced by tuberculosis antigens.[19] JAK inhibitors modulate the immune response by blocking intracellular signals on the cytokine level. The downregulation of interleukin 12, IFN-γ and other relevant cytokines is discussed to enable the reactivation of latent viral infections.[20 21] A possible explanation for the specific attenuated immune response to tofacitinib may be found in a recent basic science work when keratinocytes and synovial cells were exposed to tofacitinib and the immune response was measured after stimulation with bacterial lipopolysaccharides or viral varicella-zoster virus (VZV).[22] The results confirm what we also see in our data, namely that antiviral immunity is downregulated after exposure to JAK inhibitors.

The more unstable influence of the other bDMARDs remains to be further investigated. EAERs and EAIRs were very comparable and adjusted HRs were significant for all modes of actions except for abatacept. The numbers were not very dissimilar for this drug particularly with IPW. After IPW adjustment, only monoclonal TNF and B cell depletion remained significant, but in the sensitivity analysis B cell depletion was no longer significant. A clear difference in terms of the underlying mechanisms of action cannot be derived from this.

Age and glucocorticoids have previously been described as risk factors for HZ[4–6] and were confirmed in our data. The clear dose dependence with a 3.5-fold higher risk at doses above 10 mg should be emphasised here.

Although only 11% of the HZ events reported in our cohort were considered as being serious by the local investigator, the impact of HZ on patients' burden of disease and on healthcare in general is considerable.[23] Healthcare utilisation and costs are about doubled in patients with RA and HZ compared with persons with HZ without immunosuppression.[24]

In terms of a risk assessment with regard to vaccination, this suggests that especially elderly patients with higher glucocorticoid doses and patients for whom tsDMARD therapy is planned should be considered for vaccination. Preliminary data from tofacitinib-treated patients with RA indicate the possibility of reducing the risk of HZ by vaccination.[4]

Limitations and strengths

The query of the vaccination status has only recently been added to the questionnaire used in the RABBIT register and could not be taken into account in this analysis. Since we do not know the proportion of patients who may have been vaccinated, there is a possibility of unequal distribution in the groups. Future analyses, however, will allow adjusting for vaccination status. Concomitant methotrexate was not adjusted for as its use differs among the bDMARDs included in this analysis. Moreover, a clear association between HZ events and use of methotrexate in patients with RA has not been confirmed.[25]

The strength of our study is the large prospective register that includes all available DMARD therapies in RA, enabling direct risk comparison of different treatments within one cohort. Furthermore, we conducted a secondary analysis among a subsample of patients who were either enrolled with a certain bDMARD/tsDMARD treatment, had at least one interruption of this treatment during follow-up, and received csDMARD treatment during this interruption period or patients who were enrolled with csDMARD treatment and started a bDMARD/tsDMARD treatment during follow-up. The strength of this analysis was that each patient had been observed under different treatment episodes (bDMARD/tsDMARD vs csDMARD alone), while carrying her/his own risk factors. Thus, patients serve as their own controls in this design. The results of this secondary analysis support the findings of the main analysis.

To conclude, the risk of HZ infection in patients with RA is multiplied by age and the need for immunosuppressive therapy especially when glucocorticoids and JAK inhibitors are applied.

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Contributors IR, MS and AS had full access to all data of this study and take responsibility for data integrity and accuracy of the analysis. Study concept and design: IR, MS, AZ and AS. Acquisition of data: JK, G-RRB and JB. Analysis and interpretation of data: IR, KA, MS, AZ and AS. Drafting the manuscript: IR, KA and AS. Critical revision of the manuscript for important intellectual content: IR, MS, KA, G-RRB, JB, JK, AZ and AS. Obtaining funding: AZ and AS. All authors read and approved the manuscript.

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CLINICAL SCIENCE

Determining in which pre-arthritis stage HLA-shared epitope alleles and smoking exert their effect on the development of rheumatoid arthritis

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ABSTRACT

Objectives The human leukocyte antigen-shared epitope (HLA-SE) alleles and smoking are the most prominent genetic and environmental risk factors for rheumatoid arthritis (RA). However, at which pre-arthritis stage (asymptomatic/symptomatic) they exert their effect is unknown. We aimed to determine whether HLA-SE and smoking are involved in the onset of autoantibody positivity, symptoms (clinically suspect arthralgia (CSA)) and/or progression to clinical arthritis.

Methods We performed meta-analyses on results from the literature on associations of HLA-SE and smoking with anti-citrullinated protein antibodies (ACPAs) in the asymptomatic population. Next, we studied associations of HLA-SE and smoking with autoantibody positivity at CSA onset and with progression to clinical inflammatory arthritis (IA) during follow-up. Associations in ACPA-positive patients with CSA were validated in meta-analyses with other arthralgia cohorts. Analyses were repeated for rheumatoid factor (RF), anti-carbamylated protein antibodies (anti-CarP) and anti-acetylated protein antibodies (AAPA).

Results Meta-analyses showed that HLA-SE is not associated with ACPA positivity in the asymptomatic population (OR 1.06 (95% CI:0.69 to 1.64)), whereas smoking was associated (OR 1.37 (95% CI: 1.15 to 1.63)). At CSA onset, both HLA-SE and smoking associated with ACPA positivity (OR 2.08 (95% CI: 1.24 to 3.49), OR 2.41 (95% CI: 1.31 to 4.43)). During follow-up, HLA-SE associated with IA development (HR 1.86 (95% CI: 1.23 to 2.82)), in contrast to smoking. This was confirmed in meta-analyses in ACPA-positive arthralgia (HR 1.52 (95% CI: 1.08 to 2.15)). HLA-SE and smoking were not associated with RF, anti-CarP or AAPA-positivity at CSA onset. Longitudinally, AAPA associated with IA development independent from ACPA and RF (HR 1.79 (95% CI: 1.02 to 3.16)), anti-CarP did not.

Conclusions HLA-SE and smoking act at different stages: smoking confers risk for ACPA and symptom development, whereas HLA-SE mediates symptom and IA development. These data enhance the understanding of the timing of the key risk factors in the development of RA.

Key messages

What is already known about this subject?

- The HLA-shared epitope (HLA-SE) and smoking are the most important genetic and environmental risk factors for rheumatoid arthritis (RA), particularly for anti-citrullinated protein antibody (ACPA)-positive RA. It is unknown at which pre-arthritis stage HLA-SE and smoking exert their effect.

What does this study add?

- HLA-SE and smoking act at different pre-RA stages.
- Smoking confers risk for the development of ACPA and symptoms, whereas HLA-SE mediates symptom and arthritis development.

How might this impact on clinical practice or future developments?

- This study enhances the understanding of the timing of HLA-SE and smoking in the development of RA. This knowledge can guide pathophysiological studies seeking to determine the mechanisms in the trajectories leading to RA.
- The results could guide health-promoting behaviours: current results imply that smoking cessation can be helpful in preventing RA development especially in the asymptomatic phase, while this might be less effective in preventing RA in the symptomatic phase.

INTRODUCTION

The human leukocyte antigen-shared epitope (HLA-SE) is the most well-known and strongest genetic risk factor for the development of rheumatoid arthritis (RA), especially for anti-citrullinated protein antibody (ACPA)-positive RA.[1–14] Similarly, smoking is the strongest environmental risk factor for autoantibody-positive RA[2 9 10 12 15]; multiple studies have shown this effect is mostly present in people carrying HLA-SE alleles.[1 3 5 6 8 14 16] This knowledge is mostly obtained from case–control studies comparing patients with RA and healthy

controls. During the last decade, research attention has shifted to the stages that precede clinical arthritis and RA and several pre-RA stages have been discerned. However, so far it remains undetermined at which stage(s) HLA-SE alleles and smoking exert their effect.

The following stages are distinguished. An asymptomatic stage in which autoimmune responses can develop, resulting in autoantibody positivity. Then, autoimmune responses can mature and a symptomatic stage develops. The pattern of symptoms that is considered specific for an increased risk of RA is called clinically suspect arthralgia (CSA). Patients with CSA can progress to clinically apparent inflammatory arthritis (IA), the stage when RA is generally diagnosed.[17] This model suggests that genetic factors exert their influence first, followed by smoking with subsequent autoantibody development.[17 18] However, this time order has never been shown.

In addition to a nested case–control study,[19] several longitudinal studies assessed genetic factors and/or smoking and provided data either from healthy to IA but not the intermediate stages or from mixed populations of asymptomatic and symptomatic people.[20–24] These approaches do not allow determination of stage-dependent effects. As for the asymptomatic stage, contrasting findings are reported on associations between HLA-SE alleles and smoking and the presence of ACPA in the general population.[2 14 25–28] To the best of our knowledge, only one study evaluated the effect of smoking on the progression from ACPA positivity to CSA.[29] Furthermore, longitudinal studies within arthralgia are scarce and their findings varied.[30 31] The mentioned studies focused on ACPA; however, HLA-SE and smoking might also interact with other autoantibodies such as rheumatoid factor (RF), anti-carbamylated protein antibodies (anti-CarP) and anti-acetylated protein antibodies (AAPA), the time effects of which have not yet been studied.

We aimed to determine at which pre-RA stage HLA-SE and smoking exert their effect by studying both original and previously reported data. More specifically, we performed meta-analyses on the literature from the general population, analysed our own data at CSA onset and during progression to IA and finally performed meta-analyses using data from different longitudinal arthralgia cohorts. In doing this, we focused on fine staging the effects in the development of ACPA-positive RA. Analyses were repeated for ACPA-negative RA and associations of RF, anti-CarP and AAPA.

METHODS

Summarising the literature obtained from the general population

The literature was reviewed on studies reporting associations between HLA-SE and/or smoking with the presence of ACPA in the asymptomatic population, as described supplementary. Results were pooled in meta-analyses. Although these studies were cross-sectional in nature, observed findings were considered to reflect the influence of HLA-SE/smoking on ACPA development, as this is most likely the first event in the development of ACPA-positive RA.

The symptomatic phase

Associations of HLA-SE and smoking with autoantibodies at CSA onset were investigated in the Leiden CSA cohort, we did not identify large cohorts for validation since most arthralgia cohorts did not include autoantibody-negative patients. Additionally, the role of HLA-SE and smoking in progression from arthralgia to IA was investigated in the Leiden CSA cohort. Results obtained in the ACPA-positive subgroup were validated in ACPA-positive arthralgia/at-risk patients from two independent cohorts (Amsterdam, Leeds).

Measurements at CSA onset

Patients presenting with CSA to the Leiden rheumatology outpatient clinic between April 2012 and September 2019 were studied. As described in detail previously,[32] patients had recent-onset (<1 year) arthralgia of small joints and were, according to the clinical expertise and pattern recognition of the rheumatologist, at risk for progression to RA. Patients were excluded if clinical arthritis was already present or if a different explanation for the joint pain was more likely. At baseline smoking status (present/past/never) was obtained through questionnaires. The presence of IgM RF (in-house ELISA, cut-off >3.5 IU/mL) and IgG ACPA (anti-cyclic citrullinated peptide 2 (anti-CCP2), Phadia, Nieuwegein, the Netherlands, cut-off >7 IU/mL) was determined during routine laboratory measurements in all patients and the presence of IgG anti-CarP and IgG AAPA was determined with in-house ELISA in a subset of patients. Detailed methods are described in online supplemental material. The HLA-SE alleles were extracted from whole-genome sequencing data; the HLA region was isolated and imputed using the SNP2HLA software and T1DGC reference panel.[33] HLA-SE positivity was subsequently defined as the presence of one or two of the HLA-DRB1 alleles *0101, *0102, *0401, *0404, *0405, *0408 and *1001 (see online supplemental material).[34]

Measurements on the progression from CSA to IA

Patients in the Leiden CSA cohort were prospectively followed (median (IQR) 106 weeks (43–114)) for the development of IA, which was defined as ≥1 swollen joints at physical examination by a rheumatologist. Treatment with disease-modifying antirheumatic drugs (including systemic or intra-articular corticosteroids) was not allowed before IA development. Analyses evaluating progression to IA were stratified for ACPA status and results from the ACPA-positive subgroup were studied in meta-analyses with the results from ACPA-positive patients included in the Amsterdam and Leeds cohorts. The Amsterdam cohort included ACPA-positive and/or RF-positive patients; for this study, the data from patients with ACPA-positive arthralgia were obtained and studied.[31] Data on smoking history, presence of HLA-SE, RF, ACPA and anti-CarP were collected previously and are described in online supplemental material. In addition, IgG AAPA was determined in baseline serum samples simultaneous with Leiden CSA samples. Results on predictive value of HLA-SE and smoking in ACPA-positive patients from the Leeds cohort were obtained from Rakieh *et al*,[30] detailed methods are described in online supplemental material. Anti-CarP and AAPA were not determined in the Leeds cohort.

In subanalyses, the association of HLA-SE and smoking with RA development was studied using Leiden CSA data; RA was defined as the development of IA plus fulfilment of the 1987 and/or 2010 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) criteria at that time.[35 36]

Statistics

Results from the literature on associations of HLA-SE and smoking with ACPA in the asymptomatic population were pooled in inverse-variance weighted meta-analyses.

Associations of HLA-SE and smoking with autoantibody positivity at CSA onset were investigated with logistic regression analyses. Results of smoking were also stratified for HLA-SE.

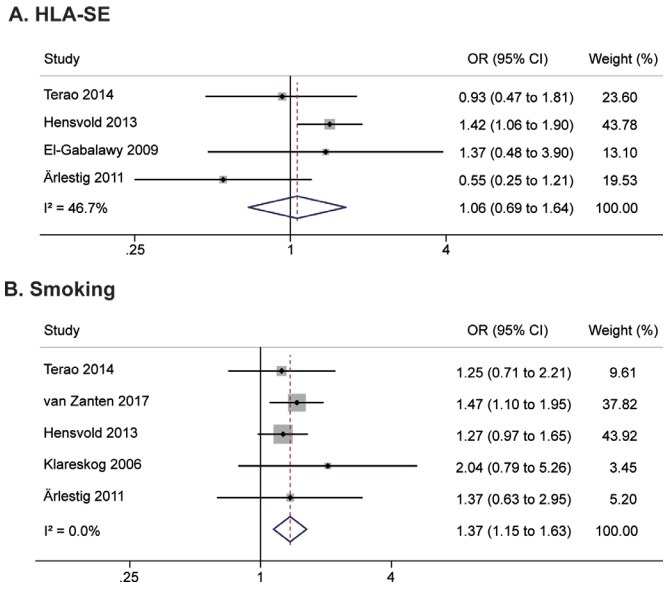

Figure 1 Meta-analyses on HLA-SE (A) and smoking (B) in asymptomatic healthy individuals and first-degree relatives, showing associations with the presence of ACPA for smoking but not for HLA-SE. ACPA, anti-citrullinated protein antibody; HLA-SE, human leukocyte antigen-shared epitope.

Associations of HLA-SE and smoking with ACPA level in ACPA-positive patients were evaluated with Mann-Whitney U tests and logistic regression.

Associations with IA development were studied with Cox regression, also stratified for ACPA. Results in ACPA-positive arthralgia were summarised in inverse-variance weighted meta-analyses.

Associations of anti-CarP and AAPA with IA development were corrected for concomitant ACPA and RF positivity in multivariable analyses with the autoantibody-negative group as reference in the Leiden data (the Amsterdam cohort did not include autoantibody-negative patients). The additional value of anti-CarP and AAPA to ACPA and RF positivity for prediction of IA development was determined in the ACPA+RF+ subgroup from the Leiden and Amsterdam cohorts.

P values<0.05 were considered statistically significant. IBM SPSS Statistics (V.25) and STATA (V.16) were used.

RESULTS

Summarising the literature obtained from the asymptomatic stage

Four studies were identified on the association of HLA-SE with ACPA and five on smoking (online supplemental file 1). Meta-analyses revealed that HLA-SE was not associated with ACPA positivity (OR 1.06 (95% CI: 0.69 to 1.64)), whereas smoking was associated (OR 1.37 (95% CI: 1.15 to 1.63)), figure 1. This suggests that smoking, but not HLA-SE, conferred risk for ACPA development in the asymptomatic stage.

Associations with ACPA at CSA onset

Characteristics of patients presenting with CSA (n=577) are provided in the online supplemental materials. HLA-SE-positive patients with CSA were more often ACPA positive (OR 2.08 (95% CI: 1.24 to 3.49), this relation was dependent on the number of alleles (table 1). Patients who smoked were also more often ACPA positive (OR 2.41 (95% CI: 1.31 to 4.43)), which was also dose dependent with a higher OR for current smokers than ex-smokers (table 1). In addition, within smokers, it was dependent on number of packyears, because the odds for being ACPA positive increased per increase in packyear (OR 1.03 (95% CI: 1.00 to 1.06)). As it has been reported in RA that the association of smoking is dependent on HLA-SE status, we stratified the analyses of smoking (ever vs never) for HLA-SE; smoking was associated with ACPA status in both HLA-SE-negative and HLA-SE-positive patients with CSA (table 1). The association of HLA-SE and smoking with ACPA positivity was present for both ACPA double positivity (ACPA+RF+) and single positivity (ACPA+RF−), and thus independent from RF (online supplemental table 2). Studying the levels of ACPA within ACPA-positive patients at CSA onset revealed that HLA-SE-positive patients tended to have higher levels than HLA-SE-negative patients (median (IQR) 236 (72–340) vs 144 (32–340), p=0.12), while no effect on ACPA levels was present for smoking (229 (64–340) vs 222 (52–340), p=0.89), see online supplemental table 3 for results from regression analyses.

Table 1 Associations of HLA-SE and smoking with the presence of ACPA in patients newly presenting with CSA

	ACPA positive, n (%)	ACPA negative, n (%)	OR (95% CI)	P value
All patients				
HLA-SE				
Absent	27 (39)	259 (57)	Reference	
Present	42 (61)	194 (43)	2.08 (1.24 to 3.49)	0.006
HLA-SE				
0	27 (39)	259 (57)	Reference	–
1	31 (45)	161 (36)	1.85 (1.06 to 3.21)	0.029
2	11 (16)	33 (7)	3.20 (1.45 to 7.04)	0.004
Smoking				
Never	15 (23)	185 (42)	Reference	–
Ever	49 (77)	251 (58)	2.41 (1.31 to 4.43)	0.005
Smoking				
Never	15 (23)	185 (42)	Reference	–
Ex-smoker	28 (44)	161 (37)	2.15 (1.12 to 4.16)	0.024
Current smoker	21 (33)	90 (21)	2.88 (1.42 to 5.85)	0.003
HLA-SE-positive subgroup				
Smoking				
Never	10 (27)	77 (45)	Reference	–
Ever	27 (73)	95 (55)	2.19 (1.00 to 4.80)	0.051
Smoking				
Never	10 (27)	77 (45)	Reference	–
Ex-smoker	13 (35)	57 (33)	1.76 (0.72 to 4.29)	0.22
Current smoker	14 (38)	38 (22)	2.84 (1.15 to 6.98)	0.023
HLA-SE-negative subgroup				
Smoking				
Never	4 (18)	99 (43)	Reference	–
Ever	18 (82)	130 (57)	3.43 (1.12 to 10.45)	0.030
Smoking				
Never	4 (18)	99 (43)	Reference	–
Ex-smoker	11 (50)	89 (39)	3.06 (0.94 to 9.95)	0.063
Current smoker	7 (32)	41 (18)	4.23 (1.17 to 15.22)	0.027

Numbers on smoking in HLA-SE strata do not add up to numbers in the total CSA group as some patients with data on smoking have missing data on HLA-SE.
ACPA, anti-citrullinated protein antibody; CSA, clinically suspect arthralgia; HLA-SE, human leukocyte antigen-shared epitope.

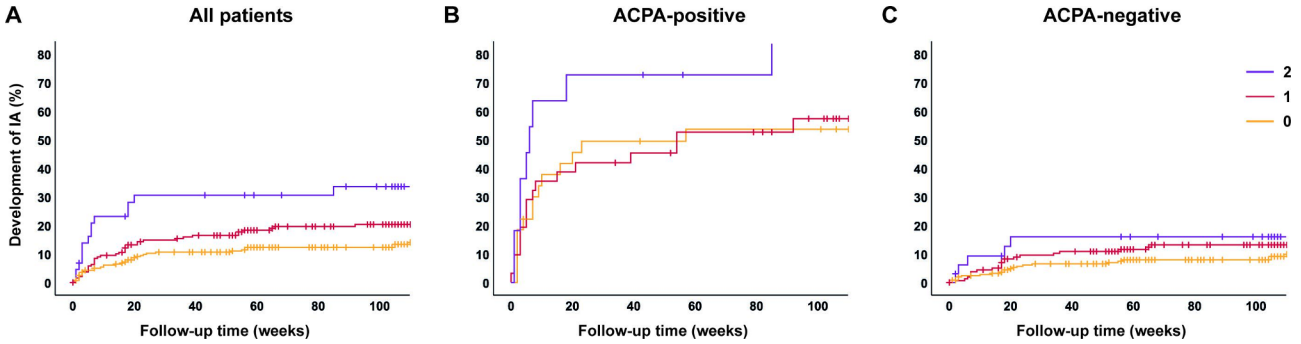

Figure 2 Associations of number of HLA-SE alleles (0/1/2 alleles present) with progression from CSA to inflammatory arthritis (IA). Corresponding HRs, with 0 HLA-SE alleles as reference category were: (A) HR 1.65 (95% CI: 1.06 to 2.56) and HR 3.03 (95% CI: 1.64 to 5.61) for 1 and 2 HLA-SE alleles, respectively, (B) HR 1.05 (95% CI: 0.52 to 2.13) and HR 2.32 (95% CI: 1.00 to 5.41) and (C) HR 1.66 (95% CI: 0.94 to 2.94) and HR 2.00 (95% CI: 0.76 to 5.28), see online supplemental table 4. ACPA, anti-citrullinated protein antibody; CSA, clinically suspect arthralgia; HLA-SE, human leukocyte antigen-shared epitope.

Progression to IA in ACPA-positive CSA

Patients were followed for the development of IA; median time till IA was 16 weeks (IQR 3–36), non-progressors were followed for median 109 (62–116) weeks. The presence of HLA-SE was significantly associated with IA development in all patients with CSA (HR 1.86 (95% CI: 1.23 to 2.82)), also here a dose–response relation was present (figure 2A,(online supplemental table 4). Within the ACPA-positive subgroup the HR was 1.29 (95% CI: 0.67 to 2.47, figure 2B, online supplemental table 4). Because of the small sample size after stratification and risk of type II error, we performed meta-analysis including ACPA-positive patients from two other arthralgia cohorts. This showed that HLA-SE significantly associated with IA development in ACPA-positive patients (HR 1.52 (95% CI: 1.08 to 2.15), figure 4A).

Smoking was not associated with IA development, neither in the total CSA population (HR 1.40 (95% CI: 0.90 to 2.18), figure 3A, online supplemental table 5) nor in the ACPA-positive subgroup (HR 0.59 (95% CI: 0.29 to 1.18), figure 3B, online supplemental table 5) and nor in meta-analysis including ACPA-positive patients from three cohorts (HR 0.94 (95% CI: 0.67 to 1.33), figure 4B).

Thus, HLA-SE, but not smoking, influenced the risk to progress from ACPA-positive CSA to RA.

Associations of HLA-SE and smoking in ACPA-negative CSA

The presence of HLA-SE was associated with IA development in ACPA-negative patients (HR 1.71 (95% CI: 0.99 to 2.96)), although the CI just included 1 (figure 2C, online supplemental table 4). Within ACPA−/RF− and ACPA−/RF+ CSA patients associations of HLA-SE with IA development were HR 1.64 (95% CI: 0.90 to 2.99) and HR 2.07 (95% CI: 0.55 to 7.75), respectively.

The tendency of HLA-SE to associate with IA development in ACPA-negative patients disappeared in sensitivity analyses with the outcome RA, in contrast to the effect that remained within ACPA-positive patients (online supplemental figure 3). Hence, HLA-SE was not convincingly associated with progression from symptoms to IA in ACPA-negative patients.

Smoking did also not associate with progression to IA in ACPA-negative patients (HR 1.30 (95% CI: 0.73 to 2.33)), figure 3C, online supplemental table 5.

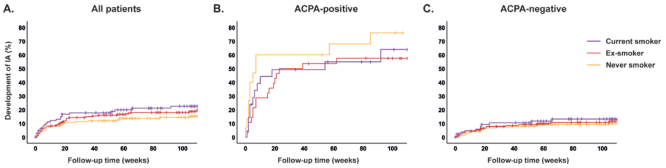

Figure 3 Associations of smoking with progression from CSA to inflammatory arthritis (IA). Corresponding HRs with never smoker as reference category were: (A) HR 1.25 (95% CI: 0.76 to 2.06) and HR 1.66 (95% CI: 0.97 to 2.83) for ex-smoker and current smoker, respectively, (B) HR 0.55 (95% CI: 0.26 to 1.19) and HR 0.64 (95% CI: 0.28 to 1.45) and (C) HR 1.17 (95% CI: 0.61 to 2.24) and HR 1.56 (95% CI: 0.76 to 3.18), see online supplemental table 5. ACPA, anti-citrullinated protein antibody; CSA, clinically suspect arthralgia.

Associations of HLA-SE and smoking with anti-CarP and AAPA at CSA onset

Neither HLA-SE positivity nor smoking was associated with a higher frequency of RF, anti-CarP or AAPA at presentation with CSA, both in univariable analyses and after correction for concomitant presence of ACPA (online supplemental table 6).

Associations of anti-CarP and AAPA with IA development

In univariable analyses, anti-CarP and AAPA were associated with IA development (table 2). Correcting for ACPA and RF in the Leiden cohort revealed that AAPA was significantly associated with RA development, but anti-CarP was not. Similar multivariable analyses were not possible in the Amsterdam cohort because of the lack of an autoantibody-negative reference group. Instead, we studied the association of both AMPA's in the ACPA+/RF+ subgroups. Meta-analyses of data from the two cohorts revealed a significant association for AAPA (HR 1.53 (95% CI: 1.02 to 2.28)), but not for anti-CarP (HR 1.29 (95% CI: 0.85 to 1.97), figure 5).

DISCUSSION

Although it has been extensively shown that HLA-SE and smoking are risk factors for RA, it was thus far unclear in which pre-arthritis stage these factors exert their effect. We aimed to fine stage the effects of HLA-SE and smoking, taking advantage of our own cohort data, as well as published data. Results from meta-analyses in people in the asymptomatic stage indicated that smoking, but not HLA-SE, is involved in the development of ACPA. At CSA onset, both HLA-SE and smoking were associated with the presence of ACPA, although only HLA-SE associated with progression towards arthritis and RA. Presuming that autoantibody development as a proxy for the emerging autoimmune response is the first event, these results imply that smoking is involved in autoantibody development and possibly symptom

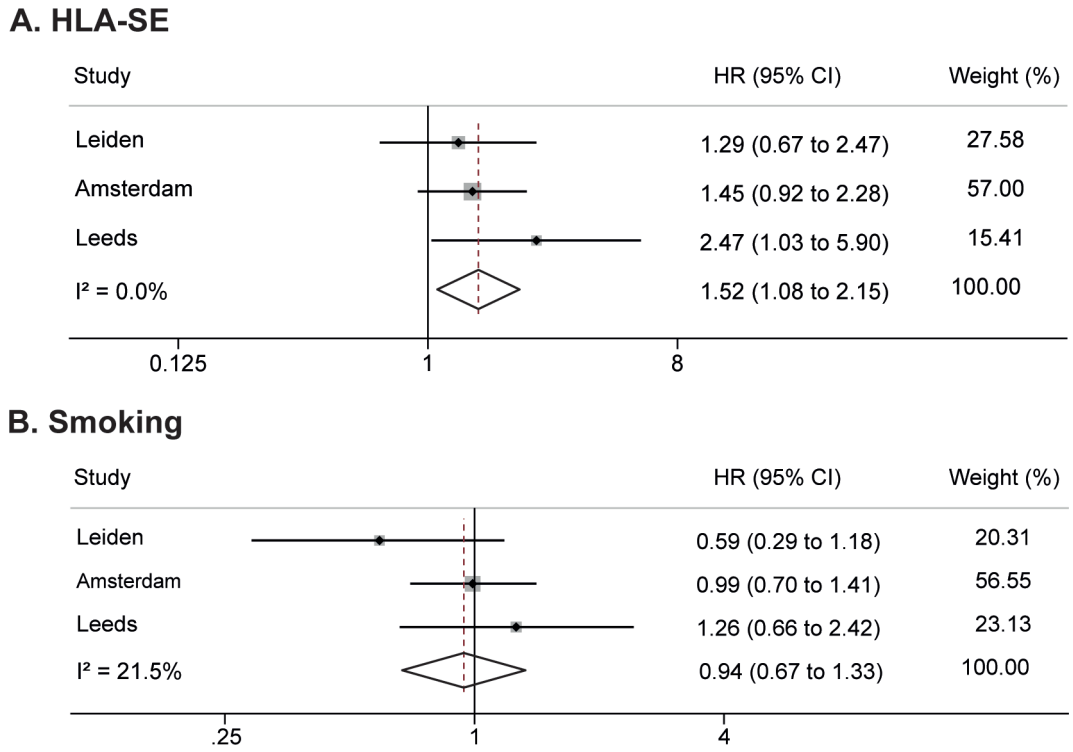

Figure 4 Meta-analyses on HLA-SE (A) and smoking (B) in three cohorts of patients with ACPA-positive arthralgia, showing an association with clinical arthritis development for HLA-SE but not for smoking. Raw data from ACPA-positive patients from the Amsterdam cohort as described by van de Stadt *et al* were obtained and analysed. Results from the Leeds cohort were obtained from Rakieh *et al* (table 2 from reference 30). ACPA, anti-citrullinated protein antibody; CSA, clinically suspect arthralgia; HLA-SE, human leukocyte antigen-shared epitope.

development, but not with further IA development. In contrast, HLA-SE is not involved in initial autoantibody development, but rather associated with autoantibody maturation and symptom development as implied by results found at CSA onset. Furthermore, it associates with further progression to clinical disease (figure 6).

To evaluate the role of HLA-SE and smoking in the asymptomatic phase, we reviewed the literature following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic literature reviews as much as possible (online supplemental file 1).[37] The results of identified studies performed in asymptomatic populations were combined in meta-analyses. These revealed an effect for smoking and absence of an association of HLA-SE with ACPA positivity. Recent data in patients with RA indicated that smoking does not associate with ACPA as such, but rather with RF or autoantibodies in general.[6 15 16 38 39] Although not all of the studies included in the meta-analyses contained data on RF, pooled analysis did not identify an association between smoking and RF in the asymptomatic population (online supplemental material). Also in patients with CSA no association between RF and smoking was found. All included studies were cross-sectionally performed in the general population. As we presumed that ACPA positivity is the first event in the development of ACPA-positive RA, we believe the observed findings reflect effects of HLA-SE and smoking on autoantibody development.

For smoking an association with ACPA was found at the asymptomatic stage and at CSA onset. Our analyses at CSA onset were cross-sectional in nature; therefore, we cannot definitely conclude whether smoking truly associates with progression from autoantibody positivity to symptom development (alternatively, the association found at CSA onset could be reflective of the association with ACPA development). However, one longitudinal study evaluated ACPA-positive individuals from the general population until the development of CSA and showed a significant association of smoking with CSA development.[29] Together with our data this suggests that smoking plays a role in the development of ACPA, further maturation and symptom development.

The absence of an association of HLA-SE with ACPA in the asymptomatic population, the presence of this association at CSA onset and the finding that ACPA levels tended to be higher

Table 2 Associations of autoantibodies with the development of inflammatory arthritis in patients newly presenting with arthralgia

	Univariable Cox regression		Multivariable Cox regression		Multivariable Cox regression	
CSA cohort	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
ACPA IgG	3.29 (2.11 to 5.13)	<0.001	2.55 (1.44 to 4.53)	0.001	2.97 (1.73 to 5.10)	<0.001
RF IgM	1.72 (1.11 to 2.67)	0.015	1.01 (0.61 to 1.69)	0.96	0.98 (0.58 to 1.67)	0.95
AAPA IgG	3.07 (1.90 to 4.98)	<0.001	1.79 (1.02 to 3.16)	0.043	–	–
Anti-CarP IgG	2.85 (1.59 to 5.11)	<0.001	–	–	1.47 (0.75 to 2.87)	0.26

AAPA, anti-acetylated protein antibody; ACPA, anti-citrullinated protein antibody; anti-CarP, anti-carbamylated protein antibody; CSA, clinically suspect arthralgia; RF, rheumatoid factor.

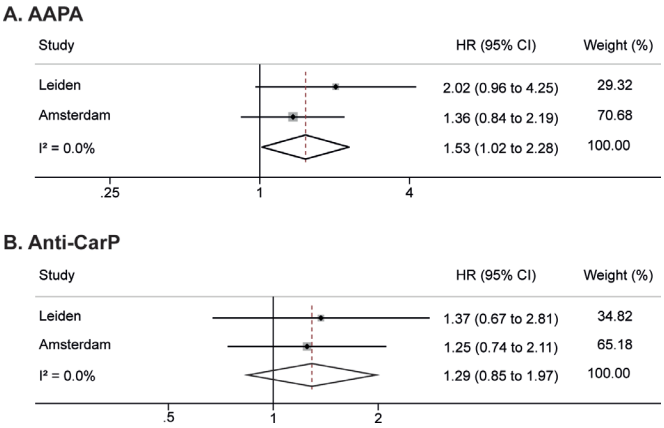

Figure 5 Meta-analyses on AAPA (A) and anti-CarP (B) in two cohorts of patients with ACPA-positive/RF-positive arthralgia, showing an association with IA development for AAPA but not for anti-CarP. Raw data from ACPA-positive patients from the Amsterdam cohort as described by van de Stadt *et al* were obtained and analysed. AAPA, anti-acetylated protein antibody; ACPA, anti-citrullinated protein antibody; anti-CarP, anti-carbamylated protein antibody; RF, rheumatoid factor.

in HLA-SE-positive patients with CSA (which is in line with a previous study on ACPA levels in arthralgia[40]) suggest that HLA-SE associates with maturation of the ACPA response and/or symptom onset. However, the latter implication is based on deductions from cross-sectional data, longitudinal data from ACPA positivity to symptom onset would have been preferable.

Several nested case–control studies have shown that autoantibody development and the increase in levels can occur years before disease onset.[41–43] The current study and previous studies on CSA showed that the period between CSA onset and clinical arthritis development is on average 4–6 months.[44] We recently showed that the autoantibody response had already matured at CSA onset and did not mature further towards RA development.[45] Together these results indicate that autoantibody-response maturation took place before symptom onset and was influenced by smoking and HLA-SE. However, although case–control studies have found gene–environment interactions,[6 9 10 14] we found no statistically signification interaction between HLA-SE and smoking for the presence of ACPA at CSA onset (p=0.52). Interestingly, in the asymptomatic phase ACPA positivity can seroreverse to negativity, as is shown in symptom-free relatives of patients with RA.[23] This is in contrast to what is described in the symptomatic phases of CSA and clinical RA,[45–48] where autoantibody status and levels were shown to be stable and seroreversion was infrequent. Regarding timelines, this suggests that the autoimmune response is no longer reversible at symptom onset. However, disease chronicity is then not yet established; only a proportion of patients with CSA develop RA and both joint symptoms and subclinical inflammation can resolve spontaneously, also in ACPA-positive patients.[49] The final processes resulting in irreversible ACPA-positive RA remain to be elucidated. However, the current data also suggest that this final step is influenced by HLA-SE.

This is not the first longitudinal study on HLA-SE and smoking and the progression from arthralgia to clinical arthritis. We took advantage of existing data to strengthen the findings and show consistency in the ACPA-positive group. Furthermore, the fact that the Leiden CSA cohort included patients based on the clinical phenotype and not on autoantibody status ensured inclusion of also autoantibody-negative patients with CSA. This served to explore the role of HLA-SE and smoking in ACPA-negative RA. Although HLA-SE seemed to promote IA development in ACPA-negative patients, this effect was not present for RA development as outcome. Large case–control studies have suggested a role for HLA-SE also in ACPA-negative RA although with a smaller effect size than in ACPA-positive RA.[50] The present longitudinal data on ACPA-negative IA or RA development were insufficient to support a role for HLA-SE in the symptomatic pre-RA stage.

This study focused on associations of ACPA as measured with anti-CCP2, associations with other ACPA tests (eg, anti-CCP3) were not studied. However, in addition to ACPA, we did evaluate other AMPAs. Although different studies have shown cross-reactivity between ACPA and other AMPAs,[51 52] associations

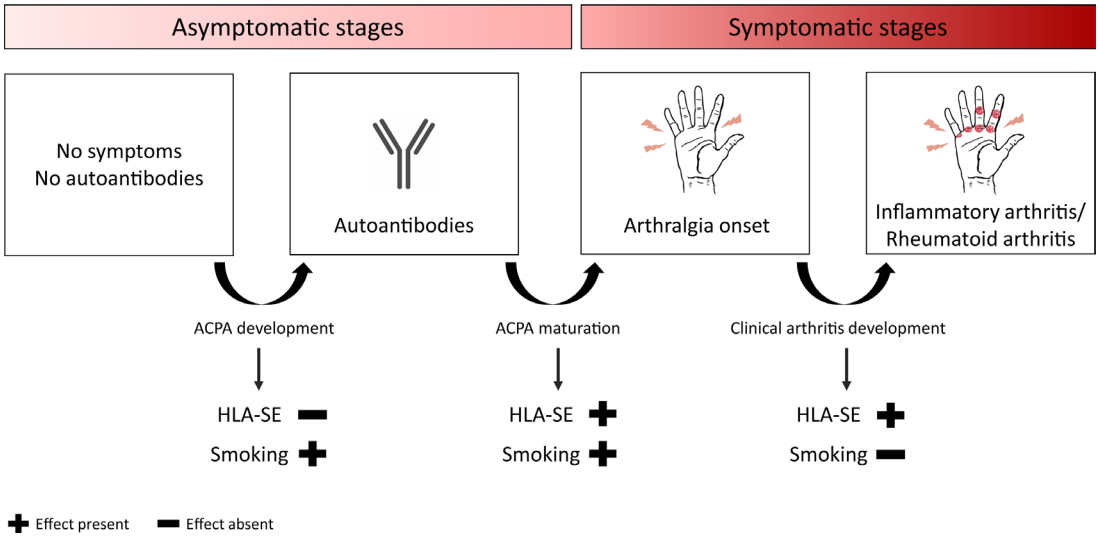

Figure 6 Summary of results on the role of HLA-SE and smoking in the asymptomatic and symptomatic phase of rheumatoid arthritis development. Meta-analyses in the asymptomatic stage indicated that smoking, but not HLA-SE, is involved in the development of ACPA. At CSA onset, both HLA-SE and smoking were associated with the presence of ACPA. Only HLA-SE further stimulated progression towards arthritis and ACPA-positive RA. Together these data imply that smoking is involved in autoantibody and symptom development, HLA-SE plays a role in autoantibody maturation, symptom development and progression to clinical disease. ACPA, anti-citrullinated protein antibody; CSA, clinically suspect arthralgia; HLA-SE, human leukocyte antigen-shared epitope.

with HLA-SE and smoking at CSA onset seemed to be specific for ACPA as no such associations were found for AAPA and anti-CarP in our patient population. This is in line with findings in RA, where anti-CarP was also not associated with HLA-SE and smoking.[53]

We aimed to fine stage the effects of HLA-SE and smoking. Identification of predictive markers for IA or RA development in CSA was not our primary aim. Nonetheless, we included an exploration and observed that AAPA, but not anti-CarP, associated with IA, independent of ACPA and RF. Further research is needed to ascertain the diagnostic value of these autoantibodies, especially their relevance on top of ACPA and RF that are measured in daily practice.

This study has extended knowledge on the timing of HLA-SE and smoking in the different stages of RA development. Intriguingly, HLA-SE and smoking exert their effect in partly different phases. Although requiring further biological exploration, it is tempting to speculate that initial autoantibody development is stimulated by smoking, whereas further expansion of the autoimmune response is promoted differently, by an HLA-SE-restricted T-cell reaction that drives further ACPA-response maturation. As such, smoking may contribute to the development of autoantibodies in general.[6 15 16 38 39] This initial antibody development does, most likely, require T-cell help as the antibodies are of the IgG isotype and hence the antibody producing B cells have undergone isotype switching, a T-cell dependent process. However, as no association with the HLA system is observed at this stage, these T cells most likely act in an HLA-SE-independent manner. In contrast, the subsequent expansion of the ACPA response does associate with HLA-SE, indicating that another, second, T-cell response is involved in the further expansion of the ACPA response. These T cells are associated with HLA-SE and, conceivably, recognise other antigens than the ones involved in the T-cell response underlying the 'initial' ACPA response. Thereafter, ACPA-positive persons with HLA-SE are particularly prone for further progression towards RA. These insights in timing of environmental and genetic factors support a further refinement of the SE hypothesis; the HLA-SE-specific T-cell response may not promote the initial break of tolerance to citrullinated antigens, but rather promotes the expansion of the (already existing) ACPA response prior to disease onset. Conceptually, this would explain why ACPA-positive patients with HLA-SE develop RA more often than ACPA-positive patients without HLA-SE and why HLA-SE does not associate with the other autoantibodies.

The findings of our study can guide future prevention studies. Prevention often concentrates on health-promoting behaviours. Our results on smoking not only imply that cessation of smoking might be able to influence the risk of ACPA development and/or symptom onset but also imply that it may not be effective in reducing the risk of progression from CSA to clinical arthritis. This would mean that trials on smoking cessation might preferably assess the efficacy in disease prevention in the asymptomatic population (primary prevention), rather than in patients with arthralgia (secondary prevention).

To conclude, HLA-SE and smoking act in partly different pre-RA stages. Smoking confers risk for the development of ACPA and/or joint symptoms, but does not further associate with IA development. In contrast, HLA-SE does not associate with ACPA in the general population, but does mediate symptom development and progression to IA. Even though the underlying time-specific biological pathways need further exploration, these data enhance understanding of timing of key genetic and environmental risk factors in the development of RA.

Contributors FW and AvdH-vM were involved in study conception and design. FW, MPM, RK, LvB and ALD contributed to collection of the data. FW and MV performed the data analyses. FW, AvdH-vM and REMT interpreted the results and wrote the first version of the manuscript. All authors critically revised the manuscript and approved the final version.

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Competing interests None declared.

Patient and public involvement statement Patient partners were involved in the design of the clinically suspect arthralgia cohort.

Patient consent for publication Not required.

Ethics approval Clinically suspect arthralgia cohort Leiden: Local Medical Ethics Committee, named 'Commissie MedischeEthiek', approval number NL38832.058.11. Amsterdam cohort: The Ethics Committee of the Slotervaart Hospital and Reade, Amsterdam.

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TRANSLATIONAL SCIENCE

Splicing machinery is impaired in rheumatoid arthritis, associated with disease activity and modulated by anti-TNF therapy

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ABSTRACT

Objectives To characterise splicing machinery (SM) alterations in leucocytes of patients with rheumatoid arthritis (RA), and to assess its influence on their clinical profile and therapeutic response.

Methods Leucocyte subtypes from 129 patients with RA and 29 healthy donors (HD) were purified, and 45 selected SM elements (SME) were evaluated by quantitative PCR-array based on microfluidic technology (Fluidigm). Modulation by anti-tumour necrosis factor (TNF) therapy and underlying regulatory mechanisms were assessed.

Results An altered expression of several SME was found in RA leucocytes. Eight elements (*SNRNP70, SNRNP200, U2AF2, RNU4ATAC, RBM3, RBM17, KHDRBS1* and *SRSF10*) were equally altered in all leucocytes subtypes. Logistic regressions revealed that this signature might: discriminate RA and HD, and anti-citrullinated protein antibodies (ACPAs) positivity; classify high-disease activity (disease activity score-28 (DAS28) >5.1); recognise radiological involvement; and identify patients showing atheroma plaques. Furthermore, this signature was altered in RA synovial fluid and ankle joints of K/BxN-arthritic mice. An available RNA-seq data set enabled to validate data and identified distinctive splicing events and splicing variants among patients with RA expressing high and low SME levels. 3 and 6 months anti-TNF therapy reversed their expression in parallel to the reduction of the inflammatory profile. In vitro, ACPAs modulated SME, at least partially, by Fc Receptor (FcR)-dependent mechanisms. Key inflammatory cytokines further altered SME. Lastly, induced *SNRNP70*-overexpression and *KHDRBS1*-overexpression reversed inflammation in lymphocytes, NETosis in neutrophils and adhesion in RA monocytes and influenced activity of RA synovial fibroblasts.

Conclusions Overall, we have characterised for the first time a signature comprising eight dysregulated SME in RA leucocytes from both peripheral blood and synovial fluid, linked to disease pathophysiology, modulated by ACPAs and reversed by anti-TNF therapy.

Key messages

What is already known about this subject?

⇒ Although there is recent evidence demonstrating the relevance of alternative splicing in tumorous and inflammatory pathologies, and some studies have shown association between the presence of splice variants and clinical profile of patients with rheumatoid arthritis (RA), alterations of the splicing machinery and their involvement in this disease have not been analysed so far.

What does this study add?

⇒ A signature comprising eight dysregulated splicing machinery elements (SME) has been identified in RA leucocytes subsets from peripheral blood, linked to key clinical features of this disease.

⇒ SME are further altered in mononuclear cells from RA synovial fluid, synovial tissue and ankle joints of K/BxN-arthritic mice and modulated in vivo by anti-tumour necrosis factor therapy.

⇒ Mechanistic studies have identified underlying mechanisms promoting SME alteration, involving both inflammatory mediators and autoantibodies (anti-citrullinated protein antibodies). Moreover, the reversion of their aberrant expression levels ameliorated the pathogenic RA phenotype of immune cells and synovial fibroblasts.

How might this impact on clinical practice or future developments?

⇒ The characterisation of new molecular mechanisms associated with the pathogenesis of RA, such as the presence of altered SME, might drive the development of potential biomarkers of disease and new therapeutic avenues for the management of this and other related immune-mediated disorders.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterised by polyarthritis, joint damage and functional disability. Patients with RA exhibit increased frequency of cardiovascular disease, higher susceptibility to infections and increased risk for certain malignancies.[1] Complex networks of pro-inflammatory cytokines, chemokines and growth factors play a fundamental role in its pathogenesis. Nevertheless, patients with RA display high heterogeneity in their clinical evolution and response to therapy, so that the precise mechanisms underlying the pathophysiology of the disease need further elucidation.

The onset of RA seems to be triggered by genetic environmental interactions that foster autoimmunity, based on genetic predisposition combined with repeated activation of the innate and adaptive immune systems.[1] Genetic analyses have identified specific loci associated to RA onset and/or related comorbidities.[2] Besides, a considerable number of genes differentially expressed on several cell populations have been characterised as predictors of clinical evolution and therapeutic response.[3 4] Epigenetic studies based on DNA methylation[5] and microRNAs[6] have also provided novel mechanisms underlying the RA pathogenesis. Despite these findings, the information generated by genomic analyses is incomplete and shows certain limitations.

Gene transcription is tightly coupled to the subsequent splicing process, whereby introns are excised and exons are pasted together in mature RNAs by an intricate nuclear molecular machinery, the spliceosome, which consist on a discrete set of ribonucleoproteins and proteins, aided by more than 300 splicing factors.[7] Alternative splicing may generate different mature RNA arising from the same gene, which precisely defines the final quantitative outcome of gene expression and impacts the functional diversification of proteins. Although the role of alternative splicing in RA has received limited attention to date, some studies have shown association between the presence of splice variants and clinical features of RA. Events related to alternative splicing previously reported in RA include the presence of splice variants of adhesion molecules such as fibronectin (*FN1*) in the microvasculature of the synovium,[8 9] proangiogenic factors (*VEGF* and *CXCL12*), on the synovial tissue,[10] regulators of cell transcription (*FOXP3*) in synovial lymphocytes[11] and on synovium fibroblasts (*TNFAIP3, BRAF* and *BIRC5*),[12 13] as well as genes involved in adhesion and cell metabolism in peripheral mononuclear cells (*CD44* and *MAP2K4*),[14 15] and monocytes (*STEAP4*).[16] As well, increased circulating levels of protein isoforms generated by alternative splicing, such as *TNFR2*, *PTPN22*, *SELE*, *ILR6* and *ILR7* have been also demonstrated in RA.[17–21] Most of these events have been associated with diverse RA features, thus supporting that the RNA splicing process might be severely altered in these autoimmune patients.

Nevertheless, alterations of the splicing machinery (SM) and their involvement in RA disease have not been analysed so far. Thus, the aim of this study was to explore and characterise the potential alterations of the SM components in peripheral blood leucocytes of patients with RA, and to define their influence on disease activity, its inflammatory and atherothrombotic profiles and the response to therapy.

Our results identified, for the first time, a signature comprising eight altered SM components in RA leucocytes, associated with key clinical features and therapy effectiveness.

PATIENTS AND METHODS

One hundred patients with RA and 29 healthy donors (HD) were included in the study (during a 24-month period), and involving two patients' cohorts. The first cohort comprised 72 patients with RA and 29 HD, whose clinical and laboratory details are displayed in table 1. All patients with RA fulfilled the American College of Rheumatology criteria for the classification of RA.[22] Patients and HD provided written informed consent. None of the HD had a history of other autoimmune diseases, atherosclerosis or thrombosis. The second cohort consisted of 38 patients with RA treated with anti-tumour necrosis factor (TNF)α drugs (TNF inhibitor (TNFi)) at standard dosage for 3 and 6 months. TNFi response was assessed by European League Against Rheumatism criteria.[23] The study was conducted in accordance with the Declaration of Helsinki principles.

Blood sample collection, assessment of clinical and biological parameters and B-mode ultrasound IMT measurements (see online supplemental materials).

Table 1 Clinical details of the patients with rheumatoid arthritis and healthy donors recruited to the study

	Rheumatoid arthritis (n=72)	Healthy donors (n=29)	P value
Clinical parameters			
Female/male, n/n	46/26	17/12	0.096
Age, years	54.0±11.7	50.2±10.2	0.078
Evolution time, years	7.4±7.5	–	
DAS28	3.2±1.4	–	
Rheumatoid factor positivity, n/n (%)	47/72 (65)	0/29 (0)	<0.001*
Anti-CCPs antibodies positivity, n/n (%)	61/72 (85)	1/29 (3)	<0.001*
Pathological CIMT, n/n (%)	27/72 (38)	0/29 (0)	
Obesity, n/n (%)	12/72 (17)	3/29 (12)	0.846
Diabetes mellitus, n/n (%)	0/72 (0)	0/29 (0)	
BMI (kg/m^2)	26.3±5.1	24.2±3.7	0.115
Hypertension, n/n (%)	18/72 (25)	0/29 (0)	
Menopause, n/n (%)	28/72 (40)	0/29 (0)	
Smoker, n/n (%)	18/72 (25)	5/29 (19)	0.500
Radiological involvement, n/n (%)	28/72 (38)	0/29 (0)	
Laboratory parameters			
Total cholesterol, mg/dL	202.6±39.3	193.0±41.0	0.155
HDL-cholesterol, mg/dL	54.24±17.9	54.0±22.0	0.348
LDL-cholesterol, mg/dL	126.8±33.0	127.0±31.0	0.607
Apolipoprotein A, mg/dL	151.2±31.3	147.1±28.1	0.553
Apolipoprotein B, mg/dL	84.1±19.5	84.9±26.9	0.537
Triglycerides, mg/dL	106.3±52.2	73.0±29.0	0.101
CRP, mg/dL	10.4±14.8	0.8±0.975	<0.001*
ESR, mm/hour	11.3±14.6	7.3±4.7	0.070
Treatments			
Corticosteroids, n/n (%)	42/72 (58)	0/29 (0)	
Antimalarials, n/n (%)	19/72 (26)	0/29 (0)	
NSAIDs, n/n (%)	58/72 (80)	0/29 (0)	
Methotrexate, n/n (%)	41/72 (57)	0/29 (0)	
Leflunomide, n/n (%)	18/72 (25)	0/29 (0)	
Vitamin D, n/n (%)	15/72 (22)	0/29 (0)	

*Denotes significant changes, P<0,001.
Anti-CCP, anti cyclic citrullinated protein; BMI, Body Mass Index; CIMT, carotid intima media thickness; CRP, C-reactive protein; DAS28, Disease activity score-28; ESR, erythrocyte sedimentation rate; HDL, high density lipoproteins; LDL, low density lipoproteins; NSAIDs, non-steroidal anti-inflammatory drugs.

Analysis of SM components by qPCR microfluidic Dynamic Array

A 48.48 Dynamic Array (Fluidigm) was used to assess the expression of 45 selected transcripts of the major and minor spliceosome and associated splicing factors as previously reported.[24–28]

Briefly, an 'integrated fluidic circuit' (IFC) is connected to reagent input wells to perform quantitative PCR (qPCR, which is detected by fluorescence.

Separated tests were developed for each cohort of patients previously detailed, including, respectively, 72 and 38 patients with RA, and 29 HD (see online supplemental materials for further details).

RNAseq analysis of public data set to gain insight in the splicing alteration

RNA-seq data of an external cohort of 44 patients (E-MTAB-6141)[29] was analysed as an independent cohort to explore enriched gene pathways, splicing variants and several splicing events associated to the dysregulation of the SM, as well as to validate the differential alteration of synovium and blood. The study of splicing variants and events was performed using Salmon, DESeq and SUPPA2 softwares[30–32] which allowed to assess the relative abundances of the splicing events as Percent Spliced In Index (PSI or Ψ) and explore their association with gene expression levels (low/high expression) (see online supplemental materials).

Bio-Plex assay of the inflammatory profile

Secreted levels of cytokines/chemokines/adhesion molecules in plasma of the two cohorts of 72 and 38 patients with RA, respectively, were determined using a 27-plex panel in a multiplex bead-based assay system (Bio-Plex multiplex immunoassays, Bio-Rad; California, USA) (see online supplemental materials).

SM analysis in K/BxN mice

Arthritis was induced in 6–8 week-old mice by intraperitoneal injection of 100 µl of K/BxN (KRNxNOD) serum on days 0 and 2. Characteristic of mice, arthritis induction and microarray analysis have been previously described.[33] Briefly, total RNA was obtained from ankle joints of three male arthritic mice and three control, non-arthritic mice. The joints were taken 7 days after serum transfer and immediately frozen in liquid nitrogen. To this end, hind limbs were prepared by dissecting the skin and muscle, and then sectioning ankle joints. Genome-wide microarray analysis was performed with the Mouse Gene 1.0 ST array (Affymetrix, Santa Clara, California, USA) at Progenika BioPharma SA (Bilbao, Spain) and SM components were identified.

In vitro studies

Four sets of in vitro experiments were developed to interrogate mechanistically the role of the altered SM in RA:

1. Treatment of HD leucocytes subsets with IgG-anti-citrullinated protein antibodies (ACPAs) or IgG-depleted ACPAs—purified from serum of active patients with RA—to evaluate their effects on the expression of both, the eight commonly altered SM elements (SME) and several inflammatory mediators. In addition, these treatments were also performed in the presence of FcR blocking.
2. Treatment of HD leucocytes subsets with key cytokines linked to the pathophysiology of RA (TNFα, interleukin (IL)-6 and CCL2), in order to assess their involvement in the aberrant expression of the eight commonly altered SME.
3. Transfection studies with *KHDRBS1* and *SNRNP70* in RA purified leucocytes subsets, to evaluate changes in cell activity promoted by overexpression of these SM components.
4. Treatment of RA-purified synovial fibroblasts (SF) with supernatants of the KHDRBS1 and SNRNP70-transfected lymphocytes, to analyse the functional consequences of SME modulation (see online supplemental materials for further details).

Cultured neutrophils were treated for 6 hours, and monocytes, lymphocytes and SF were treated for 24 hours before the respective analysis.

Identification of the citrullinome in PBMCs by LC-MS/MS

Citrullinome was evaluated by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in pooled cell lysates from peripheral blood mononuclear cells (PBMCs) isolated from two sets of five patients with RA each, including those with severe SME alteration (low expression levels) and those with mild SME alteration (high expression levels). Mass spectrometry raw files were processed with PEAKS Studio 10.6 build 20201221 (Bioinformatics Solutions). For post-translational modification quantification, citrullinated peptides with AScore >20 (p value<0.01) were considered[34] (see online supplemental materials for further details).

Statistical analyses

Data were expressed as mean±SEM or median ±IQR according to data distribution, evaluated using Kolmogorov-Smirnov test. Student's t-test or Mann-Whitney rank-sum test were used to assess statistical differences in unpaired data, and paired t-tests and Wilcoxon matched-pairs signed-rank tests for paired data. The χ^2 test was used to associate qualitative variables. Correlations were evaluated by Spearman's correlation test. Statistically significant differences were considered at p value<0.05 and false discovery rate (FDR)<0.15.

Logistic regression models and receiver operating characteristic curves were performed to evaluate the specificity and sensibility of the different discriminating models (see online supplemental materials for further details).

RESULTS

The SM is profoundly altered in RA peripheral blood leucocytes

Twenty-one components of the SM out of the 45 total analysed were found differentially expressed in RA monocytes (figure 1A). All of them, including major and minor spliceosome components and splicing factors, were found reduced in monocytes from patients with RA. Remarkably, only *RNU4ATAC*, a key component of the minor spliceosome,[35] was overexpressed. Similarly, 14 components of the SM were differentially expressed in lymphocytes from RA (figure 1B). All of them were found reduced and, in line with monocytes, *RNU4ATAC* and *NOVA1* were found overexpressed. Twenty-three components of the SM were differentially expressed in neutrophils from patients with RA (figure 1C). Similarly, most of them were found reduced, being overexpressed *RNU4ATAC*, *NOVA1* and *CELF1*.

Eight components of the SM, simultaneously altered in the three RA leucocyte subtypes, are related to key clinical features

Eight SM components were found simultaneously altered in the three leucocyte subtypes. These components included major (*SNRNP70, SNRNP200 and U2AF2*) and minor (*RNU4ATAC*) spliceosome components and four splicing factors (*RBM3, RBM17, KHDRBS1* and *SRSF10*). All of them were significantly reduced in the three leucocyte subsets of patients with RA, except for *RNU4ATAC*, which was consistently overexpressed (figures 1 and 2A). Of note, a significant relationship among all those components was identified (online supplemental figure 1).

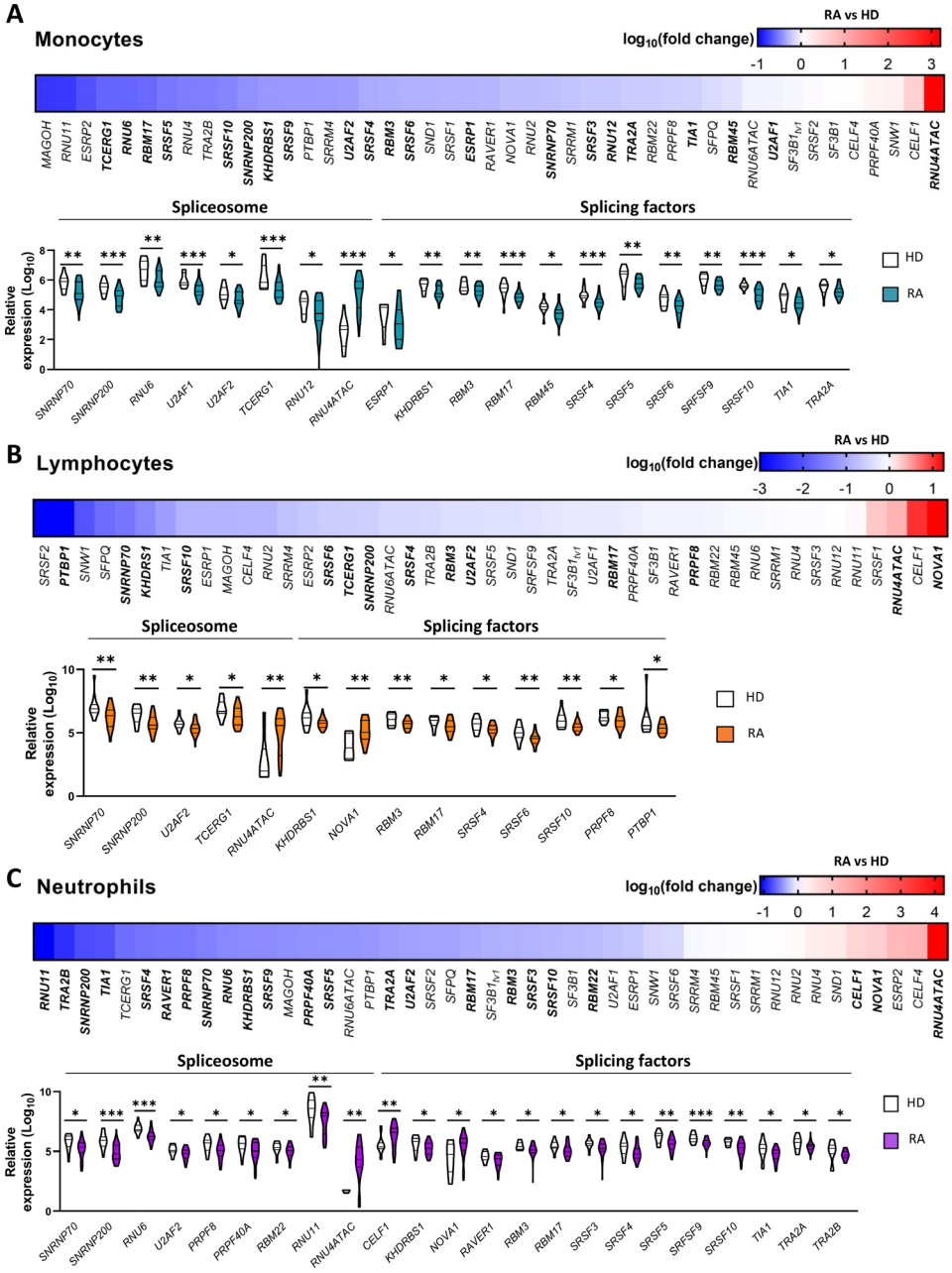


Figure 1 Splicing machinery is highly altered in leucocytes from patients with RA. Expression levels of major and minor spliceosome and associated splicing factors were quantified through a 48.48 Dynamic Array (Fluidigm) in monocytes (A), lymphocytes (B) and neutrophils (C) from 29 peripheral blood of healthy donors (HD) and 72 patients with rheumatoid arthritis (RA). Heat map are displayed on top of each panel showing differential expression in the splicing machinery between RA and HD (log_{10} fold change). Blue and red colours represent downregulated and upregulated splicing machinery elements, respectively, while those showing significant differences (p<0.05) are highlighted in bold. Violin plots are also displayed at the bottom of each panel representing the expression levels of the differentially expressed spliceosome and splicing factors in RA compared with HD. *p<0.05, **p<0.01, ***p<0.001.

Consequently, we next sought to ascertain if these eight components might be used as potential biomarkers of disease. Thus, we developed different mathematical models by applying logistic regressions on the data sets.

First, we generated a model that clearly discriminated between patients with RA and HD, with area under the curve always above 0.9 and with high specificity (figure 2B). Then, we created models to categorise different RA subsets, allowing to classify: (1) high disease-activity patients—that is, those presenting a DAS28-score higher than 5.1—(figure 2C); (2) patients suffering radiological involvement (figure 2D); (3) patients exhibiting atheroma plaques identified by doppler ultrasonography (figure 2E); and (4) patients positive for ACPAs versus those negative for these autoantibodies (figure 2F). All these models showed high specificity, particularly those generated in monocytes and lymphocytes.

The eight components of the SM, simultaneously altered in the three RA leucocyte subtypes, are closely related to their inflammatory profile

Bio-Plex analyses recognised an inflammatory profile in plasma of patients with RA, on which patients displayed altered expression

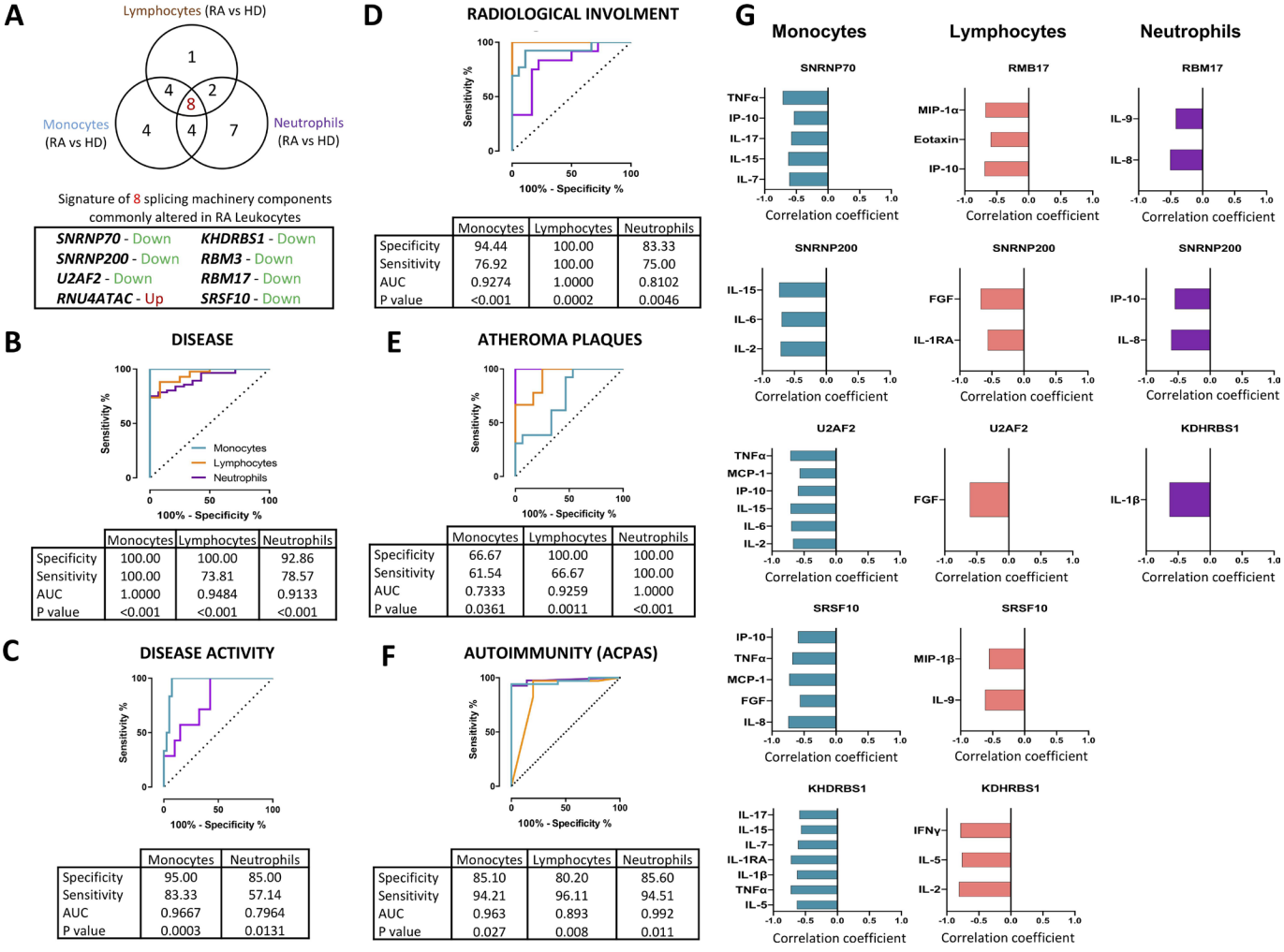


Figure 2 A signature of eight components of the splicing machinery is commonly altered in RA leucocytes and associated with clinical features of RA. (A) Venn diagram of differentially expressed splicing machinery elements in RA versus HD in leucocyte subtypes (monocytes, lymphocytes and neutrophils). A signature of eight spliceosome components commonly altered in all cell types are also highlighted indicating the direction of that alteration. The potential of this signature in each cell type as biomarkers of disease (B), disease activity (C), radiological involvement (D), atheroma plaques (E) and ACPAs positivity (F) were further demonstrated through logistic regression and receiver operating characteristic curve analysis. Area under the curve (AUC), specificity, sensitivity and p value are displayed in each analysis. (G) Correlation analysis between the signature of eight spliceosome components in each leucocyte subtype and the plasma pro-inflammatory profile was performed, and those showing a $p<0.05$ are shown. Spearman correlation coefficient is displayed where appropriate. ACPAs, anti-citrullinated protein antibodies; FG, fibroblast growth factor; HD, healthy donors; IFN, interferon; IL, interleukin; IP, interferon gamma-induced protein; MCP, monocyte chemoattractant protein; RA, rheumatoid arthritis; TNF, tumour necrosis factor.

of several ILs, chemokines and growth factors involved in inflammation and migration to inflamed tissues (online supplemental figure 2). Interestingly, a number of those inflammatory mediators were closely linked to the altered expression of the eight common components, although in a specific way on each leucocyte subtype (figure 2G).

The SM was deeply altered in the joints of both patients with RA and a RA mouse model

To reinforce the biological relevance of our findings, we evaluated those eight common components in mononuclear cells isolated from the synovial fluid of 15 patients with RA and compared their expression with that of mononuclear cells from peripheral blood of the same patients with RA. Four out of the eight common components were even more altered in synovial fluid mononuclear cells (figure 3A,B), so that *U2AF2*, *KHDRBS1* and *SRSF10* were significantly lower in mononuclear cells from synovial fluid than from peripheral blood and, consistently, *RNU4ATAC* was even higher.

These results were further validated externally using an independent public RNA-seq data set (E-MTAB-6141). A significant match with our results regarding the expression of the eight-SME signature was demonstrated. Thus, *U2AF2*, *RNU4ATAC*, *KHDRBS1* and *SRSF10* expression were altered in the same fashion, while downregulation and upregulation of *SNRNP200* and *RBM3*, respectively, were observed in this cohort (figure 3C,D). Likewise, the analysis of the 37 remaining SME evaluated in our study in this new cohort showed that a significant proportion of them displayed a lower expression in synovial tissue samples when compared with whole blood (online supplemental figure 3).

In line with this, the expression of SM components in ankle joints of the K/BxN-arthritic mice showed a marked dysregulation compared with control mice. Thus, 11 out of the 16 genes

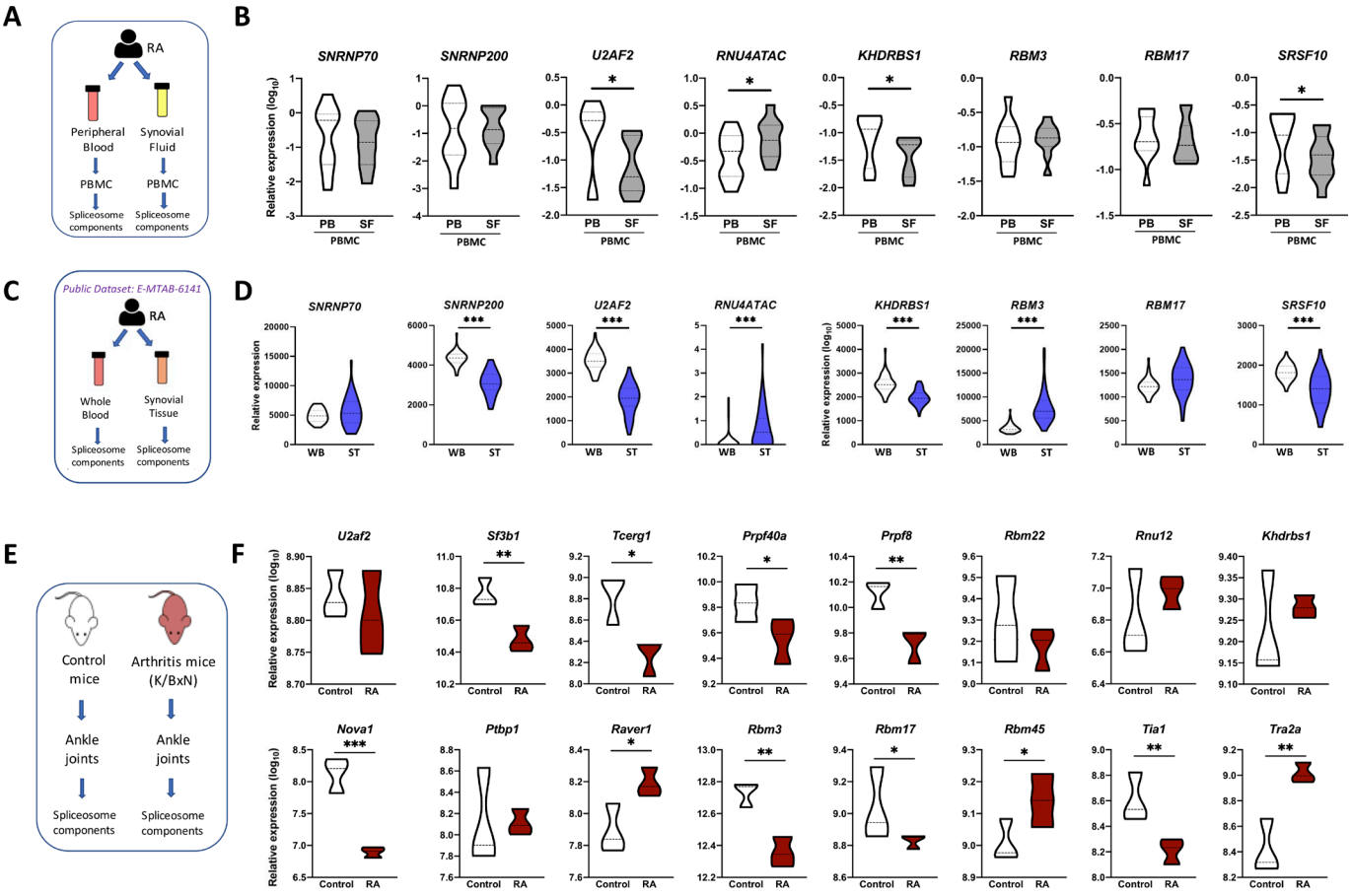


Figure 3 The splicing machinery is deeply altered in the joints of both patients with RA and RA mouse model. (A) Schematic representation of the analysis of the spliceosome signature in paired samples of RA PBMC from peripheral blood and synovial fluid. (B) Violin plots representing the expression levels of the spliceosome signature in PBMC of 15 patients with RA. (C) Schematic representation of the analysis of spliceosome signature in paired samples of whole blood and synovial tissue from patients with RA using public RNA-seq data set (E-MTAB-6141). (D) Violin plots representing the expression levels of the spliceosome signature in 44 patients with RA. (E) Schematic representation of the analysis of the spliceosome machinery in joints from K/BxN arthritis mouse model. (F) Violin plots representing the expression levels of the mouse spliceosome machinery components. *p<0.05, **p<0.01. HD, healthy donors; PB, peripheral blood; PBMC, peripheral blood mononuclear cells; RA, rheumatoid arthritis; SF, synovial fluid; ST, synovial tissue; WB, whole blood.

belonging to the SM assessed in the gene array were found altered in the ankles of K/BxN-arthritic mice (figure 3E,F). Hence, although arthritic mice did not fully mimic that found in humans, they display parallel alterations on the SM. Moreover, some components are altered in the same way in both, human and animal models.

The SME expression pattern is associated with differential splice events, splice variants and gene pathways

The public RNA-seq data set of patients with RA was divided according to the high or low expression of each altered SME. First, we analysed the differential generation of splicing variants. Next, we evaluated the impact of differential SME levels in the global performance of the splicing process. Finally, we explored enriched gene pathways associated with the differential SME expression.

The unsupervised analysis of splicing variants revealed the presence of distinctive levels of specific isoforms when comparing high or low expression of the studied components (online supplemental figure 4A). Interestingly, the differential expression profiles of the splicing variants were specific to each of the SME analysed.

RBM17 comprise the highest number of differentially expressed splicing variants (104), while SNRNP200 showed the lowest number of them (21) (online supplemental figure 4C). It should be also noted that three splicing variants were differentially expressed among all the analysed elements. Remarkably, we observed that two out of these three splicing variants belonged to the *ITGA11* gene, an alpha integrin that acts as a collagen receptor, playing a potential role in RA development.

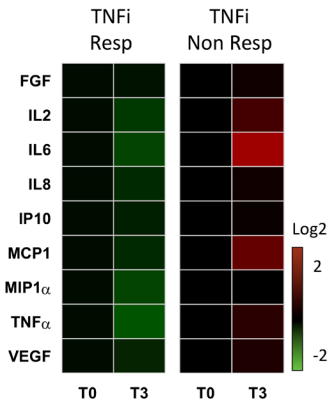
Besides, when comparing high and low expression of selected components, we observed a global alteration in the generation of alternative splicing events. Specifically, retained intron, skipped exon and mutually exclusive exons events occurred differentially. Particularly, in the case of *U2AF2*—a member of the core of the spliceosome—those samples presenting lower *U2AF2* levels displayed: (1) more exon skipping, (2) alternative 3'-splicing and 5'-splicing site, (3) less mutually exclusive exon and (4) alternative first and last exons, than those samples presenting high U2AF2 levels (online supplemental figure 4B).

Additionally, to elucidate the particular pathways potentially affected by the dysregulation of these SME, we assessed enrichment analyses on both, differentially spliced variants

A

	Responders (n=25)			Non-Responders (n= 13)		
	Before TNFi	After TNFi	p	Before TNFi	After TNFi	p
Clinical parameters						
Female/male, n/n	21/4	-		11/2	-	0,959
Age, y	53 ± 11	-		53 ± 10	-	0,923
Evolution time, y	10 ± 8	-		10 ± 8	-	0,900
Swollen joints (n)	4,7 ± 3,9	0,9 ± 1,1	**0,000**	5,2 ± 6,1	2,8 ± 3,2	**0,044**
Tender joints (n)	8,9 ± 6,4	1,9 ± 2,4	**0,000**	5,7 ± 5,7	4,7 ± 4,6	0,172
DAS28	4,8 ± 1,1	3,1 ± 1,1	**0,000**	3,7 ± 1,3	4,0 ± 1,3	0,181
VAS	60,9 ± 27,0	25,7 ± 25,6	**0,001**	64,7 ± 33,0	43,5 ± 30,3	0,305
HAQ	1,4 ± 0,8	1,1 ± 0,7	**0,007**	1,8 ± 0,3	1,1 ± 1,1	0,145
Serological assessments						
ACPA levels (U/mL)	296,4 ± 281,2	225,5 ± 145,7	0,210	253,8 ± 179,5	210,0 ± 248,2	0,389
Rheumatoid Factor levels (IU/mL)	65,4 ± 77,6	65,0 ± 75,6	0,975	75,51 ± 54,7	91,5 ± 65, 9	0,432
CRP, mg/dL	10,3 ± 8,2	8,5 ± 18,1	0,599	6,4 ± 5,6	8,8 ± 10,27	0,489
ESR, mm/h	29,9 ± 15,9	24,2 ± 17,4	0,084	13,3 ± 9,1	24,9 ± 14,5	**0,038**

C



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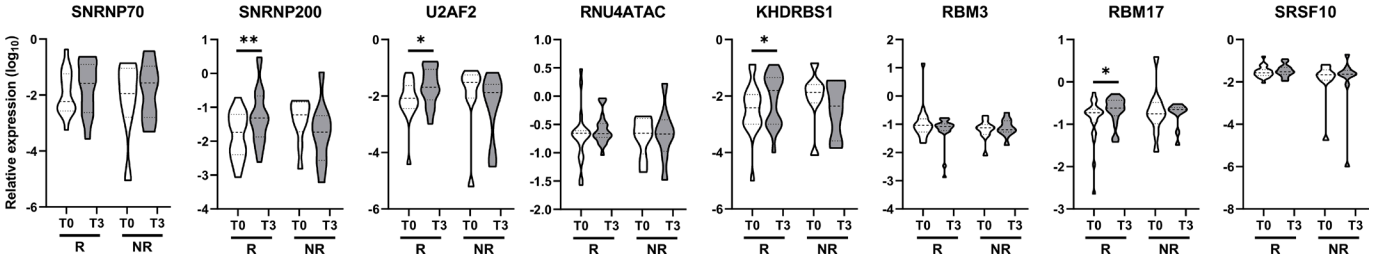

Figure 4 Anti-TNF therapy reverse the altered spliceosome signature of lymphocytes along with the inflammatory and clinical profile of patients with RA. (A) Table showing clinical and serological characteristics of 38 patients with RA before and after 3 months of TNFi therapy. Data are divided in responders and non-responders based on European League Against Rheumatism guidelines. (B) Heat map showing levels of circulating inflammatory molecules in plasma of patients with RA before and after 3 months of TNFi therapy. Levels of inflammatory molecules are expressed as log 2 and normalised to time 0 (T0), before therapy, in responders and non-responders' patients with RA. (C) Violin plots representing the expression distribution of the eight spliceosome components in lymphocytes before and after 3 months of TNFi therapy in responders and non-responders' patients with RA. *p<0.05, **p<0.01. ACPAs, anti-citrullinated protein antibodies; CRP, C-reactive protein; DAS28, disease activity score-28; ESR, erythrocyte sedimentation rate; FGF, fibroblast growth factor; HAQ, health assessment questionnaire; IL, interleukin; IP, interferon gamma-induced protein; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; R, responders, patients with RA; NR, non-responders, patients with RA; TNF, tumour necrosis factor; TNFi, TNF inhibitor; T0, time before TNFi therapy; T3, time 3 months after TNFi therapy; VAS, visual analogue scale; VEGF, vascular endothelial growth factor; Y, years.

and differentially expressed genes associated to the studied SME.

Concerning differentially expressed spliced variants, pathways related to RA pathogenesis, such as 'immune response', 'response to TNF', 'interferon signalling' and 'toll-like receptor signalling' were frequently observed (online supplemental figure 5). Similarly, among the enriched pathways according to differentially expressed genes, several pathways involved in immune function and inflammation, such as 'T-helper immune response', 'interferon signalling', 'interleukin-1 beta biosynthesis' or 'interleukin-2 production' were noticed (online supplemental figure 6).

Anti-TNF therapy modified expression of altered SM genes in RA leucocytes

Within the cohort of patients with RA treated with TNFi, according to DAS28-response,[23] 66% were responders, while 34% were non-responders (figure 4A). TNFi therapy for 3 months reverted the alteration observed in four commonly dysregulated SME in lymphocytes from responder patients (*SNRNP200*, *U2AF2*, *KHDRBS1* and *RBM17*) (figure 4B), promoting a significant upregulation, while in non-responders no changes were observed. No effects were observed in monocytes or neutrophils (data not shown).

As previously reported,[36] these alterations in responder's patients with RA paralleled the downregulation of key inflammatory mediators in plasma such as FGF, IL-2, IL-6, IL-8, TNFα and IP-10, while no changes were observed in non-responders patients with RA (figure 4C). Changes in SME did not correlate with those of ACPAs, which did not significantly change after TNFi therapy.

To further confirm the novel role of TNFi therapy as modulator of SME expression, we analysed the changes promoted by a longer period of TNFi treatment in whole blood samples from responders' patients of the same RA cohort. These analyses showed the reversion in five SME (*SNRNP200*, *KHDRBS1*, *RBM17*, *SNRNP70 and SRSF10*) after 6 months of TNFi therapy. Accordingly, along with the clinical disease improvement, a simultaneous downregulation of the same inflammatory mediators in plasma was confirmed in these patients after 6 months of treatment (online supplemental figure 7).

Lastly, statistical analyses did not confirm a potential role of SME levels as predictors of TNFi response (data not shown).

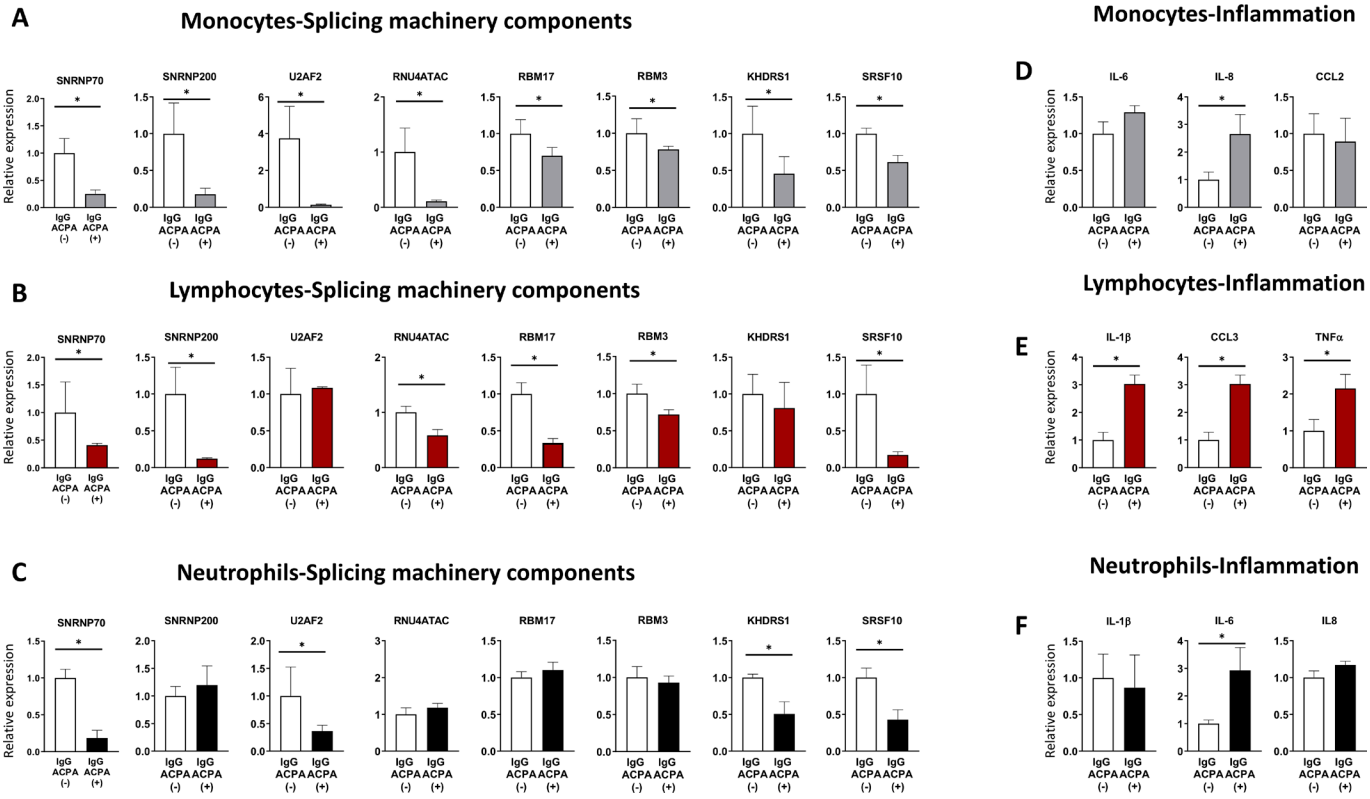

Figure 5 In vitro treatment of healthy leucocytes with ACPAs modify the expression of the spliceosome signature along with their associated inflammatory profile. Monocytes (A,D), lymphocytes (B,E) and neutrophils (C,F) from healthy donors were treated with 10 µg/mL of either IgG-ACPA purified from patients with RA through CCP-affinity column chromatography (IgG-ACPAs(+)) or the flow through depleted in Ig-ACPAs (IgG-ACPAs(-)) for 24 in monocytes and lymphocytes and 6 hours in neutrophils. Spliceosome components (A,B,C) and inflammatory molecules (D,E,F) were analysed by RT-PCR. Data from five independent experiments carried out in triplicate are shown. *p<0.05, **p<0.01. ACPAs, anti-citrullinated protein antibodies; IL, interleukin; TNF, tumour necrosis factor.

In vitro stimulation of healthy leucocytes with ACPAs mimicked the alteration of the SM trough FcR-dependent mechanisms

Given the key role of ACPAs in the pathophysiology of RA,[37] we wondered if they could have a role in the dysregulated expression of the SM components observed in vivo. Indeed, in vitro treatment of HD peripheral blood leucocytes with purified ACPAs obtained from RA serum through CCP-affinity columns, clearly altered the expression of the eight commonly altered components, although in a specific way in each leucocyte subtype, being the changes promoted in lymphocytes and monocytes the most relevant (figure 5A–C). Likewise, the inflammatory profile of leucocytes was upregulated (figure 5D–F).

To gain insight in the mechanisms related to the modulation of SME in leucocyte subsets by ACPAs, we performed in vitro studies involving blocking the FcR. The downregulation of several SME induced by ACPAs and the parallel upregulation of several inflammatory mediators was prevented by the blockage of FcR (online supplemental figure 8), thus suggesting that the effects of these autoantibodies are mediated, at least partially, by FcR-dependent mechanisms.

To further confirm this specific alteration promoted by ACPAs, we run in parallel another set of experiments where HD leucocytes were treated with monoclonal ACPAs, and IgG control (online supplemental figure 9). Similar results were obtained using this experimental approach, thus supporting the key role of ACPAs in the SME alteration and inflammation.

Next, to explore the potential role of citrullination in the dysregulation of the SM, we evaluated by LC-MS/MS the citrullinome in PBMCs from two sets of patients with RA, including those with severe SME alteration (lower levels) and those with mild SME alteration (higher levels) (online supplemental figure 10A,B).

By this approach, a total of 233 citrullinated peptides in RA were recognised (online supplemental table 2). Patients with severe SME alterations displayed higher degree of global citrullination than those with mild altered SM (online supplemental figure 10C).

Moreover, we further identified a higher degree of citrullination in three of the main established autoantigens in RA: collagen, vimentin and alpha enolase[38 39] in patients with RA with severely altered SME compared with patients with RA with mild altered SME (online supplemental figure 10D).

In vitro treatment with inflammatory cytokines dysregulated the SM in HD leucocytes

To evaluate the potential influence of inflammation on the SME dysregulation observed in patients with RA, leucocyte subsets were treated with key cytokines involved in the immune-mediated pathogenesis of RA. TNFa, IL-6 and CCL2 promoted a significant dysregulation in the eight commonly altered SM components, specific for each leucocyte subset, pointing at a relevant role of inflammatory mediators in the control of SM (online supplemental figure 11).

Overexpression of *KHDRBS1* and *SNRNP70* promoted the downregulation of key inflammatory mediators and functional endpoints in RA

Next, we aimed to prove whether restoration of altered levels of SM factors might have a positive impact in the altered

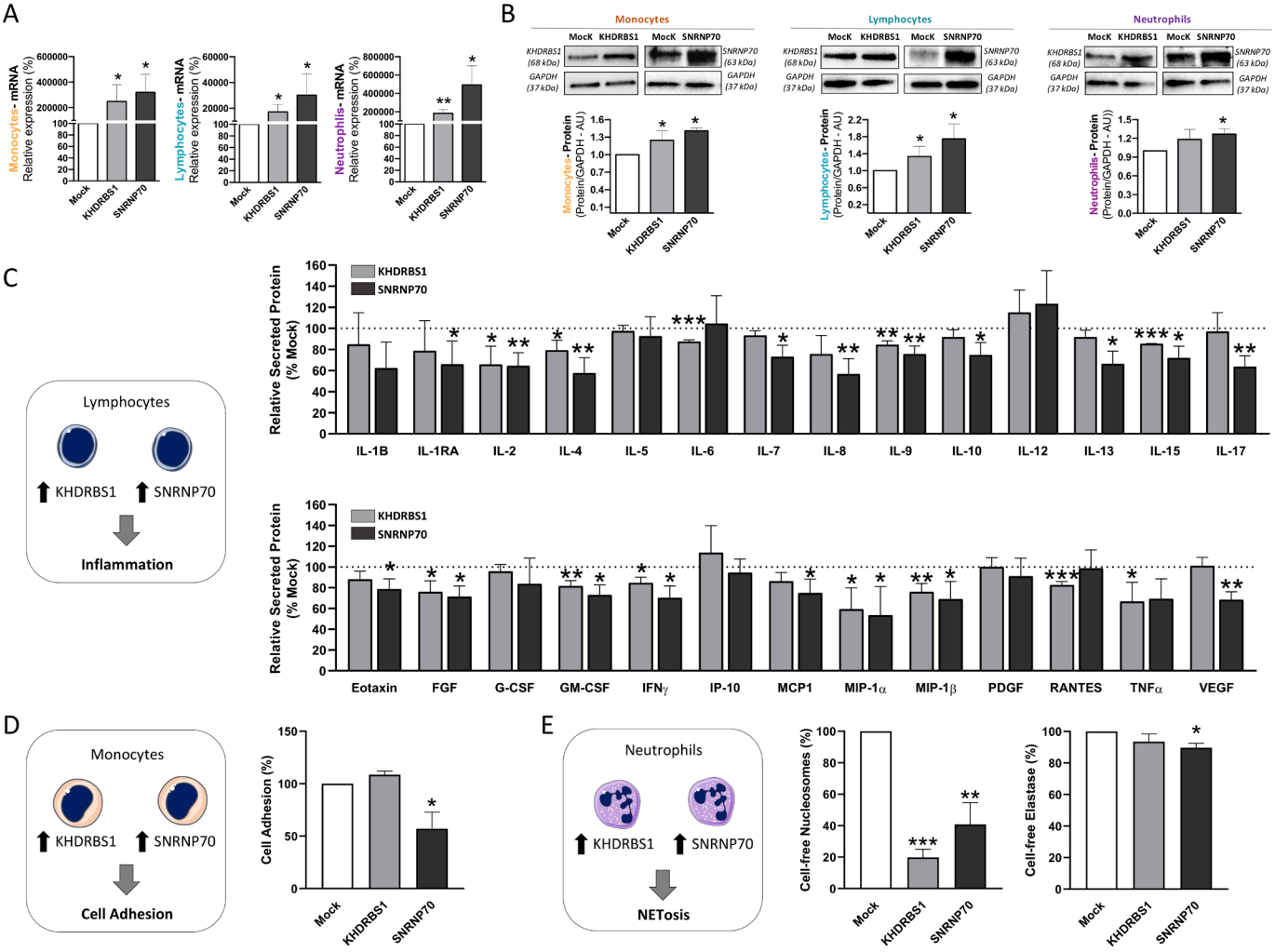

Figure 6 In vitro, the induced overexpression of *KHDRBS1* and *SNRNP70* promote the downregulation of key inflammatory mediators and functional endpoints in RA. Monocytes, lymphocytes and neutrophils from patients with RA were transiently transfected using KHDRBS1 and SNRNP70 plasmid and empty vector (mock) used as control. (A) mRNA expression of KHDRBS1 and SNRNP70 after transfection by RT-PCR. (B) Protein expression of KHDRBS1 and SNRNP70 after transfection by Western Blot analysis. (C) Pro-inflammatory molecules were analysed in lymphocytes' supernatant using a multiplex assay 24 hours after transfection. Cell adhesion (D) was assessed on monocytes 24 hours after transfection. Cell-free nucleosomes and elastase (E) were evaluated in neutrophils' supernatant after 6 hours. All experiments were compared with mock transfected cells, which was used as control and set at 100% in each panel. *p<0.05, **p<0.01, ***p<0.001. FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP, interferon gamma-induced protein; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; mRNA, messenger RNA; PDGF, platelet derived growth factor; RA, rheumatoid arthritis; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

activity of RA leucocytes. Specifically, we evaluated the effects of *KHDRBS1* and *SNRNP70* overexpression (figure 6A). These genes were selected based on their low expression in the three leucocyte subsets and their clinicals' associations. The overexpression of these genes reverted inflammatory features to normal-like levels. Specifically, lymphocytes showed a reduction of 10 and 18 inflammatory mediators after *KHDRBS1* and *SNRNP70* overexpression, respectively, compared with mock transfected cells (figure 6B). Patients with RA-derived monocytes showed reduced cell adhesion after *SNRNP70* transfection, (figure 6C). Lastly, neutrophils displayed a downregulation in NETosis features, involving reduced cell-free nucleosomes in response to the overexpression of both genes and a reduction of elastase after *SNRNP70* overexpression (figure 6D).

Modulation of the leucocyte-SME impact the global function of RA SF

Lastly, we evaluated the potential impact of modulating dysregulated leucocyte-SME in the function of RA SF. The supernatants generated by the induced overexpression of KHDRB1 and SNRNP70 in lymphocytes from patients with active RA ameliorated, in vitro, the aberrant activation status of RA SF through: (1) the reduction of cell migration capacity (online supplemental figure 12B); (2) the decrease of the proliferation rate (online supplemental figure 12C); and (3) the downregulation in the levels of both inflammatory mediators and extracellular matrix components (online supplemental figure 12D).

DISCUSSION

The present study demonstrates, for the first time, that the SM is profoundly altered in RA leucocytes and closely linked to the

pathophysiology of the disease. Importantly, we identified eight components commonly altered in leucocytes subsets, whose expression levels enabled distinguishing patients with RA from HD, and identifying patients with high disease activity, articular involvement and early atherosclerosis. Moreover, we extended these observations by examining the relationship among altered levels of SM components and those of inflammatory mediators notably involved in the clinical profile of these patients. These results were further validated in mononuclear cells obtained from synovial fluid of patients with RA, where inflammatory damage is more pronounced, and on the articular joints of a mouse model of RA, thus reinforcing the clinical relevance of the data obtained. Ex vivo and in vitro studies further identified potential mechanisms underlying these processes. Finally, significant effects of in vivo TNFi therapy on the reversion of SME dysregulation was demonstrated.

We have previously reported that the SM is altered in tumorous, metabolic and chronic inflammatory diseases.[24–28] However, this is the first study focused on the analysis of these alterations in patients with RA and their influence in its pathophysiology. The eight elements of the SM found commonly altered in the three leucocyte subsets evaluated included several molecules belonging to the major and minor spliceosome and four splicing factors. All of them are functionally interrelated, but no coordinated alterations had been reported hitherto in the setting of RA.

Several studies have established relevant (dys)functions of some of these factors in leucocytes, including an aberrant expression of *SNRNP200* (an essential component of the U5 spliceosome complex) in the cell membrane of leukaemic blasts,[40] and an activating role of *U2AF2* in CD4 + T cells,.[41] *RBM3* has shown roles in erythropoietic differentiation and in immune response, inducing the overexpression of cytokines such as IL-6 or TNFα in infection and non-infection conditions.[42] Similarly, *RBM17* has been identified as modulator of apoptosis, proliferation and cell adhesion.[43] *Also, SRSF10* has been established as a key modulator of metabolic pathways critical for obesity and related metabolic phenotypes, including adipocyte differentiation[44] and atherosclerosis development,[45] all of them closely related to the establishment of a chronic inflammatory status. In contrast, the role of *RNU4ATAC* has been scarcely explored in disease, having been only reported mutations in developmental rare diseases.[46–48]

The role of SNRNP70 in leucocytes activity has been only superficially explored to date. It has been reported the presence of a protein codified by *SNRNP70* (U1-70K autoantigen-specific) in T cells of mixed connective tissue disease (MCTD), which may be used for its diagnosis, distinguishing MCTD from systemic lupus erythematosus.[49]However, its potential role in RA has not been fully explored. Lastly, *KHDRBS1* is overexpressed in fibroblast-like synoviocytes of patients with RA, wherein it was involved in invasion, migration and proliferation.[50] Conversely, our RA cohort showed a lower expression of *KHDRBS1* in the three leucocyte subsets, accompanied by an even lower expression in synovial fluid leucocytes. Nevertheless, in our cohort, an inverse relationship among reduced levels of this splicing factor and both, a higher disease activity and the overexpression of a number of circulating inflammatory mediators was demonstrated, thus supporting its potential involvement in the pathophysiology of RA and pointing to a specific dysregulation in different cells and tissues.

To improve the significance of our results, we used a public available RNA-seq data set where matching whole blood cells and synovial biopsies of patients with RA were analysed. Even being slightly different samples, involving not only immune cells but the whole blood cell population and synovial tissue, we observed a clear correspondence with our results regarding both, the eight SME signature identified and even the whole set of SME evaluated. Thus, our results, in conjunction with the new evaluated database, support the presence of wide alterations of several SME in immune cells and synovial tissue, involving even more dysregulation in the last one. This prominent alteration in the synovium might be associated to the enhanced local inflammation widely reported in the RA joints.

Next, we analysed the biological consequences of the SME differential expression. These analyses identified, first, a distinctive alteration on the generation of alternative splicing events among patients displaying high or low SME levels. Besides, we recognised differentially expressed splicing variants among patients with RA with high or low SME expression levels, further showing a high specificity linked to each SME. Moreover, enrichment analyses on both, differentially spliced variants and differentially expressed genes associated to the studied SME, identified relevant pathways involved in RA pathogenesis.

That overall data strongly supports the notion that the dysregulation of the studied SME might have a deep impact in the splicing process and the generation of splicing variants and, consequently, might play a key role on the progression of this autoimmune disorder.

Increased protein citrullination is a hallmark of RA, closely associated with the generation of ACPAs. Interestingly, intracellular citrullinated proteins are involved in RNA splicing. In fact, some of them act as components of the RNA SM—including several heterogeneous nuclear ribonucleoproteins and *SNRNP200*, an essential component of the U5 spliceosome complex[51]—implying that citrullination modulates RNA biology. In the present study, the analysis of the citrullinome in RA PBMCs by LC-MS/MS identified a number of citrullinated peptides, which showed, a higher degree of citrullination in patients with more severe SM alteration. Moreover, we identified three well-established autoantigens in RA (alpha enolase, vimentin and collagen),[38 39] as highly citrullinated in patients with severely altered SM. These results suggest a clear relationship between this post-translational protein modification and the dysregulation of SM in the immune cells of patients with RA.

In addition, the involvement of citrullination in the dysregulation of the SM in RA was in line with both, the relationship among this alteration and ACPA positivity in leucocytes in patients with RA leukocytes, and the in vitro effects of ACPA on SME expression levels, thus suggesting a pivotal role for these autoantibodies in the identified SME alteration in RA. ACPAs have been closely related to joint damage, inflammation, oxidative stress and atherosclerosis in RA, and we previously reported that purified polyclonal ACPAs induced the expression of pro-inflammatory cytokines.[52] Accordingly, in vitro treatment of HD leucocytes with ACPAs upregulated a number of cytokines, chemokines and growth factors in distinct immune cells. Notably, in vivo, circulating levels of these pro-inflammatory proteins inversely correlated with the reduced levels of several SM components in leucocytes, pointing at a complex regulatory role of ACPAs in these processes.

In the search for a potential mechanism underlying the SME modulation by ACPAs, we evaluated the potential involvement of FcR, previously reported to mediate the effects of these autoantibodies in leucocyte activation.[53] The blockage of FcR prevented the downregulation of SME induced by ACPAs, underlying the role of these receptors on such effects.

In line with this, we also evaluated the influence of key cytokines linked to the pathophysiology of RA—widely reported to be both, associated in vivo to the positivity for ACPAs, and induced in vitro after treatment with these autoantibodies[52]—in the expression of the dysregulated SME. The in vitro treatment of HD leucocytes with either, TNF, IL-6 or CCL2, promoted a significant dysregulation in the eight commonly altered SME. That data suggested that both, ACPAs and inflammation might contribute either jointly or independently to the SME dysregulation associated to the pathogenesis of this chronic disorder.

Lastly, we evaluated the potential involvement of some dysregulated SME in the pathogenic activity of RA leucocytes. The overexpression of *KHDRBS1 and* SNRNP70 in monocytes, lymphocytes and neutrophils promoted a downregulation of several inflammatory proteins secreted by lymphocytes, decreased cell adhesion in monocytes and reduced NETosis in neutrophils. These effects were acuter after overexpression of *SNRNP70*, probably due to its central function in the core of the spliceosome, as responsible for most of splicing processes, while the functional role of *KHDRBS1* may be constrained to the splicing of certain genes.

Moreover, in our hands, the modulation of these SME in lymphocytes positively stuck the aberrant activation status of RA SF, reducing their inflammatory profile, along with their proliferative and migration capacities. Overall, these results demonstrated for the first time that the modulation of the SM in leucocytes from patients with RA directly impacts relevant pathogenic functions associated with the disease. Thus, the pharmacological intervention of these components might have a therapeutic potential role in patients suffering this and other immune-mediated diseases.

Anti-TNF therapy has significantly improved the outlook for patients suffering from RA.[36] With that premise, we evaluated in a new cohort of patients with RA the in vivo effects of TNFi on the altered expression of SME. Interestingly, in parallel to the early (3 months) and established (6 months) reduction of the disease activity and the efficient downregulation of their inflammatory profile, TNFi significantly reversed the levels of the SME altered in peripheral blood leucocytes. The presence of isoforms of the soluble TNF receptor 2, produced by alternative splicing in RA, has been demonstrated to maintain a prolonged therapeutic response to TNF.[17] Overall, although a role of SME as biomarkers for predicting or monitoring therapeutic response was not confirmed, our data support that their reversed expression might constitute an additional and/or complementary mechanism underlying the clinical response to TNFi in RA.

This study has some limitations. First, the specificity of the eight SME signature as biomarker of disease in RA was not confirmed by comparison with other chronic or autoimmune diseases. Second, new extensive cohorts of patients with RA should be evaluated to confirm and validate the alterations observed in the splicing machinery, and the effects promoted by TNFi and other biological therapies. Lastly, despite we have provided several mechanistic insights related to the regulation of the SME, the deep understanding of the mechanisms underlying their pathogenic role and modulation in disease context is still to be fully characterised by the scientific community.

Altogether, we have identified a signature composed of eight elements of the SM, simultaneously dysregulated in immune cells and closely related to key clinical features of patients with RA. Each of these components displays widespread effects on the transcription of multiple genes. Thus, most probably their coordinated altered expression, rather than a unique or specific alteration, would be responsible for the development of clinical profiles, and might jointly influence the therapeutic response to TNFi. Overall, our results reveal, for the first time, the involvement of specific SME on the pathogenesis of RA, their relationship with the inflammatory and autoimmunity status of the disease and their modulation by TNFi therapy, which jointly invite to further explore the targeting of altered splicing as a novel source of therapeutic tools in this autoimmune disorder.

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Correction notice This article has been corrected since it published Online First. The author's name, Justo P Castaño, has been corrected.

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Contributors AI-C, AMP-T, ML-T, CR-R, SP-A and MdR-M developed the in vivo assays, performed the experiments and solved technical problems; AE-C, ROC, JC, PS and EC-E followed-up with patients and contributed useful discussion and suggestions; CC, AG and NB developed the mouse models, performed statistical analysis and discussed results; MCA-A and IAdlR performed some experiments and analysed the data; AI-C and RB-E performed bioinformatic and biostatistical analyses. AI-C, CP-S, RML, JPC-F and CL-P formed the hypothesis, directed and coordinated the project, designed the experiments, analysed the data and wrote the manuscript. EC-E and CL-P are shared last authorship. EC-E is also a senior contributor.

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CLINICAL SCIENCE

Biological disease-modifying antirheumatic drugs may mitigate the risk of psoriatic arthritis in patients with chronic plaque psoriasis

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ABSTRACT

Objective To estimate the incidence of psoriatic arthritis (PsA) in patients with psoriasis who had received a continuous treatment with biological disease-modifying antirheumatic drugs (bDMARDs) compared with phototherapy.

Methods A retrospective non-randomised study involving patients with moderate-to-severe plaque psoriasis, who were prescribed at least 5 years of bDMARDs or at least three narrow-band ultraviolet light B (nb-UVB) phototherapy courses, and did not have a diagnosis of PsA at enrolment. Development of PsA in each patient was assessed by a rheumatologist according to the Classification for Psoriatic Arthritis criteria. The annual and cumulative incidence rate of PsA was estimated by using an event per person-years analysis. Cox proportional hazards models were undertaken to assess the hazard risk (HR) of PsA after adjustment for confounders.

Results A total of 464 psoriatic patients (bDMARDs, n=234 and nb-UVB, n=230) were followed between January 2012 and September 2020 (corresponding to 1584 and 1478 person year of follow-up for the two groups, respectively). The annual incidence rate of PsA was 1.20 cases (95% CI 0.77 to 1.89) versus 2.17 cases (95% CI 1.53 to 3.06) per 100 patients/year in the bDMARDs versus phototherapy group, respectively (HR 0.29, 0.12–0.70; p=0.006). The variables independently associated with higher risk of PsA were older age (adjusted HR 1.04, 1.02–1.07), nail psoriasis (adjusted HR 3.15, 1.63–6.06) and psoriasis duration >10 years (adjusted HR 2.02, 1.09–3.76); notably, bDMARDs treatment was associated with a lower risk of incident PsA (adjusted HR 0.27, 0.11–0.66).

Conclusions bDMARDs treatment may delay or reduce the risk of incident PsA in patients with moderate-to-severe chronic plaque psoriasis.

Key messages

What is already know about this subject?
- In most cases, diagnosis of plaque psoriasis may precede that of psoriatic arthritis (PsA) by an average of 5–10 years, and in only a minority of cases PsA precedes the skin disease or occurs simultaneously.
- The transition from psoriasis to PsA may evolve across various phases, that is, preclinical, subclinical and prodromal phases.

What does this study add?
- The major finding of this non-randomised intervention study was that the incidence of PsA was lower in patients treated with biological disease antirheumatic modifying drugs (bDMARDs) compared with narrow-band ultraviolet light B phototherapy.

How might this impact on clinical practice or future developments?
- Early therapeutic intervention with bDMARDs may delay or reduce the risk of PsA development in patients with moderate-to-severe chronic plaque psoriasis.

INTRODUCTION

Chronic plaque psoriasis is associated with psoriatic arthritis (PsA) in up to nearly 20%–25% of adult cases.[1] Epidemiological studies have reported that the diagnosis of psoriasis may often precede the development of PsA by several years.[2] In particular, psoriatic skin lesions can emerge before the development of musculoskeletal manifestations in around 75% of patients with PsA in a time frame of about 10 years.[2 3] Major unmet needs in the field of PsA include improving our understanding of the natural history of disease, defining those patients with psoriasis are at increased risk of developing arthritis and characterising the immune, environmental and molecular subclinical events that precedes PsA onset. Factors that may confer an increased risk of PsA include the genetic background, the psoriatic involvement of selected body areas (such as the scalp, nails and intergluteal areas), as well as obesity and psoriasis severity, whereas no protective factors have been identified so far.[4–6] The delay between the onset of skin manifestations of psoriasis and joint disease may provide a therapeutic window of clinical opportunity for preventing the progression from psoriasis to PsA. Whether a continuous systemic treatment with biological disease-modifying antirheumatic drugs (bDMARDs) in patients with psoriasis could prevent the development of PsA has not been yet extensively investigated. Thus, the main aim of this non-randomised intervention study was to estimate the incidence of PsA in patients with moderate-to-severe chronic plaque psoriasis, who had received a continuous treatment with bDMARDs compared

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with those treated with narrow-band ultraviolet light B (nb-UVB) UVB phototherapy.

METHODS

We have undertaken a retrospective non-randomised intervention study, involving psoriatic patients consecutively attending the Dermatology outpatient service of the University Hospital of Verona between 1 January 2012 and 30 September 2020, who were receiving either bDMARDs or nb-UVB phototherapy for the treatment of moderate-to-severe chronic plaque psoriasis.

We initially identified in our electronic database a total of 982 potentially eligible psoriatic patients. Among these, we selected patients, who met the following inclusion criteria: (1) age >18 years; (2) a clinically confirmed diagnosis of moderate-to-severe chronic plaque psoriasis; (3) a continuous treatment with a bDMARD for at least 5 years (between 2012 and 2020); patients with treatment interruptions up to 3 months between two consecutive courses were also included. In the case of a switch to another bDMARD of the same or different classes, the patient was still included in the analysis if the treatment interruption was less than 3 months; and (4) a treatment for at least three courses of nb-UVB phototherapy (each course consisting of 24–32 sessions at a frequency of 2–3 sessions/week) among those psoriatic patients, who did not receive any other synthetic agents or bDMARDs for treatment of pasoriasis (ie, these patients formed the control group).

Exclusion criteria were as follows: (1) patients with a diagnosis of PsA in the past or at the time of the inclusion in the study (n=234); (2) those who did not have a continuous treatment with a bDMARD for at least 5 years (n=187); (3) those who did not receive at least three courses of nb-UVB phototherapy (n=69); or (4) those with mild psoriasis, those using a combination therapies (eg, methotrexate associated with tumour necrosis factor alpha (TNF-α) inhibitors, or nb-UVB *plus* bDMARDs) (n=28). bDMARDs used in this study were TNF-α inhibitors (ie, etanercept, infliximab, adalimumab), the IL-12/23 inhibitor (ustekinumab) or the IL-17A inhibitor (secukinumab). More details about the selection procedure of the study are summarised in online supplemental figure 1, and online supplemental file 1.

At baseline, the presence of PsA was excluded in all included patients by a rheumatologist. All patients were initially screened for PsA by administering them the Early ARthritis for Psoriatic patients (EARP)-10 questionnaire.[7] This questionnaire was routinely administered to all psoriatic patients who attended our dermatology outpatient service with a frequency of once every 6 months for our internal standard procedure. In the case of a positivity to three or more items of this questionnaire, the patient was sent to rheumatologist for a specialist visit. A rapid referral to the rheumatologist is guaranteed by the fact that we set up the joint dermatological and rheumatological clinics about 10 years ago. Each patient was classified as having PsA if he/she fulfilled the Classification Criteria for Psoriatic Arthritis criteria.[8] The number of swollen and tender joints and the subset of PsA (ie, peripheral arthritis, axial involvement, enthesitis or dactylitis) were recorded. Demographics variables, smoking status, family history of PsA in the first degree relative, metabolic comorbidities (ie, obesity, diabetes, hypertension and dyslipidaemia), psoriasis duration and severity (by using the Psoriasis Area and Severity Index (PASI)), as well as involvement of nail, scalp, folds (ie, inguinal, anal, axillary or submammary) were collected in all patients at baseline. In particular, patients were diagnosed with obesity in the case of a body mass index (BMI) ≥30 kg/m^2; diabetes in the case of fasting plasma glucose ≥126 mg/dL (≥7.0 mmol/L), or prior history of disease, or use of any antidiabetic medications; arterial hypertension in the case of systolic/diastolic blood pressure ≥130/85 mm Hg, or prior history of disease, or use of any antihypertensive agents; dyslipidaemia in the case of serum triglycerides ≥150 mg/dL (≥1.7 mmol/L), or total cholesterol ≥200 mg/dL (≥5.2 mmol/L), or low-density lipoprotein cholesterol ≥100 mg/dL (≥2.6 mmol/L), or use of any lipid-lowering drugs. The local ethics committee approved the study protocol. The ethics committee exempted such kind of research from the informed consent requirement because we only accessed retrospectively a deidentified database for the purpose of data analysis.

Statistical analysis

The results of continuous and categorical variables are presented as means±SD and proportions, respectively. The Pearson's χ^2 test for categorical variables and the unpaired Student' t test for continuous variables were used to analyse the baseline differences between the groups of psoriatic patients. The number of person years at risk was calculated as the time between the date of enrolment and the last contact date. This period was used to estimate the annual incidence rate of PsA per 100 patients in the two treatment groups. The cumulative incidence of PsA over time was estimated by Kaplan-Meier method. The log-rank test was used to compare the cumulative incidence curves in the two treatment groups. The mean exposure time to phototherapy was calculated and included in the analysis. Cox proportional hazards models were used to analyse the predictors of incident PsA. Explanatory variables included smoking history, family history of PsA, metabolic comorbidities (ie, obesity, diabetes, hypertension and dyslipidaemia), psoriasis duration and severity (ie, PASI), as well as involvement of nail, scalp, folds at baseline. The effects of these included risk factors on risk of incident PsA were expressed in terms of HRs along with their 95% CI. First, an age-adjusted and sex-adjusted Cox regression model was constructed from univariate analyses and then following a directed acyclic graph only those variables presenting $p<0.15$ and/or biologically plausible were included in two progressive multivariable regression models (see adjusted model 1 and adjusted model 2 of table 1). A propensity score matching (PSM) procedure was also performed in all patients by fitting a logistic regression model in which the baseline PASI was used as the matching variable and the treatment received (bDMARDs or phototherapy) was the dependent variable. In logistic regression analysis, baseline PASI was included as a continuous variable. After fitting the logistic regression model, the logit transformation of PSM for all patients was stored for subsequent use in matched groups. By the PSM procedure, patients treated with bDMARDs were matched with those treated with phototherapy using a 1:1 nearest neighbour algorithm without replacement and a calliper width of 0.50 SD of the logit transform of the propensity score. The analysis was conducted using the STATA software (V.13 StataCorp, Collage Station, Texas, USA).

RESULTS

In this retrospective, non-randomised intervention study, we enrolled a total of 464 psoriatic patients treated with either bDMARDs (n=234) or nb-UVB phototherapy (n=230), who were followed for a mean of 6.76±1.37 years per person follow-up. In the bDMARDs group, 39 (17%) patients were treated with infliximab, 17 (7%) with etanercept, 67 (29%) with adalimumab, 50 (21%) with ustekinumab and 61 (26%) with secukinumab, respectively. A total of 35 (15%) out of

Table 1 Multivariate Cox regression model assessing associations with psoriatic arthritis (PsA)

	Univariate analysis		Adjusted model 1*		Adjusted model 2†	
Variable	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
bDMARDS	0.53 (0.30 to 0.94)	0.029	0.31 (0.13 to 0.74)	0.008	0.27 (0.11 to 0.66)	0.004
Sex, male	1.54 (0.86 to 2.77)	0.145	1.46 (0.83 to 2.58)	0.188	1.24 (0.69 to 2.24)	0.465
Age	1.04 (1.02 to 1.07)	<0.001	1.04 (1.03 to 1.07)	<0.001	1.04 (1.02 to 1.07)	<0.001
Folds psoriasis	0.72 (0.40 to 1.30)	0.281			0.73 (0.39 to 1.33)	0.301
Scalp psoriasis	2.10 (1.07 to 4.10)	0.030			1.94 (0.93 to 4.02)	0.076
Nail psoriasis	1.74 (1.00 to 3.03)	0.049	4.44 (2.29 to 8.60)	<0.001	3.15 (1.63 to 6.06)	0.001
Psoriasis duration >10 years	2.67 (1.48 to 4.83)	<0.001			2.02 (1.09 to 3.76)	0.026
Family history of PsA	2.30 (1.04 to 5.12)	0.040			1.36 (0.58 to 3.18)	0.482
Baseline PASI ≥10	0.47 (0.27 to 0.82)	0.007	0.54 (0.26 to 1.14)	0.106	0.61 (0.29 to 1.33)	0.216
Diabetes	0.69 (0.33 to 1.54)	0.364				
Dyslipidaemia	1.46 (0.84 to 2.54)	0.174				
Hypertension	1.36 (0.79 to 2.36)	0.269				
Current smoker	0.98 (0.53 to 1.80)	0.936				

Long history of psoriasis, longer than the mean psoriasis history duration
*Multivariate model 1 adjusted for age, sex, nail localisation and baseline Psoriasis Area and Severity Index (PASI) ≥10.
†Multivariate model 2 adjusted for age, sex, scalp, fold and nail localisation, psoriasis duration >10 years, baseline PASI ≥10 and family history of PsA.
bDMARDS, biological disease-modifying antirheumatic drugs.

234 patients on bDMARDs switched therapy. In particular, 10 patients switched from a TNF-α inhibitor to another, 8 patients from a TNF-α to IL-12/23 inhibitor and 12 patients to an IL-17A inhibitor. Five patients switched from IL-12/23 inhibitor to IL-17A inhibitor. In the nb-UVB phototherapy group, 120 (52%) patients had received three courses of phototherapy, 98 (43%) received four courses and 12 (5%) received five courses (with a mean exposure time to phototherapy of 53 weeks). The bDMARDs and phototherapy groups had a total of 1584 and 1478 person year of follow-up with a mean 6.91±1.06 and 6.60±1.62 years per person follow-up, respectively. Descriptive characteristics of the study population are reported in table 2. At baseline, patients who developed incident PsA were older, and more likely to have longer psoriasis duration, greater family history of PsA and higher psoriasis localisation at the level of scalp and nails compared with patients who did not developed PsA over the follow-up. No significant differences were found in BMI, smoking status, psoriasis localisation at folds, as well as prevalence of diabetes, dyslipidaemia or hypertension between the two patient groups.

Table 2 Descriptive characteristics at baseline of participants who develop psoriatic arthritis (PsA) or not at the end of the follow-up

	No PsA (n=413)	PsA (n=51)	P value*
Age, years	46.59±0.58	53.73±1.51	<0.001
Sex, male, n (%)	210 (45)	28 (55)	0.143
BMI, kg/m^2	26.83±0.13	26.45±0.52	0.357
Current smoker, n (%)	125 (27)	14 (27)	0.930
Psoriasis duration, years	22.38±0.40	25.33±0.82	0.013
Baseline PASI	12.48±0.27	11.14±0.69	0.098
Family history of PsA, n (%)	32 (7)	7 (14)	0.046
Body areas affected by psoriasis			
Scalp, n (%)	306 (66)	40 (78)	0.051
Folds, n (%)	193 (42)	16 (31)	0.116
Nails, n (%)	190 (41)	28 (55)	0.032
Comorbidities			
Type 2 diabetes, n (%)	79 (17)	7 (14)	0.506
Dyslipidaemia, n (%)	207 (45)	26 (51)	0.332
Hypertension, n (%)	195 (42)	25 (5)	0.283
Obesity, n (%)	53 (11)	9 (18)	0.340

Continuous and categorical variables are presented as means±SD and proportions, respectively.
*Unpaired t-test for quantitative variables; χ^2 test for qualitative variables
BMI, body mass index; PASI, Psoriasis Area and Severity Index.;

Over the follow-up, a total of 51 (11% of total) patients developed incident PsA, including 19 (8%) and 32 (14%) in the bDMARDs and nb-UVB phototherapy groups (p=0.046), respectively. In particular, the annual incidence rate of PsA was 1.20 cases (95% CI 0.77 to 1.89) versus 2.17 cases (95% CI 1.53 to 3.06) per 100 patients/year in the bDMARDs and phototherapy groups, respectively (p=0.006). The cumulative incidence curves of incident PsA in the two patient groups are shown in figure 1 (p=0.027 by log-rank test as assessed by the Kaplan-Meir survival method). The most frequent pattern of PsA was peripheral arthritis (84%), followed by dactylitis (20%), enthesitis (16%) and axial involvement (6%). More than one pattern could be observed in a single psoriatic patient. A significantly higher proportion of peripheral arthritis (p=0.003) and

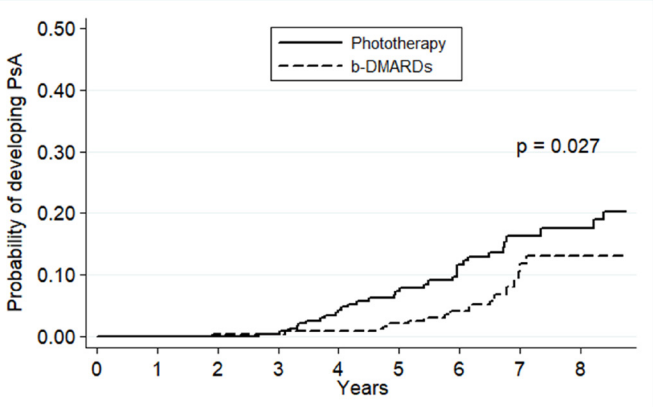

Figure 1 Cumulative incidence rates of psoriatic arthritis (PsA) in the biological disease-modifying antirheumatic drugs (bDMARDs) versus narrow-band ultraviolet light B phototherapy groups, estimated by the Kaplan-Meier method (p=0.027 by the log-rank test).

Table 3 Psoriatic arthritis (PsA) subsets in the two treatment patient groups

	bDMARDs	Phototherapy	P value
Incident PsA (any type), n (%)	19 (8)	32 (14)	0.046
Peripheral arthritis, n (%)	16 (7)	27 (12)	0.003
Axial arthritis, n (%)	1 (0)	2 (1)	0.712
Dactylitis, n (%)	2 (1)	7 (3)	0.361
Enthesitis, n (%)	3 (1)	7 (3)	0.361
Swollen joint, n	2.61±0.40	3.21±0.62	0.417
Painful joints, n	2.34±0.38	2.95±0.64	0.424

Continuous and categorical variables are presented as mean±SD and proportion, respectively.
*Unpaired t-test for quantitative variables; χ^2 test for qualitative variables
bDMARDs, biological disease-modifying antirheumatic drugs.

dactylitis (p<0.001) was observed in the phototherapy group of patients (table 3).

The univariable Cox regression analyses (table 1) showed that the baseline variables that were significantly associated with a higher risk of incident PsA were older age (HR 1.04, 95% CI 1.02 to 1.07), presence of nail psoriasis (HR 1.74, 95% CI 1.00 to 3.03), scalp psoriasis (HR 2.10, 95% CI 1.07 to 4.10;), family history of PsA (HR 2.30, 95% CI 1.04 to 5.12), psoriasis duration >10 years (HR 2.67 95% CI 1.48 to 4.83) and baseline PASI≥10 (HR 0.47, 95% CI 0.27 to 0.82). Notably, in univariable regression analysis, treatment with bDMARDs was significantly associated with a lower risk of incident PsA (HR 0.53, 95% CI 0.30 to 0.94) compared with nb-UVB phototherapy. The aforementioned significant variables were included in two progressive multivariable Cox regression models, as shown in table 1. Treatment with bDMARDs remained significantly associated with a lower risk of incident PsA after adjustment for age, sex, nail psoriasis and baseline PASI ≥10 (adjusted model 1), and even after additional adjustment for presence of folds psoriasis, scalp psoriasis, family history of psoriasis and psoriasis duration >10 years (adjusted model 2). Other variables that were independently associated with a higher risk of incident PsA were older age and psoriasis duration >10 years, but not baseline PASI.

Finally, given the non-randomised intervention design of the study, we also performed a PSM procedure for baseline PASI. After this statistical procedure, a total of 172 patients were identified for the analysis (n=86 in each treatment group), as shown in online supplemental table 1). After this PSM procedure, compared with nb-UVB phototherapy, treatment with bDMARDs did not show any significant association with the risk of incident PsA both in univariable regression analysis and even after adjustment for age and sex (online supplemental table 2).

DISCUSSION

The major finding of our retrospective, non-randomised intervention study was that the incidence of PsA was significantly lower in patients with moderate-to-severe chronic plaque psoriasis treated with bDMARDs compared with those treated with nb-UVB phototherapy.

It is known that the diagnosis of plaque psoriasis often precedes the development of PsA by an average of nearly 5–10 years, and in only a minority of cases PsA precedes the skin disease or occurs simultaneously.[9] Genetic predisposition may dictate a different development time of the two disorders, or skin inflammation may foster the development of joint disease in genetically predisposed patients. Experimentally, it has been shown that specific epidermal alterations, such as the abrogation of JunB/activator protein 1, which is a well-known regulator of cytokine production and keratinocyte proliferation, may be sufficient to initiate both skin lesions and arthritis in mouse models of psoriasis.[10] Possibly, a persistent and greater skin inflammation related to psoriasis may favour the development of PsA at both close and distant sites.[11] Psoriasis and PsA share several immuno-inflammatory pathways, with a key role of TNF-α, IL-17 and IL-23 in both conditions. Different murine models of arthritis, enthesitis or psoriasiform skin lesions have documented that IL-23 administration leads to enthesis-centred inflammatory arthritis, with bone erosion and new bone formation that are mostly mediated by levels of IL-17 and TNF-α.[12] The transition from psoriasis to PsA may evolve across various different phases, including preclinical, subclinical and prodromal phases.[13 14] We have previously reported, and then confirmed by others, that lower limb enthesopathy is a common ultrasonographic finding among patients with psoriasis without clinical signs of arthropathy.[15–19] Ultrasonographic enthesopathy may predict PsA development and it is likely related to a subclinical entheseal psoriatic inflammation.[20]

In the present study, we reasoned that bDMARDs targeting TNF-α or the IL-23/IL-17 axis could attenuate, delay or prevent the transition from psoriasis to PsA. In contrast, nb-UVB phototherapy dampers only skin inflammation, and it is regarded as a specific skin targeted therapy. Moreover, we admit that there is a difference in the exposure time between patients treated with either bDMARDs or phototherapy, because the latter is commonly used in intermittent courses and not continuously as bDMARDs.[21] In both patient groups, there may have been some subclinical cases of incident PsA who evolved into the clinical phase more frequently in the phototherapy group than in the bDMARDs group, because these treatments are different in their mechanisms of action. Accordingly, the interception in very early PsA study showed that IL-17A inhibition led to a decline in subclinical joint inflammation, arthralgia, and skin lesions in patients with subclinical PsA.[22] Conversely, oral retinoids targeting mainly keratinocytes differentiation and hyperproliferation have been associated with increased risk of PsA.[23]

In the present study, we found that in addition to the use of bDMARs, which had a protective effect on risk of PsA, other independent predictors of PsA development were older age, psoriasis duration >10 years and psoriasis localisation at nails. Different predictors of PsA have been identified in literature among patients with psoriasis, including genetic and environmental factors, metabolic comorbidities and psoriasis localisation.[13] Genome-wide association studies and studies of human leucocyte antigen (HLA) alleles have reported that PsA has a strong genetic component.[24 25] Frequencies of HLAB*08, HLAB*27, HLAB*38 and HLAB*39 alleles are higher in patients with PsA than in general population and polymorphisms of interleukin 23 receptor (*IL23R*) and tumour necrosis factor alpha-induced protein-3 (*TNFAIP3*) genes are more strongly associated with PsA than psoriasis alone.[13 24] Cohort studies found that nail psoriasis, particularly pitting, was also a positive predictor for PsA development.[5 23] Psoriatic patients with nail disease have a greater magnitude of underlying systemic subclinical enthesopathy than those with normal nails. Nail matrix and bed are in close proximity with the entheses of the distal interphalangeal joints.[26] Among psoriasis-related metabolic comorbidities, obesity has been recognised as a strong risk factor for PsA, because of its proinflammatory load and greater biomechanical stresses on joints and entheses.[9 27] In this study, we found that duration of psoriasis correlated positively with a higher risk of developing

PsA. To date, few studies have examined the association between psoriasis duration and risk of PsA.[28 29] Although PsA incidence remains constant following initial diagnosis of psoriasis among patients seen in European dermatology clinics, conflicting data were found with regards to an association between age of psoriasis onset and risk of PsA.[30]

Our study has some important limitations that should be mentioned, including its retrospective, non-randomised intervention design with consequent possible selection, observational and immortality biases. Confounding by indication is another major limitation of the study. Since the study is not randomised, the patient's likelihood of receiving treatment with bDMARDs or nb-UVB phototherapy was different and clinically influenced by the extent and localisation of skin lesions, but also by the patient's willingness to come to the hospital 2–3 times a week for nb-UVB phototherapy, or by some coexisting comorbidities (for example, prior history of melanoma or non-melanoma skin cancers and the use of photosensitizing drugs such as diuretics). In order to try to adjust the aforementioned confounding bias by indication due to the non-randomised intervention study design, we also performed a PSM procedure, which showed that treatment with bDMARDs failed to show any significant association with the risk of incident PsA compared with nb-UVB phototherapy. However, these latter results should be interpreted with some degree of caution, because the number of patients included in this propensity score analysis was too small to draw any firm conclusion about the long-term effect of bDMARDs on PsA development. In addition, as partly expected, we believe that the PSM for baseline PASI, which is one of most important variables to decide the type of treatment for psoriasis in clinical practice, would abolish any protective effect of bDMARDs on PsA development in this analysis.

Finally, a subgroup analysis examining the effects of different bDMARD classes on the risk of incident PsA could not be performed because of the relatively small sample size of this study. In addition, soluble markers of inflammation and genetic factors have not been investigated. Notwithstanding these limitations, the major strengths of our study are the completeness of the database, the lack of missing data and the accuracy for PsA diagnosis that was always supported by an expert rheumatologist.

In conclusion, the results of this non-randomised intervention study suggest that continue therapeutic intervention with bDMARDs may reduce the risk of incident PsA in patients with moderate-to-severe chronic plaque psoriasis. Future large prospective and intervention studies are needed to further validate these findings in independent samples.

Contributors FB performed all statistical analyses, contributed to writing the manuscript and visited the patients of the study population. PG designed the study, contributed to writing the manuscript and visited the patients of the study population. GT contributed to perform statistical analyses, and writing the manuscript. LI visited the patients of the study population and revised the manuscript. GG visited the patients of the study population and revised the final version of the manuscript.

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Competing interests PG has been a consultant and/or speaker for Abbvie, Almirall, Amgen, Janssen, Leo-pharma, Eli Lilly, Novartis, Pierre Fabre, Sandoz, Sanofi, and UCB. LI served as consultant and/or speaker for AbbVie, Amgen, Biogen, Merck Sharp & Dohme, Eli-Lilly, Novartis, Celgene, Sandoz. GG served as consultant and/or speaker for AbbVie, Abiogen, Almirall, Amgen, Biogen, Boehringer-Ingelheim, Bristol-Meyers Squibb, Celltrion, Eli-Lilly, Genzyme, Leo Pharma, Novartis, OM Pharma, Pfizer, Regeneron, Samsung Sanofi and UCB.

Patient consent for publication Not required.

Ethics approval The local ethics committee approved the study protocol. The ethics committee exempted such kind of research from the informed consent requirement because we only accessed retrospectively a de-identified database for the purpose of data analysis.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. These are deidentified participant dataAll the data are available at paolo.gisondi@univr.it. There is no embrago for the data.

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EPIDEMIOLOGICAL SCIENCE

Treating the skin with biologics in patients with psoriasis decreases the incidence of psoriatic arthritis

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ABSTRACT

Objectives To compare the incidence of psoriatic arthritis (PsA) in patients with psoriasis (PsO) according to different treatments for their skin: topics/no treatment, conventional disease-modifying antirheumatic drugs (DMARDs) (cDMARDs) or biological DMARDs (bDMARDs).

Methods Patients with PsO without PsA followed at a university hospital were included in this retrospective cohort study. Patients were classified according to their treatment in topics (topics, phototherapy or no treatment), cDMARDs (methotrexate and cyclosporine) and bDMARDs (tumour necrosis factor inhibitors (TNFi), interleukin 17 inhibitors (IL-17i) and IL-12-23i ((interleukin (IL) 12/IL-23 inhibitor))) groups. Incident cases of PsA were attributed to one treatment if developed during the administration of that treatment. A Cox proportional hazards model was used to evaluate the adjusted risk of PsA development by treatment group.

Results 1719 patients with PsO contributed a total of 14 721 patient/years (py). 1387 (81%) patients were in the topics, 229 (13%) in cDMARDs and 103 (6%) in the bDMARDs group. During follow-up, 239 patients (14%) developed PsA (231 under topics, six under cDMARDs and two under bDMARDs). Global incidence was 1.6 per 100 py. The risk of developing PsA in patients with PsO treated with bDMARDs was significantly lower (incidence rate ratio (IRR)=0.26; 95% CI 0.03 to 0.94; p=0.0111), compared with topics, but not compared with cDMARDs (IRR=0.35; 95% CI 0.035 to 1.96; p=0.1007). Adjusted Cox proportional hazards regression analysis showed that male sex, nail involvement and higher body max index were associated with increased risk of developing PsA, while biologics use was protective (HR: 0.19; 95% CI 0.05 to 0.81).

Conclusion Treatment with biologics in patients with PsO reduced the risk of PsA development.

Key messages

What is already known about this subject?

- Psoriasis usually precedes the development of psoriatic arthritis by many years, and it is the principal risk factor.
- Subclinical enthesitis is another risk factor for the development of psoriatic arthritis.
- Both psoriasis and subclinical enthesitis could be efficaciously treated with biological disease-modifying drugs, but it has not been shown that that could prevent the development of psoriatic arthritis.

What does this study add?

- In this analysis of a single-centre cohort, treating psoriasis skin involvement with biologics was associated with lower risk of developing psoriatic arthritis.
- Risk factors for the development of psoriatic arthritis in patients with psoriasis were male sex, higher body mass index and nail involvement.

How might this impact on clinical practice or future developments?

- Preventing development of psoriatic arthritis could be another factor suggesting the use of biologics early on in the treatment of the skin in patients with psoriasis at increased risk of developing psoriatic arthritis.

INTRODUCTION

The prevalence of psoriasis (PsO) in the general population ranges between 0.1% and 2.8%, and between 6% and 42% of patients with PsO can develop psoriatic arthritis (PsA).[1 2] Majority of patients develop PsO before articular involvement.[3]

PsO is a unique model for preventive medicine, as we have easy access to patients at high risk of developing PsA: patients with PsO. However, it is still not clear which patients with PsO will develop PsA, although several risk factors, such as extension of skin disease, obesity and subclinical enthesitis, have been described.[4–6] Conceptually, one of the models proposed to explain the development of PsA in patients with PsO is that chronic skin inflammation expands systemically into synovio-entheseal tissues.[7] Although biological therapies (TNF-α inhibitors (tumour necrosis factor α inhibitors) IL-12/23 (interleukin (IL) 12/IL-23), IL-17 (interleukin 17) and IL-23 (interleukin 23) inhibitors) have shown to be highly effective for PsO treatment,[8] it has not been proven that effective treatment of the skin could prevent the development of PsA.[4] On the other hand, a high percentage of subclinical enthesitis has been detected by ultrasound in asymptomatic patients with PsO[9 10] and can be observed at early stages of the articular disease.[11] Enthesitis has been implicated as the initial lesion in PsA,[12] and there is some evidence that subclinical enthesitis in patients with PsO is a risk factor for the development of PsA.[13–15] It has been shown that the use of biologics can improve the inflammatory component of subclinical enthesitis in

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patients with PsO.[16] We can assume that biological treatment being effective in diminishing chronic skin inflammation, and solving subclinical enthesitis in patients with PsO, might prevent the development of PsA in those patients. With the hypothesis that biologics prescribed for treatment of the skin will be able to prevent the development of PsA, the objective of our study was to compare the incidence of PsA in patients with PsO according to their treatment (biological disease-modifying antirheumatic drugs (bDMARDs) or topical treatment or conventional disease-modifying antirheumatic drugs (cDMARDs)) in a large university hospital-based healthcare management organisation (HMO)

PATIENTS AND METHODS

Study design

Retrospective cohort study between 1 January 2000 and 31 December 2018

Setting

The study was performed at a prepaid HMO. This HMO provides comprehensive medical and health services to around 140 000 adult members primarily located in urban areas. Since the year 1999, the HMO has a problem-oriented electronic medical record (EMR) where all medical appointments, diagnoses and procedures are registered without exceptions.[17 18]

All patients with cutaneous PsO, over 18 years old at time of entering the cohort, diagnosed by a dermatologist, without musculoskeletal symptoms and without diagnosis of PsA at study entry treated with biologics (TNF-α inhibitors, IL-12/23 and IL-17 inhibitors) because of their skin disease were included as cases. All patients with cutaneous PsO, over 18 years old at time of entering the cohort, diagnosed by a dermatologist, without musculoskeletal symptoms and without diagnosis of PsA at study entry, without treatment and/or with topical treatment and/or with systemic non-biological treatment methotrexate (MTX) or cyclosporine (CycA), were included as controls.

Cases and controls ascertainment

Cases and controls were obtained from the EMR including all patients with diagnosis of PsO, with at least two medical appointments in the EMR. The absence of PsA at study entry was checked by manual review of each one of the EMR. All medical appointments were reviewed, with special attention to pain-related problems or musculoskeletal symptoms and orthopedists, physiotherapists and rheumatologists visit. The final diagnosis impression of each one of these appointments and the presence of signs and symptoms of CASPAR (ClASsification criteria for Psoriatic ARthritis) criteria, even when the diagnosis was not recorded in the EMR, were considered to rule out the diagnosis of PsA. Also, special attention was paid to all medical appointments and signs and symptoms around the date of the prescription of a cDMARD or bDMARD looking for evidence of PsA.

A patient was considered not having PsA if the diagnosis of PsA was not recorded in the EMR, plus not fulfilling CASPAR criteria with the information collected from all the medical appointments manually reviewed from the EMR (including the evaluation by a rheumatologist ruling out PsA, when present)

Follow-up

All clinical, laboratory and diagnostic studies are recorded in the HMO EMR. In the primary analysis, incident cases were attributed to one treatment if developed during the administration of that treatment (at least 1 month of treatment should have elapsed). In this analysis, patients contributed time of exposure since the beginning of the corresponding treatment until diagnosis of PsA, loss of follow-up, end of that treatment or end of study (31 December 2018). In those patients that received more than one cDMARDs or more than one biologics, time on each one of the drugs was added to the specific treatment and to the corresponding treatment group. In the secondary analysis, it was considered that once cDMARDs or bDMARDs were received for at least 6 months, they would prevent the development of PsA forever (once the patient was exposed to a biologics, they were considered always exposed). In this secondary analysis, patients contributed time of exposure since the beginning of the corresponding treatment until diagnosis of PsA, loss of follow-up or end of the study (31 December 2018), independently of treatment discontinuation.

As treatment of PsO usually has a hierarchical order where patients are treated with topicals before cDMARDs and with cDMARDs before biologics, to avoid survival bias, time on topics (if received) was not added to that group in patients included in the cDMARDs group, and time on cDMARDs (if received) was not added to that group in patients who received biologics (by definition, these patients should not have PsA when starting cDMARDs or biologics, so by definition, all the time those patients spend on topics or cDMARDs would be free of PsA).

Definition of incident PsA

A patient was defined as developing PsA if she/he had the diagnosis of PsA confirmed by a rheumatologist and/or fulfilled CASPAR criteria[19] (by review of the EMR, even if the diagnosis was not recorded in the EMR), at any time after 1 month of study entry. If the diagnosis was made within the first month of entering the cohort, it was considered a prevalent case and excluded from the study.

Data collection

The following data were collected by manual review of the EMR for each patient with PsO: sex, age at PsO diagnosis, date of incident diagnosis of PsA (if developed), PsO duration, ungueal involvement, type of PsO, main PsO localisation, current and previous treatments including biological therapy and start and end dates of each one of the treatments received.

Statistical analysis

Sample size

The incidence of PsA in patients with PsO was calculated in 2.7 per 100 py; with a mean follow-up of 10 years, we estimated a PsA incidence of 20%.[6]

A sample size of 398 patients was required, considering an expected incidence of 20% of PsA for the controls (confidence level of 80% and alpha of 5%) with a 60% reduction in incidence rate (IR) in those patients treated with biologics.

Statistical analysis

Descriptive statistics were computed for patients at risk, overall and stratified by treatment, mean±SD for continuous variables and number and percentage for categorical variables. Pearson's χ^2 test for categorical variables and the unpaired Student' t-test for continuous variables with normal distribution or Wilcoxon rank-sum test for variables without normal distribution was used to compare baseline differences between patients that did or did not develop PsA.

IRs were calculated as the number of incident PsA events divided by the number of py at risk per 100 py, with their 95%

CIs for each one of the treatment groups using exact (Clopper-Pearson) Poisson confidence limits: (1) those with topical/no treatment, (2) those treated with cDMARDs and (3) those treated with bDMARDs. Incidence rate ratio (IRR) between treatments was also calculated.

Associations between treatment group and incident PsA were analysed using a Cox proportional hazards model. Explanatory variables included all variables collected in most patients: age at PsO onset, sex, body mass index (BMI) and presence of nail involvement. All these variables were included in the model for the primary and a secondary analysis. All variables included in the model were significantly associated with the outcome or the treatment in univariable analysis. The effects of these variables on the risk of incident PsA were expressed in terms of HRs along with their 95% CI. The goodness of fit of the null proportional hazards assumption was tested with the proportional hazards assumption test based on Shoenfeld residuals.

We also estimated the average treatment effect (ATE) and ATE on the treated (ATET) by propensity score matching (PSM) by fitting a logistic regression model in which baseline gender, age, BMI, PsO localisation, type of PsO and nail involvement were used as matching variables and the treatment received (dichotomised to biologics vs other treatments) was the dependent variable. bDMARDs were matched with other treatments (cDMARDs plus topicals) using at least two controls per treatment with replacement and a calliper width of 0.1 of the SD of the propensity score.

All analyses were conducted using the STATA software (V.14.2, StataCorp, Collage Station, Texas, USA).

The study was conducted according to the Declaration of Helsinki and local regulations. Ethical approval for the study was obtained from the hospital local ethics committee.

RESULTS

A total of 1719 patients with PsO that contributed a total of 14 721 py (median follow-up 7.3 years; IQR: 2–15) were included in the primary analysis. Patient's characteristics are shown in table 1 (primary analysis; for the secondary analysis: online supplemental table 1). One thousand three hundred eighty-seven (81%) patients were treated with topics phototherapy or no treatment, 229 (13%) with cDMARDs (77%, MTX; 13%, CycA; and 10%, both sequentially) and 103 (6%) with biologics (TNFi, n=92; etanercept, n=53; adalimumab, n=31; infliximab, n=8; IL-17i, n=47; ixekizumab, n=15; secukinumab, n=32; IL-12-23i: ustekinumab, n=19; some patients received more than one biologics). Patients treated with biologics were significantly younger and men (table 1).

Table 2 Patient's disease characteristics in those that did and did not develop psoriatic arthritis (PsA)

	Developed PsA (n=239)	Did not develope PsA (n=1480)	P value
Mean age at psoriasis (PsO) diagnosis (SD)	36 (17)	44 (20)	<0.0001
Men, n (%)	152 (64)	741 (51)	<0.0001
BMI, n; mean (SD)	182; 28.7 (5.2)	1083; 28 (5.5)	0.1177
Type of PsO: n with data	237	1439	0.024
Plaque: n (%)	221 (93)	1296 (90)	
Guttate: n (%)	4 (2)	86 (6)	
Inverse: n (%)	7 (3)	24 (2)	
Pustulosis: n (%)	0 (0)	13 (1)	
Nail involvement: n/n with data (%)	91/117 (78)	283/498 (57%)	<0.0001

BMI, body mass index; n, number; ;SD, Standard Deviation.

During follow-up, 239 patients (14%) developed PsA (231 under topics, six under cDMARDs and two under bDMARDs (table 2) in the primary analysis) with a median time between PsO onset and PsA development of 9.8 years (IQR: 3–20). Patients that developed PsA started their PsO at a significantly earlier age, were more frequently men, had significantly more frequent nail involvement and had higher BMI (table 2).

In most cases (222 patients: 93%), the diagnosis was recorded in the EMR (and confirmed by manual check of fulfilment of CASPAR criteria). In 17 cases (7%), the diagnosis was not recorded, but patients fulfilled CASPAR criteria after review of EMR 15/231 (6.5%) patients in topics, 1/6 (17%) in cDMARDs and 1/2 (50%) in bDMARDs.

PsA diagnosis was recorded by rheumatologists in 87% of the cases, by dermatologists in 3% and by others or not recorded in 10% of the cases.

Among patients who developed PsA while on cDMARDs, five were under treatment with MTX, and this was the only cDMARD they received, and one patient developed PsA while under treatment with CycA on which had been for 3 months. This patient received MTX for 4 months 13 months before starting CycA. Two patients developed PsA while under biological therapy (one secukinumab and one ustekinumab). Global IR of PsA development was 1.6 per 100 py (table 3). In the primary analysis, the risk of PsA in patients with PsO treated with bDMARDs was significantly lower than that of patients treated with topics (IRR=0.26; 95% CI 0.03 to 0.94; p=0.0111), but not that of patients treated with cDMARDs (IRR=0.35; 95%

Table 1 Patients' disease characteristic according to treatment group (primary analysis)

	Total group, n=1719 (100%)	Topics, n=1387 (81%)	cDMARDs, n=229 (13%)	bDMARDs, n=103 (6%)	P value
Mean age at psoriasis (PsO) diagnosis (SD)	43.1 (20)	43.4 (20)	44.3 (20)	35.4 (18)	0.0003
Men, n (%)	901 (52)	718(52)	116 (51)	67 (65)	0.029
BMI, n; mean (SD)	1265; 28 (5.5)	1013; 27.8 (5.4)	165; 28.6 (5)	87; 30 (6.7)	0.004
Median ears of treatment (IQR)	7 (1.9–15)	10 (3.5–15.7)	1 (0.4–2.6)	4.4 (1.1–6.7)	<0.0001
Type of PsO: n with data	1676	1351	224	101	0.042
Plaque: n (%)	1427 (90)	1217 (91)	205 (91)	95 (94)	
Gutata: n (%)	90 (5)	78 (6)	11 (5)	1 (1)	
Inverse: n (%)	31 (2)	29 (2)	2 (0.9)	0 (0)	
Pustulosis: n (%)	13 (0.8)	10 (0.7)	1 (0.4)	2 (2)	
Nail involvement: n/n with data (%)	374/615 (61)	275/460 (60)	54/94 (57)	45/61 (74)	0.084

%, percentage; bDMARDs, biological disease-modifying antirheumatic drugs; BMI, body mass index; cDMARDs, conventional disease-modifying antirheumatic drugs; n, number.

Table 3 Incidence of psoriatic arhtritis (PsA) according to treatment group, primary and secondary analysis

Primary analysis	Topics, n=1387	cDMARDs, n=229	bDMARDs, n=103	Total group, n=1719
N developed PsA (%)	231 (16.6)	6 (2.6)	2 (1.94)	239 (13.9)
Median years (IQR) between PsO and PsA	9 (2.8–20)	22.9 (11.7–24.7)	9.5 and 17.3	9.8 (3–20)
Follow-up (patient/years)	13 775	484	461	14 720
Incidence rate/100 patient/years (95% CI)	1.67 (1.5–1.9)	1.2 (0.56–2.8)	0.43 (0.11–1.7)	1.6 (1.4–1.8)
Secondary analysis	**Topics, n=1383**	**cDMARDs, n=232**	**bDMARDs, n=104**	**Total group, n=1626**
N developed PsA (%)	227 (16.4)	9 (3.8)	3 (1.3)	239 (13.9)
Median years (IQR) between PsO and PsA	9 (2.7–20)	22.6 (7.8–23.6)	9.5, 11 and 17.3	9.8 (3–20)
Follow-up (patient/years)	13 760	1116	541	15 417
Incidence rate/100 patient/years (95% CI)	1.65 (1.45–1.9)	0.81 (0.42–1.5)	0.55 (0.18–1.7)	1.55 (1.36–1.76)

bDMARDs, biological disease-modifying antirheumatic drugs; cDMARDs, conventional disease-modifying antirheumatic drugs; n, number; PsA, psoriatic arthritis; PsO, psoriasis.

CI 0.035 to 1.96; p=0.1007) (table 3). Results of the secondary analysis are shown in table 3; again, the risk of PsA was lower in patients with bDMARDs compared with patients with topical treatment (IRR: 0.34; 95% CI 0.07 to 0.99; p=0.0158).

Adjusted Cox proportional hazards regression analysis (table 4) showed that male sex, nail involvement and higher BMI were associated with increased risk of developing PsA, while biologics use was protective relative to topical/no treatment (test of proportional hazards assumption: p=0.5438). The adjusted model for the secondary analysis showed similar results (online supplemental table 2).

The ATE and ATET by PSM were −0.19, 95% CI −0.25 to −0.15 ; p<0.0001 and −0.31, 95% CI −0.43 to −0.18 ; p<0.0001, respectively. This implies that had the entire population been treated with biologics, the incidence of PsA would be 20% less than the average that would occur if none of the patients with PsO had received biologics. Distribution of variables before and after PSM is shown in online supplemental table 3.

DISCUSSION

Early detection and treatment of PsA might represent an opportunity to prevent the development of PsA. We found that patients with PsO treated with bDMARDs had a lower risk of developing PsA compared with patients treated with topicals or without treatment. There are few studies that had explored the role of treatment of PsO as prevention of PsA. Ogdie *et al* at EULAR (EUropean Alliance of Associations for Rheumatology) meeting presented a study performed in the USA with Optum de-identified Electronic Health Record dataset.[20] Using a traditional multivariable adjustment approach, they found in the fully adjusted model that treatment with biologics was protective of PsA development in patients with PsO; however, when the analysis was done after PSM, patients treated with biologics had higher risk compared with patients treated with no biologics.[20] Authors concluded that given the directional discrepancy in their results, further work is needed to understand the nature of this relationship.[20]

Table 4 Results of Cox proportional hazards model of time to onset of psoriatic arthritis in patients with skin psoriasis (PsO) with primary analysis

Variable	HR	95% CI	P value
Male sex	1.7	1.1 to 2.6	0.013
Age at PsO onset	0.99	0.98 to 1	0.085
BMI	1.05	1 to 1.1	0.014
Nail involvement	2.7	1.6 to 4.5	<0.0001
cDMARDs	0.53	0.16 to 1.7	0.285
bDMARDs	0.19	0.05 to 0.81	0.025

bDMARDs, biological disease-modifying antirheumatic drugs; BMI, body mass index; cDMARDs, conventional disease-modifying antirheumatic drugs; ;HR, Hazard Ratio.

Recently, Lindberg *et al* in a population-based study using secondary administrative registries reported the incidence of PsA in patients with PsO according to disease severity in Sweden between 2007 and 2017.[21] To classify patients into severity subgroups, they used treatment received as proxy of severity: patients receiving skin PsO-indicated biological treatments or apremilast were classified as biological-treated patients (severe disease proxy), those receiving skin PsO-indicated conventional systemics including phototherapy were classified as conventional systemic-treated patients (moderate proxy) and the remaining patients were classified as others (mild proxy). Lindberg *et al*, in contrast with our study, found an increased incidence in those patients treated with biologics (IR: 5.49 (95% CI 4.94 to 6.04) per 100 py). There are some differences between their cohort and ours. They used administrative data, while we review the electronical medical records. The percentage of patients treated with cDMARDs and bDMARDs was lower in the Lindberg study compared with ours (9.8% and 1.3% vs 13% and 6%, respectively), perhaps indicating differences in patients' characteristics.

More recently, Gisondi *et al* in a retrospective non-randomised study involving patients with moderate-to-severe plaque PsO, who were prescribed at least 5 years of bDMARDs, compared with patients treated with narrowband ultraviolet light B phototherapy, also found that bDMARDs treatment reduced the risk of incident PsA.[22] This study included a larger number of patients treated with bDMARDs, followed for a similar amount of time than our study.[22]

Interestingly, Lindenberg's IR of PsA of 1.69 per 100 py and Gisondi's IR of 1.20 per 100 py are remarkably similar to that found in our study (1.6 per 100 py). The incidence of PsA in patients with PsO in different population studies has varied between 0.27 per 100 py[23] and 2.7 per 100 py.[6] Our figures are well within this range.

Among the risk factors associated with the incidence of PsA in our study, some have already been described, such as nail involvement[6 22 24] and obesity.[23 25] A significantly increased incidence of PsA in men has been found by Green *et al*[23] and a non-significant increase in Wilson's[24] and Gisondi's[22] studies, while the incidence was higher in women in the Swedish study.[21] In our study cohort, men had slightly shorter PsO disease duration before PsA development, perhaps explaining higher incidence because of earlier onset.

Our study has several limitations. It is a retrospective study based on the review of electronical medical records. We could not completely discard that some prevalent cases were included;

however, there is no reason to think that this would have been different in the different treatment groups. On the other side, we have already proved in several studies that EMR review in our setting provides reliable incidence and prevalence data.[1 26–30] We did not have full data for adjustment for some confounders, such as severity of skin involvement. As mentioned in the Lindberg study, treatment with biologics could be considered a proxy of severe skin involvement and lead to confounding by indication. However, on the other side, patients with more severe PsO have increased risk of developing PsA,[31] so confounding by indication would have been towards higher incidence of PsA in patients treated with biologics. When we performed the analysis using matching by propensity score, biologics still protected against development of PsA. As all patients were not routinely evaluated to assess the presence of PsA, we could not rule out some PsA underdiagnosis. However, usually, patients on biological therapy are more closely followed, so it is unlikely that PsA was missed in that group and not in the others. Also, as mentioned before, our IR is similar to that of previous studies, which makes major underdiagnosis unlikely. Another potential limitation is the presence of protopathic bias that would occur if biologics were prescribed because of the presence of early manifestations of PsA that were not noticed or registered. To avoid this bias, the patients' charts were carefully reviewed around the dates of cDMARDs and bDMARDs prescription searching for potential signs or symptoms of PsA. Also, the event was attributed to that treatment only if at least 1 month after the initiation of therapy elapsed. Another limitation is the relatively low number of patients treated with cDMARDs and bDMARDs; however, low numbers are usually associated with type II error that would have prevented us from finding differences. Finally, the study was performed at a single centre; however, there is no reason to believe that results are not generalisable, as patient's characteristics are similar to those of other cohorts.

Our study has also some strengths, we used a proved methodology for incidence assessment and we have a long period of follow-up and a considerably large number of controls. On the other side, as mentioned in the introduction, there is biological plausibility for biologics administered in PsO impairing the development of PsA by subclinical enthesitis improvement and/or reducing systemic inflammation.[4]

In conclusion, in our study, we found that treating patients with PsO with biologics might reduce the risk of developing PsA. However controversial results with other studies leave this association still unresolved.

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Preliminary data from this study were previously presented at 2019 ACR Meeting: Lo Giudice L, Acosta Felquer M, Mazzuoccolo L, Galimberti M, Soriano E. Can Biologics "Prevent" the Development of Psoriatic Arthritis in Psoriasis Patients? Data from a Large University Hospital Cohort in Argentina [abstract]. Arthritis Rheumatol. 2019; 71 (suppl 10). https://acrabstracts.org/abstract/can-biologics-prevent-the-development-of-psoriatic-arthritis-in-psoriasis-patients-data-from-a-large-university-hospital-cohort-in-argentina/. Accessed May 25, 2021. And at the 2020 EULAR Meeting: Lo Giudice L, Acosta Felquer ML, Galimberti ML, et al. Sat0426 can biologics "prevent" the development of psoriatic arthritis in psoriasis patients? Data from a large university hospital cohort in Argentina. Annals of the Rheumatic Diseases 2020;79:1167-8.

Contributors MLAF and ERS: designed the study and performed data analysis and interpretation. LLG and MLG: contributed to data collection, data quality control and interpretation of the data. JR and LM: contributed to data quality control and interpretation of the data. All authors contributed intellectual content during the drafting and revision of the work and approved the final version to be published.

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Ethics approval The study was conducted according to the Declaration of Helsinki and local regulations. Ethical approval for the study was obtained from the Hospital Institutional Board: approval number 3635 (2018).

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Data availability statement Data are available upon reasonable request. Our data are not in a repository; data are available upon request to ERS.

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EPIDEMIOLOGICAL SCIENCE

Does biologic therapy impact the development of PsA among patients with psoriasis?

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ABSTRACT

Objective To examine the association of biologic therapy use for psoriasis with incident psoriatic arthritis (PsA) diagnosis.

Methods A retrospective cohort study was conducted in the OptumInsights Electronic Health Record Database between 2006 and 2017 among patients with psoriasis between the ages of 16 and 90 initiating a therapy for psoriasis (oral, biologic or phototherapy). The incidence of PsA was calculated within each therapy group. Multivariable Cox models were used to calculate the HR for biologic versus oral or phototherapy using biologics as a time-varying exposure and next in a propensity score-matched cohort.

Results Among 193 709 patients with psoriasis without PsA, 14 569 biologic and 20 321 cumulative oral therapy and phototherapy initiations were identified. Mean age was lower among biologic initiators compared with oral/phototherapy initiators (45.9 vs 49.8). The incidence of PsA regardless of therapy exposure was 9.75 per 1000 person-years compared with 77.26 among biologic users, 61.99 among oral therapy users, 26.11 among phototherapy users and 5.85 among those without a prescription for one of the target therapies. Using a multivariable adjustment approach with time-varying exposure, adjusted HR (95% CI) for biologic users was 4.48 (4.23 to 4.75) compared with oral or phototherapy users. After propensity score matching, the HR (95% CI) was 2.14 (2.00 to 2.28).

Conclusions In this retrospective cohort study, biologic use was associated with the development of PsA among patients with psoriasis. This may be related to confounding by indication and protopathic bias. Prospective studies are needed to address this important question.

Key messages

What is already known about this subject?

► Psoriasis often precedes psoriatic arthritis (PsA) development; treating psoriasis with biologic therapies reduces subclinical joint inflammation and may help to prevent or slow PsA development. Recently published studies in selected cohorts seem to support this concept.

What does this study add?

► Contrary to the study hypothesis, patients with psoriasis using biologic therapies were more likely to develop PsA. This may be related to confounding by indication or protopathic bias.

How might this impact on clinical practice or future developments?

► Prospective clinical trials are needed to address whether biologic therapies can mitigate or modify the risk for PsA among patients with psoriasis.

INTRODUCTION

Psoriasis is a chronic immune-mediated skin disease that affects approximately 2% of US adults. Up to 10%–30% of patients with psoriasis will develop psoriatic arthritis (PsA), a chronic inflammatory musculoskeletal disease, at some point during the course of the disease.[1] Despite treatment advances, patients with PsA still experience significant morbidity, functional disability, increased healthcare costs and diminished quality of life and less than half of patients achieve minimal disease activity in clinical practice.[2–5] Earlier diagnosis and treatment initiation could improve therapeutic response, but there often remains a delay in diagnosis.[6 7]

Psoriasis remains one of the strongest known risk factors for the development of PsA.[8] As the severity of psoriasis increases, the prevalence and incidence of PsA also increase.[9–12] Given the shared pathogenetic pathways (tumour necrosis factor (TNF), IL-17), one would expect the treatment of psoriasis to be associated with reduced progression to clinically overt PsA.[8 13–17] Furthermore, biologics, such as TNF alpha inhibitors, IL17i, 12/23i and IL23i, have demonstrated a clear benefit in improving the signs and symptoms of both psoriasis and PsA.[18–23] However, the relationship between a treatment for psoriasis and resulting PsA may be confusing in that some biologic therapies and retinoids have been purported to result in altered stimulation of the immune system, which has led to the paradoxical onset of PsA or the onset of pustular psoriasis among TNF inhibitor (TNFi) users.[20 24]

Studies addressing the impact of therapy for psoriasis on development of PsA are lacking. Few sufficiently large cohort studies, and no population-based studies, have addressed the impact of biologic therapy on development of PsA, and no prospective studies have been published. One of the greatest challenges in examining the impact of treatment for psoriasis on the development of PsA is confounding by indication, the concept that an

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individual receives a therapy for a reason (eg, severe psoriasis).[25] For example, patients with more severe psoriasis are more likely to receive systemic therapy for psoriasis and are also at an increased risk for PsA.[8] Additionally, 'protopathic bias', a situation in which treatment is prescribed because of a symptom or an undiagnosed disease that is also the outcome of interest, may be at play and could induce an apparent association between therapy and the *development* of the disease of interest.[26] Finally, confounding by prognosis describes the phenomenon in which clinicians prescribe more aggressive therapy when the outlook is poor, as is the case with severe psoriasis.[27] Together, these biases challenge the validity of results from retrospective cohort studies addressing this important question.

In this study, we aimed to examine the impact of biologic therapies on the development of PsA and to examine the potential role of these biases on the results. We used data from an electronic health record (EHR) database obtained from OptumInsights. We hypothesise that, theoretically, patients receiving a biologic therapy for psoriasis should be less likely to develop PsA than those receiving a non-biologic therapy for psoriasis. The alternative hypothesis is that biologic use is either not associated with or positively associated with development of PsA.

METHODS

Study design

A retrospective cohort study was performed within the OptumInsights EHR Database (USA) between 2006 and 2017.

Data source

The OptumInsights EHR database is a longitudinal EHR repository derived from dozens of healthcare organisations in the USA including more than 150 000 providers, 2000 hospitals and 7000 clinics. Data captured include demographics, medications prescribed and administered and coded diagnoses and procedures.[28] In a sensitivity analysis, we repeated the analyses in the OptumInsights Administrative Database. The administrative database contains claims data from over 85 million patients in the USA and between 15 and 20 million patients annually.

Patients

Patients with two or more International Classification of Diseases (ICD) codes for psoriasis who were aged 16–90 were identified. For cohort entry, patients were required to have at least 12 months in the dataset prior to the first code for psoriasis.

Exposures

The primary exposure of interest was biologic therapy (adalimumab, alefacept, brodalumab, certolizumab, etanercept, golimumab, guselkumab, infliximab, ixekizumab, secukinumab, ustekinumab) compared with non-biologic systemic oral therapy (acitretin apremilast, cyclosporine, etretinate, methotrexate) or phototherapy. Because it remains unclear how long exposure to a given medication could affect the patient, we used a 'once exposed, always exposed' approach in the primary analysis. In other words, once a patient was exposed to a biologic therapy, they did not transition back to the 'oral therapy' group.

Outcome

The outcome was PsA defined by a single ICD code. A single code for PsA in the setting of psoriasis and a relevant therapy has a high positive predictive value.[29] In a sensitivity analysis, we required two codes for PsA.

Time

The risk window over which the outcome was assessed varied by analysis (shown in online supplemental figure S1). In the time-varying exposure analysis, the outcome could occur at any point after diagnosis of psoriasis. In the propensity score (PS)-adjusted models, the outcome could occur any time after initiation of therapy.

Covariates

Covariates at baseline were determined in the 12 months prior to therapy initiation. The incidence of PsA was described overall and within each therapy group. Comorbidities were also identified using ICD codes. The code list was developed by two coders independently and reconciled.

Statistical analysis

Incidence was calculated among each of the key subgroups: all patients, those initiating oral therapy, phototherapy or biologic therapy (as well as subgroups including TNFi, IL12/23i and IL17i) from initiation of therapy to end of follow-up or development of PsA. We also examined incidence while on therapy (ending at end of therapy plus 90 days). We then compared incidence of PsA between patients on biologic therapy versus those not on biologic therapy and versus those initiating oral and/or phototherapy and censoring at switch to biologic therapy. The following models were used: (1) a multivariable Cox model using a time-varying exposure where the exposure was biologic initiation (once patients were exposed to a biologic, they were considered always exposed) adjusted for covariates selected using purposeful selection[30] and (2) after excluding patients with a prior history of biologic and/or oral therapy use, PS matching between patients initiating biologic therapy and oral or phototherapy. In the PS-matching analysis, the date of initiation of biologic therapy or oral or phototherapy, respectively, was the start date. PS was developed at therapy initiation using the age, sex, psoriasis duration and comorbidities and conditions (see online supplemental methods 1). Patients were matched 1:1 using a greedy-matching algorithm with calliper 0.1. Overlap of the PS and balance of covariates were checked to ensure adequate matching (online supplemental figure S2A and online supplemental figure S2B). Sensitivity analyses are described in the online supplemental methods 1. All statistical analyses were performed within Stata software, V.16.0 (College Station, Texas, USA).

Ethics approval and patient involvement

This study was considered exempt by the University of Pennsylvania Institutional Review Board. Patients were not involved in designing or analysing this study.

RESULTS

Among 193 709 patients with psoriasis without PsA at baseline, 34 890 initiated phototherapy, oral therapy or biologic therapy during follow-up. In the cohort, there were 14 569 new biologic initiations and 20 321 cumulative oral therapy and phototherapy initiations. The mean age was slightly lower in the biologics group compared with the oral/phototherapy group (45.9 vs 49.8). The proportion of women was slightly lower among biologic users (51.8% vs 57.0%), and there was a similar proportion of Caucasians (85.8% vs 85.8%) in each group (Table 1). Observation time was similar across both groups (2.6 years in the biologic therapy group and 2.5 years in the oral systemic therapy or phototherapy group). Among those with a new prescription

Table 1 Baseline characteristics of patients with psoriasis who initiated therapy

	All	Biologic therapy initiators	Oral systemic therapy or phototherapy initiators
N	193 709	14 569	20 321
Age, mean (SD)	50.0 (15.8)	45.9 (13.9)	49.8 (15.1)
Female sex, N (%)	105 999 (55%)	7540 (52%)	11 582 (57%)
Caucasian race, N (%)	166 196 (86%)	12 498 (86%)	17 432 (86%)
Observational time*, mean (SD)	5.85 (3.1)	2.60 (2.2)	2.50 (2.2)
Prior biologic therapy*, N (%)	5283 (3%)	1484 (10%)	848 (4%)
Prior oral systemic therapy*, N (%)	4706 (2%)	1128 (8%)	623 (3%)
Prior phototherapy*, N (%)	1940 (1%)	259 (2%)	257 (1%)
Primary care provider in system, N (%)	18 172 (9%)	1297 (9%)	1891 (9%)

*The index date defined for the time-varying covariate analysis was 1 year after the latest of the first diagnosis of psoriasis or the entry into the health system. These covariates are defined at that time. In the propensity score matched analysis, time started after that time point with first therapy initiation.
N, number; oral, oral therapies (including methotrexate, apremilast, cyclosporine, acitretinoin, and tretinoin).

for a new biologic therapy, 10% had a history of prior biologic therapy, 8% had a history of prior oral systemic therapy and 2% had a history of prior phototherapy at any point in the past. Among those initiating a new oral therapy, 4% had a history of prior biologic therapy, 3% had a history of prior oral systemic therapy and 1% had a history of prior phototherapy.

Among all patients, regardless of therapy exposure, the incidence of PsA was 9.8 per 1000 person years (table 2). The incidence was lower among those who did not receive therapy during follow-up (5.9 per 1000 person-years). Patients who received phototherapy also had a relatively low incidence of PsA, particularly when censoring patients at the start of either oral or biologic therapy. Patients who initiated biologic therapies or oral therapies for psoriasis had a substantially higher incidence of PsA (77.3 cases per 1000 person-years and 62.0 cases per 1000 person-years, respectively). Among patients who received oral therapy who were censored at initiation of a biologic therapy, the incidence was much lower (58.5 per 1000 person-years). The incidence decreased by increasing line of therapy and among those on a single biologic therapy for at least 5 years (37.3 per 1000 person-years) (online supplemental table S1).

Table 2 Incidence of PsA by therapy initiated

	N	Mean follow-up time (years)	Person years	Cases (PsA)	Incidence (per 1000 person years)
All	193 709	5.85	1 133 247	11 047	9.75
No therapy	160 675	6.53	1 048 590	6132	5.85
Biologic therapy	12 983	2.50	32 460	2508	77.26
TNFi	10 441	2.60	27 145	2253	83.00
IL12/23i	1946	2.09	4068	191	46.95
IL17i*	334	0.84	279	22	78.78
Oral therapy	14 259	2.38	33 908	2102	61.99
Phototherapy	6195	2.95	18 269	477	26.11
Oral censored†	14 259	1.99	28 446	1664	58.50
Phototherapy censored†	6195	2.68	16 631	393	23.63
All (two codes for PsA)‡	193 709	5.85	1 133 247	7584	6.69

*There were relatively few IL17i initiations given the end of the study in 2017 and approval of therapy in 2016 and there were not enough guselkumab (IL23i) initiations to examine this subgroup as it became available in 2017.
†In these analyses, time was censored when the patient switched to a biologic therapy.
‡In a sensitivity analysis, we used two codes for PsA as the outcome instead of a single code.
IL17i, interleukin 17 inhibitor; IL12/23i, interleukin 12/23 inhibitor (ustekinumab); N, number; oral, oral therapies (including methotrexate, apremilast, cyclosporine, acitretinoin, and tretinoin); PsA, psoriatic arthritis; TNFi, tumour necrosis factor inhibitor .

To examine whether the incidence of PsA differed between patients using biologic therapy and those using oral therapy or phototherapy (after adjusting for potential confounders), we constructed two different types of models: (a) traditional Cox proportional hazards multivariable-adjusted model with a time-varying exposure and separately (b) Cox proportional hazards models starting time at therapy initiation and in a PS-matched cohort. The unadjusted Kaplan-Meier (KM) curves are shown in figure 1A,B and the KM curves for each of the individual biologics are shown in figure 1C. In a model in which biologics are a time-varying exposure compared with no biologic therapy (including patients who have received oral therapy, phototherapy or no therapy), receiving biologic therapies was associated with a higher incidence of PsA (HR 4.84, 95% CI 4.64 to 5.05). The results are similar, though more attenuated, when restricting the cohort to patients who have received either a biologic, an oral therapy, or a phototherapy (HR 4.48, 95% CI 4.23 to 4.75). When time is started at first biologic therapy, the results are further attenuated though similar whether using multivariable adjustment (2.14, 95% CI 2.00 to 2.28) or PS matching (HR 2.17, 95% CI 2.03 to 2.33) (table 3). In examining time from therapy start to diagnosis of PsA, many patients who developed PsA did so shortly after biologic or oral therapy initiation (figure 2). In contrast, diagnosis of PsA occurred more evenly over time in patients receiving phototherapy or no therapy prescriptions.

Sensitivity analyses resulted in very similar results, including when patients who developed PsA within the first year after biologic use in the PS model were excluded (online supplemental table S2). Additionally, results were also similar, although the HR attenuated, when run within the administrative data set (online supplemental table S2).

DISCUSSION

PsA is a chronic inflammatory disease that currently has suboptimal clinical outcomes with at least 50% of patients achieving remission by any definition. One strategy for improving outcomes in PsA is to treat psoriasis more aggressively with the goal of preventing the onset of clinically overt PsA. Some proof-of-concept studies have demonstrated that treatment with biologics reduces subclinical joint and enthesial inflammation in patients with psoriasis.[31 32] This would suggest that treating patients with biologics could potentially prevent PsA. Our study sought to examine whether treatment with a biologic agent among patients with psoriasis has an impact on the development of PsA. Several approaches were used to address this question, but the key outcome was that patients on biologics seemed to have a higher incidence of PsA than patients on oral or phototherapy. We do not suggest that these results should be interpreted causally; in other words, biologics likely do not cause PsA. On the contrary, there are several biases that may play a role

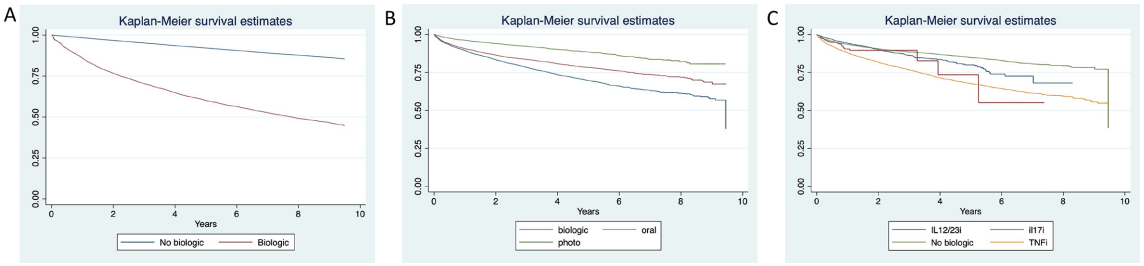

Figure 1 Kaplan-Meier curves for development of psoriatic arthritis. Kaplan-Meier curves for two separate analyses: (A) In the time-varying covariate analysis, we examined patients with psoriasis who initiated a biologic versus no therapy. In this case, time is started at first diagnosis of psoriasis (n=199 204); (B) In the propensity score analyses, time begins at the new initiators of therapy (biologic vs oral vs phototherapy) (n=29 612); (C) Finally, we examined time to diagnosis by individual biologic subclass (N=29 612).

in this finding, suggesting that caution should be used in interpreting observational studies that study the impact of biologic therapy on the development of PsA.

Two recent publications in this journal and one in another journal have addressed this topic in selected dermatology clinic-based populations (as opposed to the population-based cohorts studied here).[33–35] One demonstrated a similar HR to our PS analysis in their PS analysis comparing biologics to phototherapy (HR 2.07, 95% CI 0.87 to 4.93). In contrast, their primary analysis[34] and the analyses in a second and third study[33 35] found a decreased incidence of PsA in the biologics cohort compared with phototherapy and no therapy. Why are the results different within studies and between these studies and our study? This may in part be related to the patient population studied. The other two studies were based in dermatology populations with dermatology–rheumatology collaborations. This is a small subset of the population observed in the current study, and the opposing results may be the result of collider stratification bias where the collider is being seen in a dermatology–rheumatology centre.[36] Additionally, Gisondi *et al* required the patients studied to be on therapy for at least 5 years, essentially excluding these patients that developed PsA in the first 5 years. After we restricted the biologics cohort to that described in Gisondi *et al*, the incidence in this subgroup was reduced to approximately half the incidence when this restriction was not applied and was lower than the incidence in the oral therapy group. Thus, selection bias may be contributing to the differential results; the population observed is important to consider in interpreting the results of any of these retrospective studies, including this study. The only prospective cohort study of psoriasis and the risk for PsA, a Toronto-based cohort, found that patients with psoriasis using a TNFi had a higher risk of PsA than those who did not use a TNFi, although the results were not statistically significant (HR 1.56, 95% CI 0.19 to 12.6).[37] This was likely related to the relatively small number of patients on a TNFi in that study.

Several biases may contribute to the results of retrospective studies. First, selection of a therapy for a given patient is motivated by many factors (see figure 3); this is known as confounding by indication. Most of the factors that influence therapy selection (ie, a patient's risk tolerance) are not measured in any of the three studies and thus are potential unmeasured confounders. PS-matching and marginal structural models are two strategies for addressing confounding by indication, but these cannot be causally interpreted if important confounders, particularly those that may be differential between the exposure groups, are not measured.[25 38 39] Next, in an extension of confounding by indication, patients may be prescribed a biologic because there is either a perceived increased risk for PsA or symptoms that may suggest early PsA. This is known as protopathic bias. For example, a dermatologist may see a patient with psoriasis who complains of joint issues and the dermatologist switches the patient to a biologic (believing the patient may have PsA). When a rheumatologist then sees the patient, the rheumatologist may code for PsA. This was evident in our study as a large number of the incident PsA diagnoses occurred shortly after initiation of the biologic therapy. In another recent study, we observed that dermatologists rarely coded for musculoskeletal complaints, suggesting that misclassification of the outcome may be particularly problematic in this context.[40] In the current study, a sensitivity analysis suggested that while protopathic bias seems to be playing a role (ie, there is a considerable number of diagnoses of PsA within the first year of biologic treatment), the increased risk of PsA with biologic use seemed to persist throughout the first 5 years. Next, survival bias may be playing a role in these results. In other words, a patient has to 'survive' without PsA to receive a biologic therapy and they are, thus, closer to the development

Table 3 Primary multivariable models

Comparison	N	Model	HR (95% CI)
All: biologic initiators vs no biologics*‡	216 901	TVE†	4.84 (4.64 to 5.05)
Biologics vs oral/photo‡	51 815	TVE†	4.48 (4.23 to 4.75)
Biologics vs oral/photo (age and sex adjusted)	29 258	Traditional	2.10 (1.97 to 2.23)
Biologics vs oral/photo (fully adjusted)§	29 258	Traditional	2.14 (2.00 to 2.28)
Biologics vs oral/photo	22 638	PS match	2.17 (2.03 to 2.33)

The primary outcome for these models was a single code for PsA. N is the number of observations for each model.
*Reference group includes patients with oral, phototherapy or no therapy.
†Once exposed to a biologic, the patient is considered always exposed.
‡Covariates in the fully adjusted model include age, sex, anaemia, anxiety, cancer, congestive heart failure, depression, diabetes, fatty liver disease, hidradenitis suppurative, hyperlipidaemia, hypertension, obesity, uveitis, venous thromboembolism.
§Adjusted for all factors in PS (see online supplemental methods 1 for list).
oral, oral therapies (including methotrexate, apremilast, cyclosporine, acitretinoin, and tretinoin); PS, propensity score; TVE, time-varying exposure.

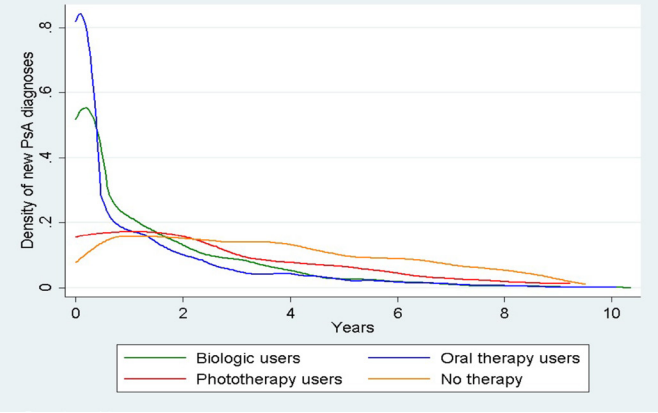

Figure 2 Timing of PsA diagnosis by therapy start. Among patients who receive a new diagnosis of PsA, we examined the time to new PsA diagnosis. Time represents the time from start in the study: initiation of the specified therapy or 1 year after psoriasis diagnosis for those who do not receive therapy. PsA, psoriatic arthritis.

of PsA if they are going to develop it, enhancing the observed risk of PsA in the biologic group. To address this concern, we adjusted for duration of disease and we performed an additional analysis within the PS-matched cohort to address the concern for survival bias and these did not meaningfully change the HR.

Besides the biases that may lead to seeing a higher incidence of PsA among biologic users, is it possible that biologics due in fact stimulate development of PsA? Some have theorised that paradoxical reactions may be the result of shifting polarisation of T cell responses, leading to inflammation in alternate tissues (ie, development of palmoplantar pustular psoriasis with TNFi and eczematous reactions with IL17i).[41] Alternatively, other theories include disruption of negative feedback loops, secondary effects of antidrug immune response (ie, drug-induced lupus erythematosus with TNFi) or non-specific interactions with Fc receptors leading to activation of innate immunity.[41] Unlike skin-related paradoxical reactions and development of new inflammatory bowel disease, development of de novo inflammatory arthritis with biologic therapies among patients with psoriasis has not been commonly reported beyond drug-induced lupus.[42] Thus, while a paradoxical reaction may explain some of these cases, we believe that the more likely explanation is the epidemiologic phenomena described above.

This study should be interpreted in light of limitations. EHR data were used in this study as the primary data source. Health

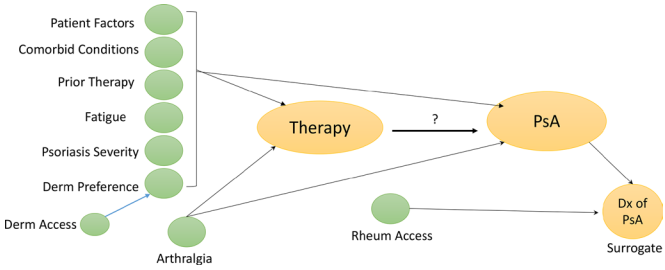

Figure 3 Directed acyclic graph: potential confounders in studying biologic therapies and the risk for PsA. Directed acyclic graphs are a graphical method of displaying relationships between variables. Shown here are the potential confounders in the relationship between therapy prescription and diagnosis of PsA, many of which are unmeasured in EHR and administrative datasets. EHR, electronic health records; PsA, psoriatic arthritis.

systems in the US function as open systems in that patients may be seen in one system for their psoriasis and also seen in another system by their primary care physician. This leads to a situation where not all codes are captured in one system. We addressed this concern in two ways. First, by restricting the study to the subpopulation of patients with a primary care physician in the system. Second, when the analyses were repeated within an administrative data set, there were no substantial differences in the results. In addition, reliance on codes as a surrogate for psoriasis and PsA diagnosis may be associated with misclassification.[29] The results were similar when using one and two codes for PsA, and that the use of a therapy for psoriasis makes the likelihood of truly having psoriasis or PsA much higher.[29] Misclassification may influence the results of this study.[43] If misclassification is balanced between the biologic and oral therapy initiators, known as non-differential misclassification, the results would bias toward the null.[44] The misclassification would not lead to excessive type I error but would lead to reduced statistical power in identification of risk factors that are associated with PsA.[45] However, it is possible that there is more misclassification in the biologic group given the larger number of new diagnoses after therapy, which could lead to excessive false-positive findings (ie, inflation of type I error).[46] Given the large number of patients, we are unable to examine these patients. Use of patient-reported outcomes or physician examination could have improved our understanding of the timing and reasons for therapy prescriptions, but unfortunately this was not available. Next, in an EHR, medications may have been prescribed but never filled. To address this limitation, we used two prescriptions for a given therapy (suggesting a refill was initiated) as the primary analysis. We also repeated the analyses in the administrative data set where the prescriptions were dispensed and the results were similar. Finally, mortality was not addressed as a competing risk in the development of PsA because we did not have data on mortality. Given that this is a relatively young and healthy patient population overall, we believe that this would not have a significant impact on the results.

There remains much to learn about the development of PsA including the best methods for studying whether interventions, such as biologic use or weight loss, may have an impact on disease prevention.[47 48] Ongoing efforts seek to define the stages of PsA development,[49] to understand the predictors of PsA development among patients with psoriasis[47] and to identify the pathophysiologic and imaging features that may signal development of PsA.[50–52] While retrospective observational studies offer several advantages, questions of therapeutic effectiveness for preventing PsA may not be ideally addressed in these data sets. While EHR data sets capture coding as it happens in the real world, the development of PsA can be insidious, subtle and challenging to recognise, particularly when assessing PsA development retrospectively. It is important to consider the population analysed; selection bias (ie, only selecting patients with moderate to severe psoriasis or those that have been continuously on therapy for several years), and resulting collider stratification bias can result in very different estimates and should be considered in all study designs. Prospective observational studies are needed to study the pathophysiology of PsA development. In addition, randomised controlled trials that remove confounding by indication are needed to better understand the effect of therapy for psoriasis on the prevention, or delayed development, of PsA.

In summary, in this retrospective cohort study, patients with psoriasis initiating biologic therapy were more likely to develop PsA than patients initiating phototherapy or oral therapies or those not receiving therapy. Caution should be used

in interpreting retrospective studies of the impact of biologic therapy on development of PsA. Future randomised controlled trials with long-term follow-up are needed to address the impact of therapy for psoriasis on the prevention of and/or the delay in development of PsA.

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TRANSLATIONAL SCIENCE

METTL3-mediated m^{6}A modification of ATG7 regulates autophagy-GATA4 axis to promote cellular senescence and osteoarthritis progression

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ABSTRACT

Objective The aim of the study was to investigate the role and regulatory mechanisms of fibroblast-like synoviocytes (FLSs) and their senescence in the progression of osteoarthritis (OA).

Methods Synovial tissues from normal patients and patients with OA were collected. Synovium FLS senescence was analysed by immunofluorescence and western blotting. The role of methyltransferase-like 3 (METTL3) in autophagy regulation was explored using N6-methyladenosine (m^{6}A)-methylated RNA and RNA immunoprecipitation assays. Mice subjected to destabilisation of the medial meniscus (DMM) surgery were intra-articularly injected with or without pAAV9 loaded with small interfering RNA (siRNA) targeting METTL3. Histological analysis was performed to determine cartilage damage.

Results Senescent FLSs were markedly increased with the progression of OA in patients and mouse models. We determined that impaired autophagy occurred in OA-FLS, resulting in the upregulation of senescence-associated secretory phenotype (SASP). Re-establishment of autophagy reversed the senescent phenotype by suppressing GATA4. Further, we observed for the first time that excessive m^{6}A modification negatively regulated autophagy in OA-FLS. Mechanistically, METTL3-mediated m^{6}A modification decreased the expression of autophagy-related 7, an E-1 enzyme crucial for the formation of autophagosomes, by attenuating its RNA stability. Silencing METTL3 enhanced autophagic flux and inhibited SASP expression in OA-FLS. Intra-articular injection of synovium-targeted METTL3 siRNA suppressed cellular senescence propagation in joints and ameliorated DMM-induced cartilage destruction.

Conclusions Our study revealed the important role of FLS senescence in OA progression. Targeted METTL3 inhibition could alleviate the senescence of FLS and limit OA development in experimental animal models, providing a potential strategy for OA therapy.

Key messages

What is already known about this subject?

⇒ The chronic presence of senescent cells is closely associated with the development of osteoarthritis (OA). However, the underlying mechanisms remain unclear.

What does this study add?

⇒ Senescent fibroblast-like synoviocytes (FLSs) affect the normal function of chondrocytes in vitro and in vivo.

⇒ We demonstrated the critical role of methyltransferase-like 3 (METTL3)/YTH N6-methyladenosine RNA-binding protein 2-mediated N6-methyladenosine (m^{6}A) modification of the autophagy-related 7 messenger RNA in regulating autophagy and cellular senescence.

⇒ Inhibition of METTL3 effectively suppresses the senescence of FLS and decelerate OA development.

How might this impact on clinical practice or future developments?

⇒ Our study highlights the functional importance of the m^{6}A methylation machinery in autophagy, which provides insights into the underlying molecular mechanisms of METTL3 in regulating cellular senescence and the development of therapeutic strategies for the treatment of OA.

INTRODUCTION

Osteoarthritis (OA), the most prevalent joint disease in late life, is primarily characterised by progressive loss of cartilage matrix, accompanied by pathological changes in other joint components, including subchondral bone sclerosis and synovial inflammation.[1] Incident symptomatic knee OA has been reported to peak between 55 and 64 years of age. Moreover, the prevalence of OA increased with age, ranging from 13% in non-obese men to 32% in obese women over 85 years of age.[2] With the ageing of the world population, the number of older adults affected by OA and in need of joint replacement will substantially increase in the following decades. In older adults, a variety of factors related to ageing may contribute to the development of OA. Mitochondrial dysfunction, oxidative stress and reduced autophagy alter chondrocyte function, promoting catabolic processes and cell death during anabolic processes.[3] Thus, improving our understanding of how ageing promotes OA progression would provide novel strategies to slow or stop the

development of the disease, which may have a major impact on public health.

The chronic presence of senescent cells is tightly associated with tissue function loss and age-related chronic diseases such as OA. Cellular senescence is an essential hallmark of ageing, and chondrocytes have various features that are characteristic of senescent cells during ageing and OA progression.[3 4] Senescent cells are characterised by inability to divide, resistance to apoptosis and robust secretome of senescence-associated secretory phenotype (SASP), which could alter the structure and function of the surrounding cells and tissues.[5] Increased production of proinflammatory mediators, including interleukin (IL)-1, IL-6 and matrix metalloproteinase (MMP)3, is a feature of SASP that overlaps with mediators that contribute to the development of OA. To date, the molecular mechanisms associated with the regulation of cellular senescence in OA remain elusive.

It has been reported that large numbers of synoviocytes are senescent in the pathogenesis of OA.[6] In our study, we found a dramatic increase in senescent cells in the synovium region 2 weeks after destabilisation of the medial meniscus (DMM) surgery, which preceded the events of chondrocyte senescence and cartilage degradation. Increased secretion of proinflammatory cytokines and MMPs by the synovium is believed to be involved in the degradation of joint cartilage.[7] Growing evidence supports the notion that the provoked SASP expression and accelerated ageing process are tightly correlated with autophagy inhibition.[8] Autophagy activation can effectively suppress the severity of experimental OA.[9] As a normal cellular metabolic process, autophagy mediates the delivery of cellular components to lysosomes and promotes cell survival under stress.[10] The factors implicated in ageing, such as the loss of proteostasis and accumulation of oxidative damage, genomic instability and epigenomic alteration, are modified through autophagy. Enhancing the autophagy process is regarded as a common characteristic of all evolutionarily conserved antiageing interventions.[11] Articular chondrocytes rely on autophagy as the primary mechanism for maintaining normal function and survival.[12 13] During ageing, autophagy gradually decreases in chondrocytes, thus inducing senescence, which ultimately results in aggravated OA severity.[14] However, the mechanisms underlying impaired autophagy in OA progression are not well understood.

Cell growth and survival depend on the fine-tuning regulation of gene expression at both the transcriptional and translational levels.[15] N6-methyladenosine (m^6A) is a widespread post-transcriptional modification of RNA that determines messenger RNA (mRNA) stability, splicing, transport, localisation and translation efficiency.[16 17] Increasing pieces of evidence suggest that m^6A participates deeply in various cellular processes, including DNA damage, autophagy and cellular senescence.[18–20] The m^6A modification is dynamic and reversible, and it can be catalysed by m^6A methyltransferases and removed by m^6A demethylases.[21 22] In addition, m^6A functions through 'reader' proteins, which selectively recognise and directly or indirectly bind to the m^6A motif to affect mRNA function. YTH N6-methyladenosine RNA-binding protein (YTHDF), a class of m^6A readers, includes YTHDF1 and YTHDF2. YTHDF1 promotes the translation of m^6A-modified mRNA, while YTHDF2 suppresses the stability and mediates alternative splicing of m^6A-modified-mRNA. Recently, it was reported that methyltransferase-like 3 (METTL3), the core component of the m^6A methyltransferase, was significantly elevated in the synovium of human rheumatoid arthritis. METTL3 knockdown effectively suppressed inflammatory and MMP factor expression in fibroblast-like synoviocytes (FLSs).[23] In addition, inhibition of METTL3 significantly reduced the IL-1β-induced degeneration of chondrocytes.[24] However, the biological significance of m^6A modification and the potential regulatory mechanisms of cellular senescence in FLS remain incompletely understood.

In this study, we demonstrated for the first time the critical role of senescent FLS in OA progression in vitro and in vivo and found a positive correlation between m^6A modification and FLS senescence. Further studies revealed that METTL3 influenced autophagy activity by affecting the stability of autophagy-related 7 (ATG7) mRNA in an m^6A-YTHDF2 dependent manner, which subsequently promoted FLS senescence and OA progression. Conversely, METTL3 suppression in FLS effectively inhibited the senescence of FLS and attenuated OA progression in the DMM-induced OA mouse model. Thus, our work implicates METTL3 as a potential therapeutic target for OA treatment.

MATERIALS AND METHODS

Detailed experimental procedures are described in the online supplemental materials and methods (see online supplemental file 1).

RESULTS

FLS senescence and impaired autophagy are closely associated with the progression of OA

To explore the role of cellular senescence in OA development, we first examined the expression of $p16^{INK4a}$ and p21, a typical biomarker of senescent cells, in human OA synovial tissues. The protein and mRNA levels of $p16^{INK4a}$ and p21 were dramatically elevated in the synovium of patients with OA (figure 1A–D). We further observed the accumulation and phenotypical characterisation of senescent FLSs in OA synovial tissues, as confirmed by double-positive immunostaining for $p16^{INK4a}$ and vimentin, a marker of FLS (figure 1E). In addition, the primary FLSs isolated from the synovium of patients with OA also exhibited various senescent phenotypes, including increased expression levels of senescence-associated β-galactosidase (SA-β-Gal), $p16^{INK4a}$ and p21, and enhanced secretion of IL-1β (online supplemental figure S1A–D and S2A). Interestingly, we found decreased accumulation of autophagic vesicles by transmission electron microscopy analyses in the synovium of patients with OA, indicating deficient autophagy in the OA synovium (figure 1F). In addition, we further measured the autophagic markers LC3B-II (a typical marker of autophagosomes) and p62 (a protein regulating autophagic clearance of dysfunctional organelles or aggregates) in the synovium and found lower expression of LC3B-II and higher levels of p62 in the synovium of patients with OA, compared with patients without OA (figure 1C).

To further verify the aforementioned findings and explore FLS senescence during OA development, we established a post-traumatic OA model by DMM and analysed the number of senescent FLSs identified by the positive expression of $p16^{INK4a}$ during OA development. Compared with sham-operated mice, we found that the number of $p16^{INK4a}$-positive FLSs in the synovium of DMM mice significantly increased in a time-dependent manner (figure 1G and online supplemental figure S1E). In addition, we found a large number of $p16^{INK4a}$-expressing cells in the synovium region 2 weeks after surgery, which occurred earlier than chondrocyte senescence (online supplemental figure S3). Meanwhile, the cartilage degradation and the Osteoarthritis Research Society International (OARSI) score were significantly aggravated with time during the course of DMM-induced OA pathogenesis (figure 1H), which was tightly correlated with enhanced FLS senescence (figure 1I). In

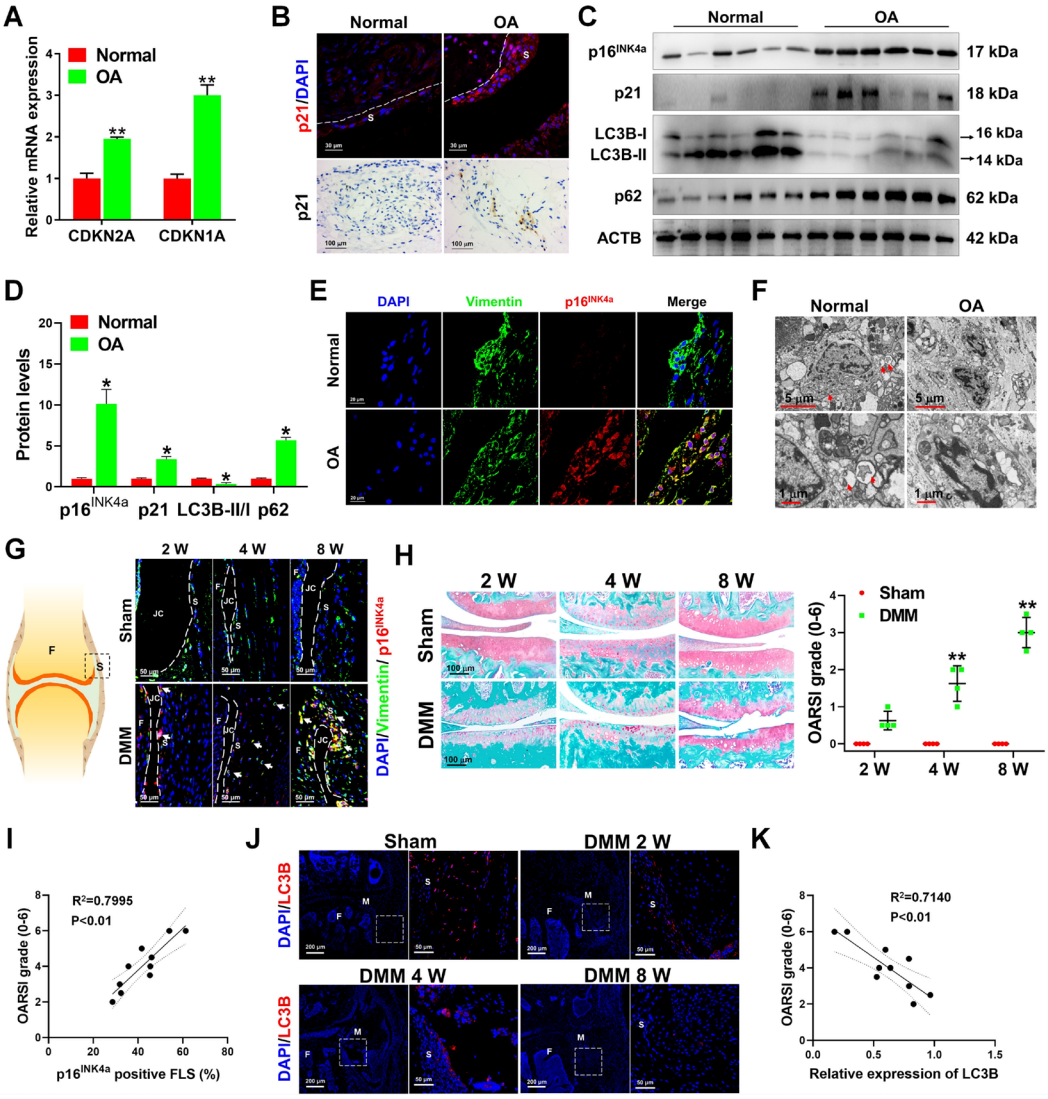

Figure 1 FLS senescence and impaired autophagy closely associates with the progression of OA. (A) Quantitativ PCR analysis of messenger RNA levels for CDKN2A and CDKN1A in synovial tissues from patients with OA (OA) and patients without OA (normal). n=10 per group. **P<0.01. (B) Representative images of immunostaining for p21 in synovial tissues from patients with OA (OA) and patients without OA (normal). (C) Western blot analysis of $p16^{INK4a}$, p21, LC3B and p62 in synovial tissues from patients with OA (OA) and patients without OA (normal). (D) Protein quantification of (C) via ImageJ. n=6 per group. *P<0.05. (E) Representative images of coimmunostaining of vimentin and $p16^{INK4a}$ in synovial tissues from patients with OA (OA) and patients without OA (normal). (F) The representative electron microscopy images of human normal and OA synovium. Red arrowheads indicate autophagic vesicles. (G) The representative images of coimmunostaining of vimentin and $p16^{INK4a}$ in the synovium from control mice (sham) or post-traumatic mice at 2, 4 and 8 weeks after DMM surgery. The dotted box indicates the amplified synovium regions. Arrowheads indicate double-positive cells. (H) The representative safranin O staining images of osteoarthritic knee joints from control mice (sham) or post-traumatic mice at 2, 4 and 8 weeks after DMM surgery. The severity of OA-like phenotype was analysed by grading histological sections in medial femoral condyles and the medial tibial plateau using the OARSI score system. n=4 of each group. **P<0.01. (I) Correlation curves between OARSI grade and the number of $p16^{INK4a}$-positive FLS in the synovium of mice suffered with DMM. (J) The representative images of immunofluorescence of LC3B in synovium of control mice (sham) or post-traumatic mice at 2, 4 and 8 weeks after DMM surgery. The dotted box indicates the amplified synovium regions. (K) Correlation curves between OARSI grade and LC3B expression in the synovium of mice that suffered from DMM surgery. All data were presented as the means±SEM. Paired t-test (A,D) and repeated-measures two-way analysis of variance (H) were used for statistical analysis. ACTB, β-actin; DAPI, 4',6-diamidino-2-phenylindole; DMM, destabilisation of the medial meniscus; F, femur; FLS, fibroblast-like synoviocyte; JC, joint cavity; M, meniscus; S, synovium; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International.

addition, we also demonstrated that the expression of LC3B was dramatically decreased during the progression of DMM-induced OA (figure 1J), which was negatively correlated with OARSI scores (figure 1K). These results indicate that FLS senescence and impaired autophagy are tightly correlated with OA progression.

Senescent FLSs contributed to the catabolic effects of chondrocytes in vivo and in vitro

To further verify whether senescent FLS could accelerate the pathological progression of OA, we cocultured human chondrocytes (C28/I2 cells) with either primary FLSs from patients with OA (OA-FLS) or FLSs from patients without OA (Con-FLS,

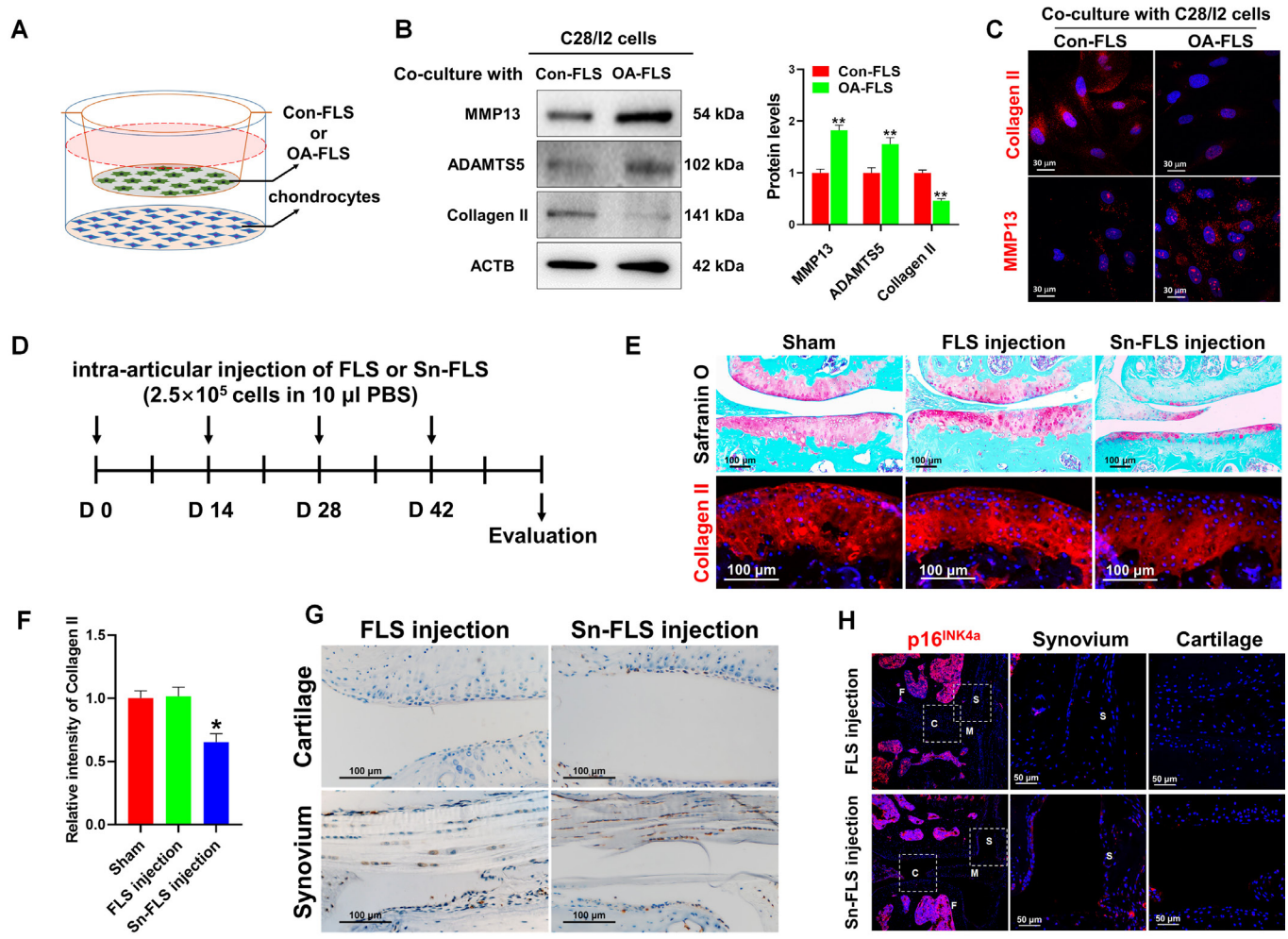

Figure 2 Senescent FLS promotes cartilage degradation in vitro and in vivo. (A) Experimental design diagram of coculture human Con-FLS or OA-FLS with chondrocytes free of direct contact. (B) Western blot analysis for the protein expression of MMP13, ADAMTS5 and collagen II in human chondrocytes (C28/I2 cells) after coculture with human Con-FLS or OA-FLS (passage 2) for 48 hours. n=3. *P<0.05. (C) Immunofluorescence staining of collagen II and MMP13 in C28/I2 cells after coculture with human Con-FLS or OA-FLS for 48 hours. (D) Experimental design diagram of intra-articular injection of normal FLS (FLS) or Sn-FLS induced by bleomycin on mice. (E) The representative images of safranin O staining (top panel) and collagen II staining (bottom panel) for joint from mice after intra-articular injection of FLS or Sn-FLS. (F) Quantification of collagen II expression in cartilage via ImageJ. n=3 per group. *P<0.05. (G,H) The representative images of immunohistochemistry (G) and immunofluorescence (H) staining of $p16^{INK4a}$ in cartilage and synovium from mice after intra-articular injection of FLS or Sn-FLS. All data were presented as the means±SEM. Paired t-test (B) and one-way analysis of variance with Dunnett's multiple comparisons test (F) were used for statistical analysis. C, cartilage; F, femur; FLS, fibroblast-like synoviocyte; M, meniscus; MMP, matrix metalloproteinase; OA, osteoarthritis; S, synovium; Sn-FLS, senescent fibroblast-like synoviocyte.

figure 2A). Interestingly, we detected increased expression of MMP13 and ADAMTS5 and decreased expression of collagen II in C28/I2 cells after coculture with OA-FLS (figure 2B,C). In addition, to further investigate the effects of senescent FLSs on cartilage degradation in vivo, we used bleomycin, a DNA-damaging chemical agent, to induce robust cellular senescence in mouse FLSs, as indexed by the induction of SA-β-Gal activity (online supplemental figure S4A). The excessive FLS senescence induced by bleomycin was further confirmed by increased expression of $p16^{INK4a}$ and p21 and elevated mRNA levels of SASP (online supplemental figure S4B,C). Then, mice without DMM surgery were intra-articularly injected with either 2.5×10^5 normal FLSs or senescent FLSs induced by bleomycin treatment (figure 2D). At day 56 after the first injection, we found decreased safranin O staining and lower expression of collagen II in mice injected with senescent FLSs compared with mice injected with normal FLSs (figure 2E,F), accompanied by elevated expression of $p16^{INK4a}$ in the synovium and cartilage (figure 2G,H). This indicated that exogenous injection of senescent FLS could trigger cartilage dysfunction and induce senescence of the synovium and cartilage.

Autophagy was impaired in senescent FLSs from patients with OA and in DMM-induced OA mice

Recently, impaired autophagy has been implicated in the ageing of various model organisms, possibly contributing to enhanced cellular senescence,[25 26] both in patients with OA and in OA mice models. We observed reduced LC3B expression and elevated levels of p62 in FLSs in both patients with OA and DMM mouse models (figure 3A,B, and online supplemental figure S5A,B). In addition, we found that LC3B was significantly decreased and p62 was dramatically elevated in $p16^{INK4a}$ positive cells in both patients with OA and DMM-induced OA models (figure 3C,D, and online supplemental figure S5C,D). To further confirm whether the reduced autophagic structures in OA-FLS were

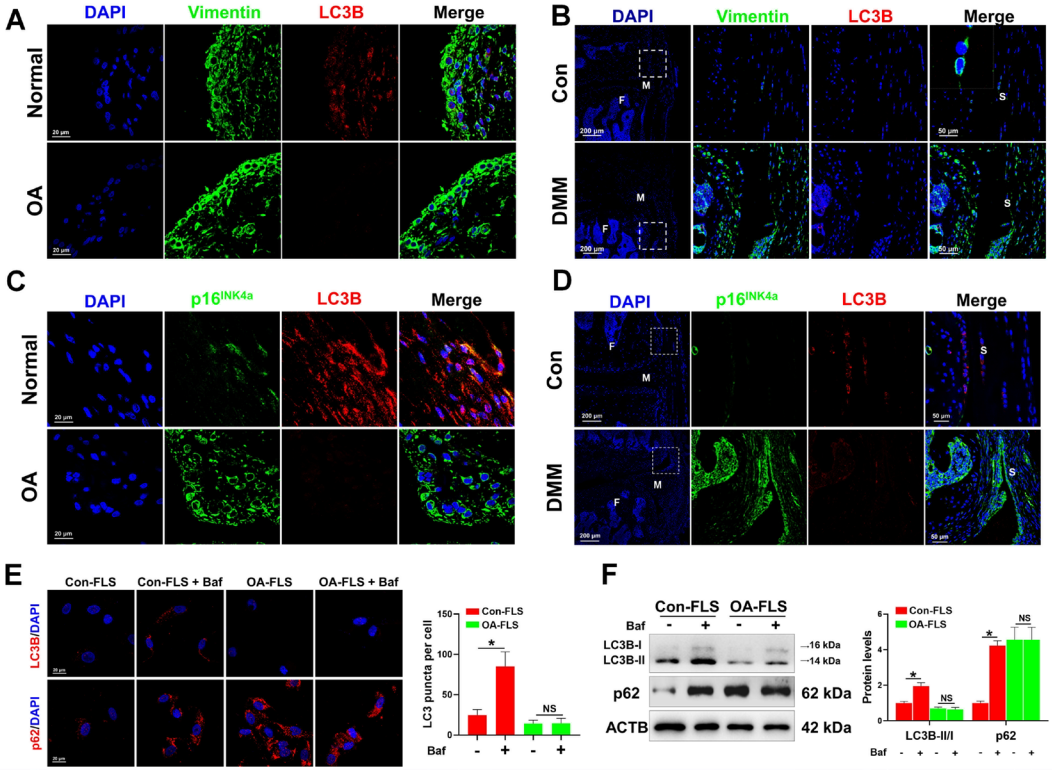

Figure 3 Autophagy is impaired in FLS from patients with OA and DMM-induced OA mice. (A, B) The representative images of double fluorescent immunostaining for vimentin with LC3B in the synovium from patients with OA (A) and post-traumatic mice (B) 8 weeks after DMM surgery. The dotted box indicates the amplified synovium regions. (C, D) The representative images of double fluorescent immunostaining for $p16^{INK4a}$ and LC3B in the synovium from patients with OA (C) and post-traumatic mice (D) 8 weeks after DMM surgery. The dotted box indicates the amplified synovium regions. (E) The representative images of immunofluorescent labelling of LC3B and p62 in human Con-FLS or OA-FLS (passage 2) with the treatment of bafilomycin A1 (Baf, 50 nM) or not; the average number of LC3B puncta per cell was quantified via ImageJ (right panel). n=3. *P<0.05. (F) Western blot analysis of LC3B and p62 in human Con-FLS or OA-FLS (passage 2) with the treatment of Baf or not. n=3. *P<0.05. All data are presented as the mean±SEM. One-way analysis of variance with Dunnett's multiple comparisons was used for statistical analysis. DMM, destabilisation of the medial meniscus; F, femur; FLS, fibroblast-like synoviocyte; M, meniscus; NS, not significant; OA, osteoarthritis; S, synovium.

caused by autophagy impairment or by enhanced autophagic degradation, we used the autophagy-flux inhibitor bafilomycin, which could increase LC3B by preventing lysosomal degradation.[27] Bafilomycin treatment increased the number of LC3B puncta and elevated the levels of p62 in Con-FLS. However, treatment with bafilomycin did not increase LC3B and p62 expression in OA-FLS (figure 3E,F), indicating that OA-FLS lacks the capacity for further autophagosome formation and that the degradation capacity of lysosomes was already at a low level in OA-FLS.

Impaired autophagy in FLS accelerated cellular senescence in a GATA4-dependent manner

To assess whether autophagy mediated FLS senescence, we performed additional autophagy activation and blockade experiments. On one hand, activation of autophagy by rapamycin effectively increased the protein levels of LC3B-II and LC3 puncta per cell (figure 4A and online supplemental figure S6) and suppressed a series of events including $p16^{INK4a}$, p21 and p62 expressions, as well as IL-1β secretion in OA-FLS (figure 4A and online supplemental figure S2B). On the other hand, inhibition of autophagy via 3-methyladenine (3-MA) significantly decreased LC3B-II levels, accompanied by elevated expression of p62, $p16^{INK4a}$ and p21 in Con-FLS (figure 4B). Furthermore, we found that GATA4, a recently described senescence regulator,[8] was significantly increased in OA-FLS both in vivo and in vitro (figure 4C,D, and online supplemental figure S7). GATA4 knockdown significantly suppressed the secretion of IL-1β (online supplemental figure S2C). In addition, recovery of autophagy via rapamycin in OA-FLS could effectively decrease the expression of GATA4, whereas 3-MA treatment significantly promoted the protein level of GATA4 in Con-FLS (figure 4A,B). To further investigate whether autophagy prevented cellular senescence by suppressing GATA4, Con-FLSs were transfected with pcDNA3.1-GATA4 or GATA4 small interfering RNA (siRNA) with or without rapamycin or 3-MA treatment. GATA4 knockdown alleviated the 3-MA-induced expression of $p16^{INK4a}$, p21 and SASP (figure 4E,F). In contrast, upregulation of GATA4 could contribute to the expression of $p16^{INK4a}$, p21 and SASP (figure 4G,H).

Elevated m^6A levels correlated with impaired autophagy in OA-FLS

Recently, an increasing number of studies have reported that m^6A modification controls autophagy activity in various physiological processes, including tumorigenesis and cell apoptosis.[18 28] To determine whether m^6A modification was involved in regulating autophagy activity in FLS during the development of OA, the levels of m^6A were measured by using immunofluorescence. We observed that m^6A expression was significantly increased in both the FLSs of patients with OA and DMM mouse models (figure 5A–C and online

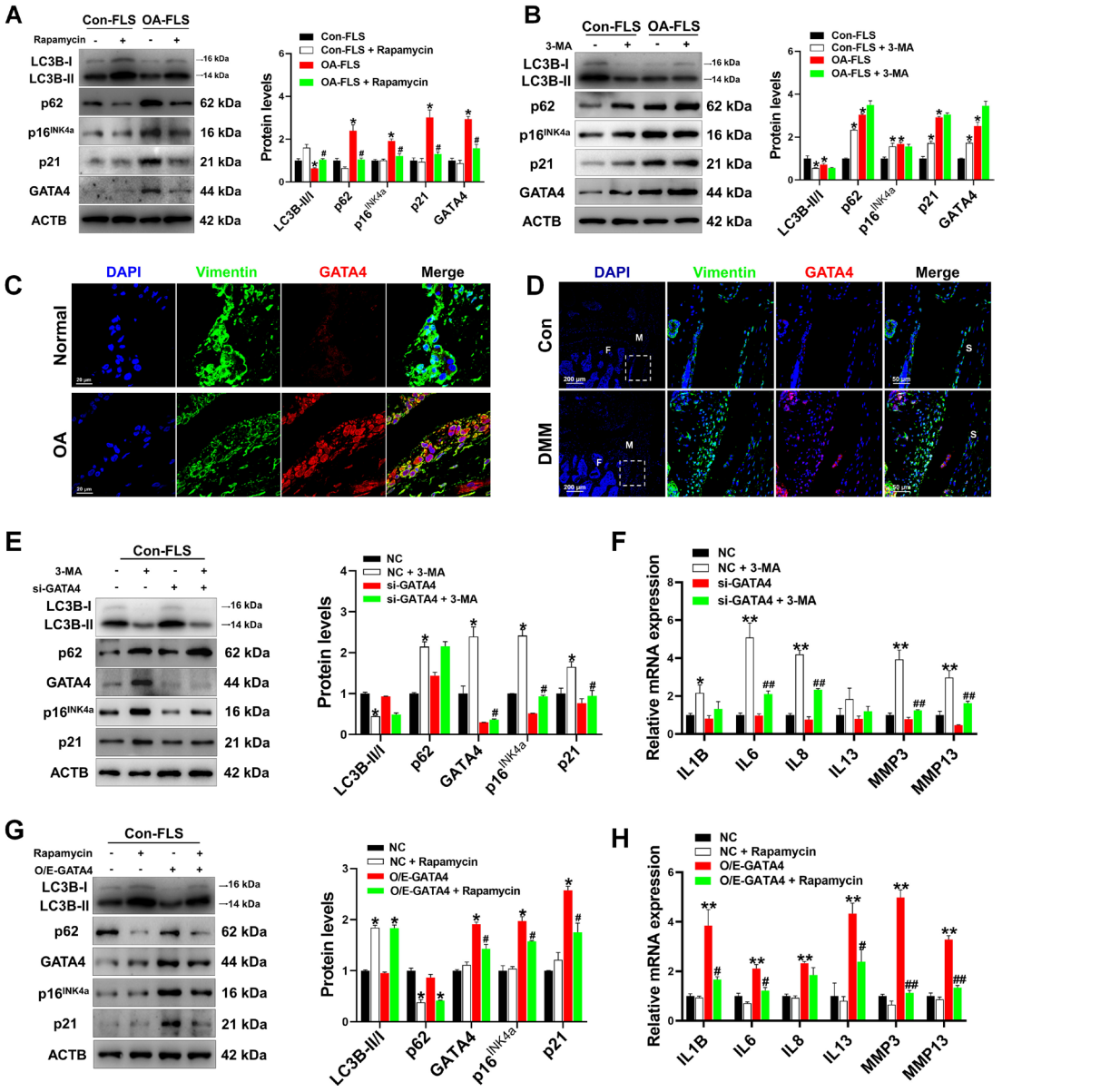

Figure 4 Autophagy regulates cellular senescence in a GATA4-dependent manner. (A) Western blot analysis of the protein levels for autophagy markers (LC3B and p62), cellular senescence markers (p16^{INK4a} and p21) and GATA4 in human Con-FLS and OA-FLS (passage 2) treated with or without rapamycin (50 nM) for 48 hours. n=3 per group. *P<0.05 vs Con-FLS; #P<0.05 vs OA-FLS. (B) Western blot and analysis of the protein levels for autophagy markers (LC3B and p62), cellular senescence markers (p16^{INK4a} and p21) and GATA4 in human Con-FLS and OA-FLS (passage 2) treated with or without 3-MA (1 mM) for 48 hours. n=3 per group. *P<0.05 vs Con-FLS. (C,D) The representative images of double immunofluorescent staining for vimentin and GATA4 in human synovium (C) and post-traumatic mice 8 weeks after DMM surgery (D). The dotted box indicates the amplified synovium regions. (E) Western blot analysis of protein levels for autophagy markers (LC3B and p62), cellular senescence markers (p16^{INK4a} and p21) and GATA4 in human Con-FLS (passage 2) transfected with siRNA targeting GATA4 (si-GATA4) followed by 3-MA treatment for 48 hours. n=3 per group. *P<0.05 vs NC; #P<0.05 vs NC +3-MA. (F) qPCR analysis of SASP-related inflammatory cytokines (IL-1β, IL-6, IL-8 and IL-13), MMP3 and MMP13 in indicated groups. n=3 per group. *P<0.05, **P<0.01 vs NC; ##P<0.01 vs NC +3-MA. (G) Western blot analysis of protein levels for autophagy markers (LC3B and p62), cellular senescence markers (p16^{INK4a} and p21) and GATA4 in human Con-FLS (passage 2) transfected with pcDNA3.1-GATA4 (O/E-GATA4) vector followed by the treatment of rapamycin for 48 hours. n=3 per group. *P<0.05 vs NC; #P<0.05 vs O/E-GATA4. (H) qPCR analysis of SASP-related inflammatory cytokines (IL-1β, IL-6, IL-8 and IL-13), MMP3 and MMP13 in indicated groups. n=3 per group. **P<0.01 vs NC; #P<0.05, ##P<0.01 vs O/E-GATA4. All data were presented as the means±SEM. One-way analysis of variance with Dunnett's multiple comparisons was used for statistical analysis. 3-MA, 3-methyladenine; DMM, destabilisation of the medial meniscus; F, femur; FLS, fibroblast-like synoviocyte; IL, interleukin; M, meniscus; MMP, matrix metalloproteinase; NC, negative control; OA, osteoarthritis; O/E, overexpression; qPCR, quantitative PCR; S, synovium; SASP, senescence-associated secretory phenotype; siRNA, small interfering RNA.

supplemental figure S9A). Given that m^{6}A modification is mainly regulated by the m^{6}A methyltransferase complex,[29] we measured the mRNA levels of METTL3, METTL14, WT1-associated protein (WTAP), fat mass and obesity-associated protein (FTO), and AlkB homolog 5 (ALKBH5) in the OA synovium and OA-FLSs. We found that the mRNA expression of METTL3 was significantly upregulated, while the mRNA expression of the other genes did not change significantly

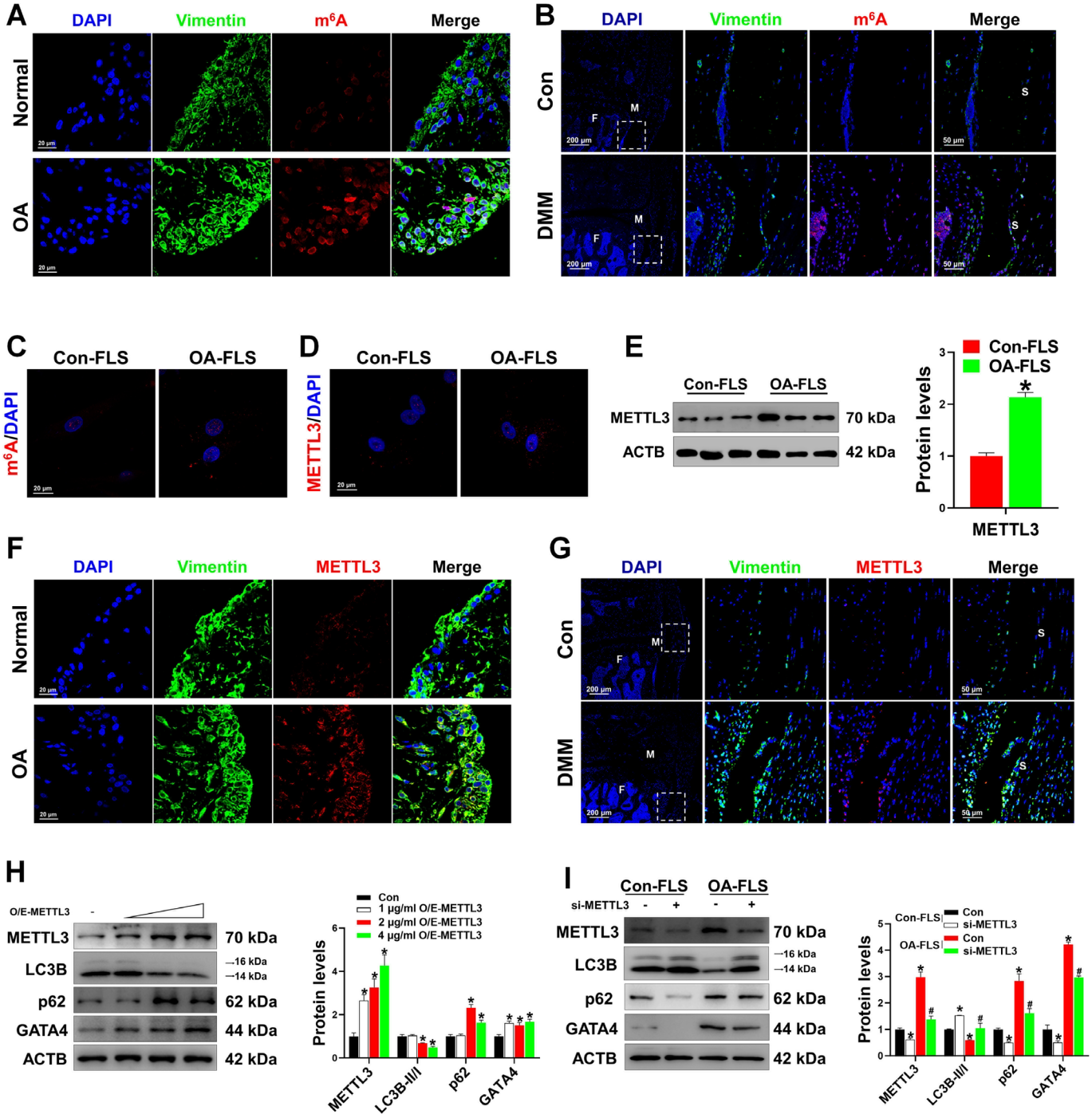

Figure 5 Elevated m^{6}A levels contribute to impaired autophagy in OA-FLS. (A,B) The representative images of double immunofluorescent labelling for vimentin and m^{6}A in synovium from patients with OA (A) and post-traumatic mice 8 weeks after DMM surgery (B). The dotted box indicates the amplified synovium regions. (C) The representative images of immunofluorescent detection for m^{6}A in human Con-FLS and OA-FLS (passage 2). (D) The representative images of immunofluorescent staining of METTL3 in human Con-FLS and OA-FLS (passage 2). (E) Western blot analysis of METTL3 protein levels in human FLS (passage 2) derived from patients with OA or patient without OA. n=3. *P<0.05. (F,G) The representative images of double immunofluorescent labelling of vimentin and METTL3 in synovium from patients with OA (F) and DMM-induced OA mice (G) at 8 weeks after surgery. The dotted box indicates the amplified synovium regions. (H) Western blot analysis of protein levels for METTL3, LC3B, p62 and GATA4 in human Con-FLS (passage 2) after transfection with pcDNA3.1-METTL3 vector (O/E-METTL3, O/E) at different dosages (1, 2 and 4 μg/mL) for 3 days or not. n=3. *P<0.05. (I) Western blot analysis of protein levels for METTL3, LC3B, p62 and GATA4 in human Con-FLS and OA-FLS (passage 2) after transfected with si-METTL3 and cultured for 3 days or not. n=3. *P<0.05 vs Con-FLS; #P<0.05 vs OA-FLS. All data were presented as the means±SEM. Paired t-test (E) and one-way analysis of variance with Dunnett's multiple comparisons (H,I) were used for statistical analysis. 3-MA, 3-methyladenine; DMM, destabilisation of the medial meniscus; F, femur; FLS, fibroblast-like synoviocyte; S, synovium; M, meniscus; m^{6}A, N6-methyladenosine; METTL-3, methyltransferase-like 3; OA, osteoarthritis; O/E, overexpression.

(online supplemental figure S8). Consistent with these findings, the protein levels of METTL3 were also profoundly increased in FLS isolated from the synovium of patients with OA (figure 5D,E). We further confirmed this finding by double labelling of vimentin and METTL3 using immunofluorescence staining in both human OA synovium and DMM-induced OA models (figure 5F,G). To further confirm whether autophagy repression in OA was due to elevated m^{6}A modification and METTL3 expression, human FLSs were overexpressed with METTL3. We observed that upregulation of METTL3 elevated m^{6}A levels (online supplemental figure S9B,C), accompanied by decreased expression of LC3B-II and enhanced expression of p62 and GATA4 (figure 5H). On the contrary, METTL3 knockdown decreased the levels of m^{6}A (online supplemental figure S9B,C), upregulated the expression of LC3B-II, and suppressed the protein levels of p62 and GATA4 in OA-FLS (figure 5I). These results revealed that METTL3-mediated m^{6}A modification plays a critical role in autophagy-regulated senescence in FLS.

METTL3-mediated m^{6}A modification induced the decay of the ATG7 transcript in a YTHDF2-dependent manner

To investigate the role of m^{6}A modification and verify METTL3 as its potential target gene in autophagy, we first employed quantitative PCR (qPCR) analysis to examine the mRNA levels of autophagy-related genes, which tightly execute and control the process of autophagy from initiation to closure.[30] Our results showed that the mRNA levels of ATG7 were significantly attenuated in both the human OA synovium and OA-FLSs (online supplemental figure S10A,B). Consistently, the ATG7 protein levels were markedly decreased in human OA-FLSs and the DMM mouse model (figure 6A,B). Upregulation of METTL3 significantly decreased the expression of ATG7 (online supplemental figure S10C and figure 6C). In contrast, METTL3 knockdown upregulated the expression of ATG7 in OA-FLS (figure 6D).

Given that we found a negative correlation between METTL3 and autophagy, we further confirmed whether METTL3 influenced autophagy by regulating the expression of ATG7. Upregulation of ATG7 effectively alleviated METTL3-induced LC3B-II reduction and decreased the expression of p62 and GATA4 in Con-FLS (figure 6E). Knockdown of ATG7 also suppressed the METTL3 knockdown-induced LC3B-II increase, and enhanced the levels of p62 and GATA4 in Con-FLS (figure 6F), suggesting that METTL3-induced autophagy defects in FLS were mediated through the inhibition of ATG7. Next, we performed sequence analysis of the ATG7 transcript and found three sites of m^{6}A modification within the coding sequence region and five m^{6}A sites in the 3′-untranslated region (UTR) (figure 6G). The m^{6}A RNA-immunoprecipitation (RIP) analyses demonstrated that m^{6}A was significantly enriched at sites 1, 2, 4, 5 and 7 (figure 6H). Compared with Con-FLS, m^{6}A enrichment at sites 4 and 7 was dramatically increased in OA-FLS (figure 6I), which was markedly decreased on METTL3 knockdown (figure 6J). These results suggest that METTL3 targets the ATG7 transcript and regulates ATG7 in an m^{6}A-dependent manner.

While METTL3 serves as a ‘writer’ for m^{6}A on ATG7, potential m^{6}A-selective-binding proteins are required to recognise m^{6}A-modified mRNA and exert regulatory functions. YTHDF1 has been reported to promote the translation of targeted m^{6}A-modified mRNA, while YTHDF2 selectively recognises and destabilises m^{6}A-modified mRNA.[31] To further illustrate whether YTHDF1 or YTHDF2 selectively targeted m^{6}A-modified mRNA of ATG7 to regulate its expression in FLS, we transfected FLSs with the METTL3 plasmid, followed by treatment with or without si-YTHDF1 and si-YTHDF2, respectively. We found that knockdown of YTHDF2 markedly alleviated the METTL3-induced reduction of ATG7 protein expression, whereas YTHDF1 knockdown did not affect ATG7 protein expression. This indicated that the m^{6}A-modified mRNA of ATG7 by METTL3 was a target of YTHDF2 (figure 6K). In addition, we performed RIP-qPCR analyses to confirm that ATG7 indeed interacted with YTHDF2 but not with YTHDF1 (figure 6L,M). Taken together, our results demonstrate that METTL3 regulates ATG7 expression in a YTHDF2-dependent manner.

METTL3 regulated cellular senescence and SASP expression in vitro

To further explore the functional role of METTL3 in regulating FLS senescence, we overexpressed METTL3 in human FLSs via transfection with the METTL3 plasmid. We demonstrated that upregulation of METTL3 significantly elevated the expression of p16^{INK4a} and p21 (figure 7A,B), and the mRNA levels of SASP (figure 7B) in human FLSs. The β-galactosidase assay also confirmed that METTL3 promoted senescence in FLSs (figure 7C). Next, we investigated whether downregulation of METTL3 by transfection with METTL3 siRNA could reverse senescence in OA-FLS. We observed that METTL3 knockdown obviously suppressed p16^{INK4a} and p21 expressions (figure 7D,E) and decreased SASP expression and IL-1β secretion (figure 7E and supplemental figure S2C) in OA-FLS. To further confirm the aforementioned findings, mouse FLSs were treated with bleomycin, followed by transfection with or without si-METTL3. We also found that knockdown of METTL3 effectively alleviated bleomycin-induced p16^{INK4a}, p21 and SASP expressions (figure 7F), as well as β-galactosidase production (figure 7G,H) in mouse FLSs. Together, these data demonstrate that METTL3 plays a critical role in promoting FLS senescence, and METTL3 may act as a potential therapeutic target for impairing cellular senescence in FLSs.

Synovium-targeted inhibition of METTL3 alleviated the progression of OA in a DMM mouse model

A previous study reported that the synovial fibroblast-targeting peptide motif (HAP-1) could effectively deliver drug-encapsulated liposomes to synovial fibroblasts in the inflamed synovium.[32] To confirm whether targeted inhibition of METTL3 in FLS could suppress OA progression, we inserted HAP-1-encoding DNA sequences into the N-terminus of the VP2 domain to construct an FLS-targeted adeno-associated virus vector (rAAV9.HAP-1) (figure 8A). To test whether HAP-1 insertion affects the cellular transfection ability of rAAV9, the vectors were infected with FLS and chondrocyte progenitor cells (ATDC5). Compared with rAAV9, rAAV9.HAP-1 showed a modest increase in transfection efficiency, and low enhanced green fluorescent protein (EGFP) expression was detected in ATDC5 cells (figure 8B). Since the rAAV9.HAP-1 engineered capsid retained full transfection activity in vitro, we next tested its FLS-targeting activity in vivo. We administered 2-month-old mice with rAAV9 or rAAV9.HAP-1 vectors via intra-articular injection. EGFP expression in the liver of mice treated with rAAV9.HAP-1 was comparatively lower than that in mice treated with rAAV9 (online supplemental figure S11A). Intriguingly, the fluorescent signal of EGFP in the joint from mice treated with rAAV9.HAP-1 was obviously stronger than that in rAAV9-treated mice (online supplemental figure S11A). The fluorescence microscopy images also confirmed that

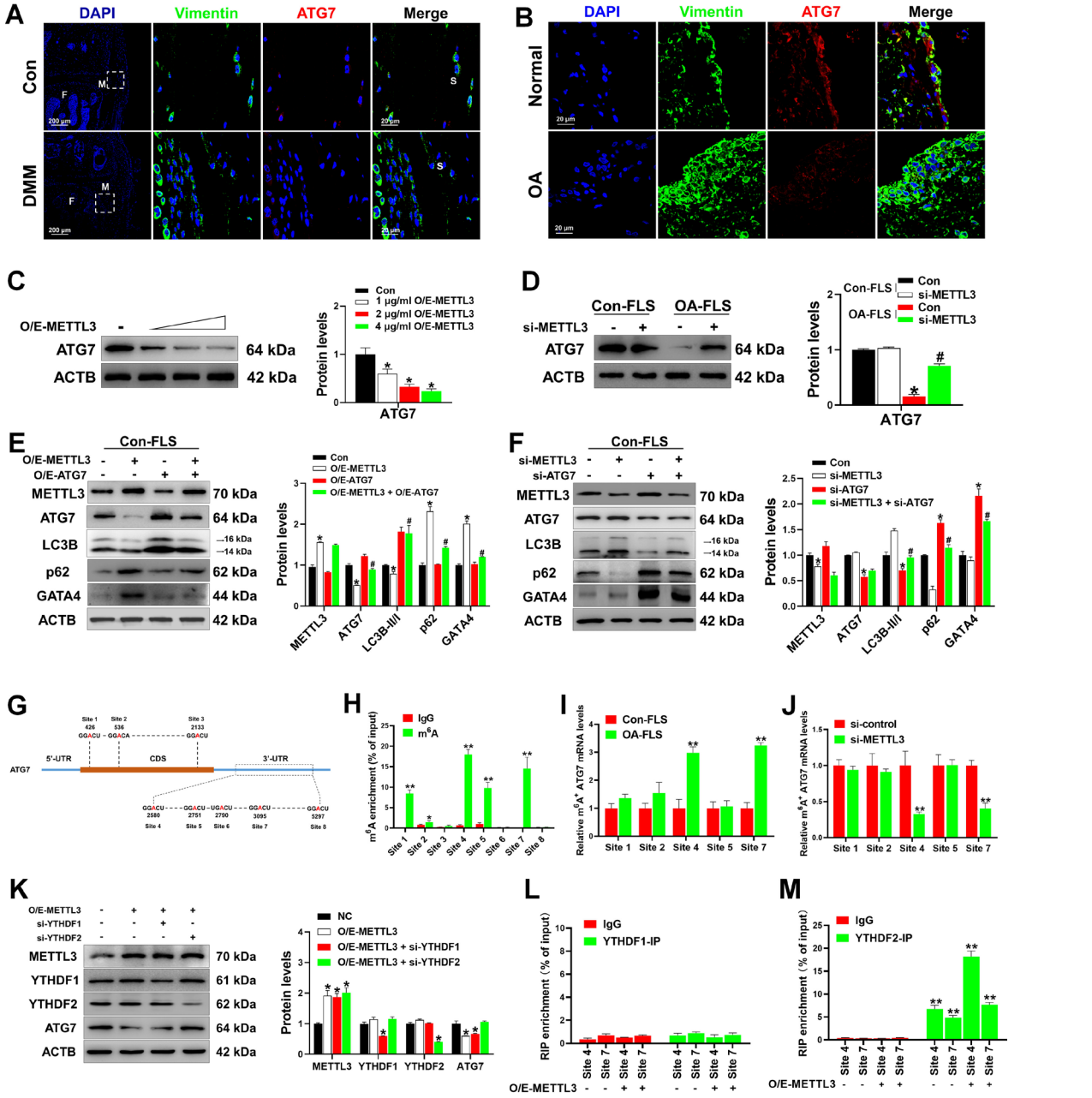

Figure 6 METTL3-mediated m^{6}A modification induces the decay of the ATG7 transcript in YTHDF2-dependent manner. (A,B) The representative images of double immunofluorescent staining of vimentin and ATG7 in synovium from patients with OA (A) and post-traumatic mice (B) 8 weeks after DMM surgery. The dotted box indicates the amplified synovium regions. (C) Western blot analysis of protein level for ATG7 in human FLS (passage 2) after transfected with pcDNA3.1-METTL3 vector (O/E-METTL3,O/E) at different concentrations (1, 2 and 4 μg/mL) for 3 days. n=3. *P<0.05. (D) Western blot analysis of protein level for ATG7 in human Con-FLS and OA-FLS (passage 2) after transfected with si-METTL3) for 3 days. n=3. *P<0.05 vs Con-FLS; #P<0.05 vs OA-FLS. (E) Western blot analysis of protein levels for indicated genes (METTL3, ATG7, LC3B, p62 and GATA4) in human Con-FLS (passage 2) after transfection with or without O/E-METTL3 followed by treatment with or without pcDNA3.1-ATG7 vector (O/E-ATG7). n=3. *P<0.05 vs Con; #P<0.05 vs O/E-METTL3. (F) Western blot analysis of protein levels for indicated genes (METTL3, ATG7, LC3B, p62 and GATA4) in human Co-FLS (passage 2) after transfection with or without si-METTL3 followed by treatment with or without siRNA targeting ATG7 (si-ATG7). n=3. *P<0.05 vs Con; #P<0.05 vs si-ATG7. (G) Schematic diagram showing the position of m^{6}A motifs within ATG7 transcript sequence. (H) MeRIP-qPCR analysis of m^{6}A levels of ATG7 at different sites in human Con-FLS (passage 2). n=3. **P<0.01. (I) MeRIP-qPCR analysis of m^{6}A levels of ATG7 in human FLS and OA-FLS. n=3. **P<0.01. (J) MeRIP-qPCR analysis of m^{6}A levels of ATG7 in human FLS (passage 2) after transfection with or without si-METTL3. n=3. **P<0.01. (K) Western blot analysis of protein levels for METTL3, YTHDF1, YTHDF2 and ATG7 in human Con-FLS (passage 2) after transfection with or without O/E-METTL3, followed by treatment with or without siRNA targeting YTHDF1 or YTHDF2. n=3. *P<0.05. (O,P) RIP-qPCR analysis of the interaction of ATG7 with YTHDF1 (O) or YTHDF2 (P) in human Con-FLS (passage 2) transfected with or without O/E-METTL3. n=3. **P<0.01. All data were presented as the means±SEM. One-way analysis of variance with Dunnett's multiple comparisons (D–F,K–M) and paired t test (H, I,J) was used for statistical analysis. ATG7, autophagy-related 7; DMM, destabilisation of the medial meniscus; F, femur; FLS, fibroblast-like synoviocyte; M, meniscus; m^{6}A, N6-methyladenosine; MeRIP, methylated RNA immunoprecipitation; METTLE3, methyltransferase-like 3; OA, osteoarthritis; O/E, overexpression; qPCR, quantitative PCR; RIP, RNA-immunoprecipitation; S, synovium; si-METTL3, siRNA targeting METTL3; siRNA, small interfering RNA; YTHDF, YTH N6-methyladenosine RNA-binding protein.

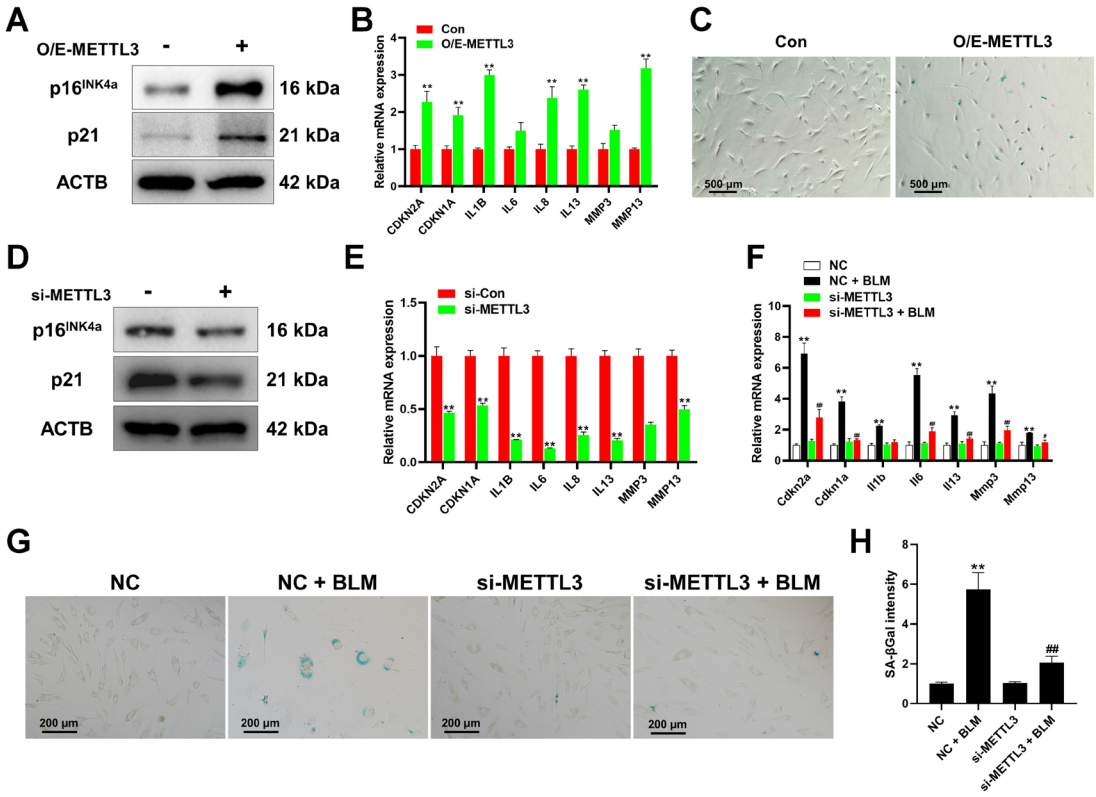

Figure 7 METTL3 regulates cellular senescence and SASP expression in FLS in vitro. (A) Western blot analysis of protein levels for p16^{INK4a} and p21 in human Con-FLS (passage 2) after transfection with or without pcDNA3.1-METTL3 vector (O/E-METTL3, O/E). (B) Q-PCR analysis of mRNA levels for SASP-related genes (CDKN2A, CDKN1A, IL-1β, IL-6, IL-13, MMP3 and MMP13) in human Con-FLS (passage 2) after transfected with or without O/E-METTL3. n=3, **p<0.01. (C) The representative images of SA-β-Gal staining for human Con-FLS (passage 2) transfected with or without O/E-METTL3. (D) Western blot analysis of protein levels for p16^{INK4a} and p21 in human OA-FLS (passage 2) after transfected with or without si-METTL3. (E) qPCR analysis of mRNA levels for SASP-related genes (CDKN2A, CDKN1A, IL-1β, IL-6, IL-13, MMP3 and MMP13) in human OA-FLS (passage 2) after transfection with or without si-METTL3. n=3. **P<0.01. (F) qPCR analysis of mRNA levels for SASP-related genes (Cdkn2a, Cdkn1a, IL-1β, IL-6, IL-13, MMP3 and MMP13) in mouse FLS (passage 2) after treatment with or without BLM (10 μM), accompanied with or without the transfection of si-METTL3. n=3. **P<0.01 vs NC, #P<0.05, ##P<0.01 vs NC +BLM. (G,H) The representative images of SA-β-Gal staining (G) and subsequent quantification of SA-β-Gal intensity (H) for mouse FLS (passage 2) after indicated treatment as in (F). n=3. **P<0.01 vs NC; #P<0.05, ##P<0.01 vs NC +BLM. All data were presented as the means±SEM. Paired t-test (B,E) and one-way analysis of variance with Dunnett's multiple comparisons (F,H) were used for statistical analysis. BLM, bleomycin; FLS, fibroblast-like synoviocyte; IL, interleukin; METTL3, methyltransferase-like 3; MMP, matrix metalloproteinase; mRNA, messenger RNA; OA, osteoarthritis; O/E, overexpression; qPCR, quantitative PCR; SA-β-Gal, senescence-associated β-galactosidase; SASP, enescence-associated secretory phenotype.

there were more EGFP-positive cells in the synovium of mice injected intra-articularly with rAAV9.HAP-1 than in mice treated with rAAV9, which were costained with vimentin (figure 8C and online supplemental figure S11B). These results demonstrate that the engineered VP2 capsid protein fused with HAP-1 improved the FLS tropism of rAAV9. In addition, as compared with chondrocytes or cartilage, the expression of METTL3 was significantly decreased in the FLSs and synovial tissues of mice treated with AAV9.HAP-1-si-METTL3, indicating that AAV9.HAP-1-si-METTL3 could specifically decrease the expression of METTL3 in FLS (online supplemental figure S12A,B).

We next examined whether intra-articular injection of AAV9.HAP-1-si-METTL3 could exert therapeutic effects in a DMM-induced OA mouse model. The results showed that the progressive cartilage degradation in DMM mice during OA development was significantly reversed after intra-articular injection of AAV9.HAP-1-si-METTL3 (figure 8D,E). In addition, we found that the levels of METTL3 and p16^{INK4a} in the synovium of mice treated with AAV9.HAP-1-si-METTL3 were markedly reduced, relative to those in mice treated with AAV9.HAP-1-NC (figure 8F and online supplemental figure S12C). Taken together, these results demonstrate that delivery of si-METTL3 by the synovium-tropic AAV9.HAP-1 capsid could counteract OA progression in DMM-induced OA mouse models.

DISCUSSION

To date, ageing has always been considered an essential aetiological agent for OA, which is characterised by cellular senescence and progressive loss of tissue and organ function over time.[3 33] It has been demonstrated that local clearance of senescent cells could attenuate the progression of OA and create a proregenerative environment.[6] However, the molecular mechanisms underlying the relationship between ageing and OA pathogenesis remain unclear. In this study, we found extensive numbers of senescent FLSs in the progression of OA, which could promote cartilage dysfunction in vitro and in vivo (figures 1 and 2), indicating the critical role of senescent FLSs in OA pathogenesis. Thus, uncovering the mechanism of FLS senescence may provide new key targets for the clinical treatment of OA.

It has been reported that OA deregulates common molecular and cellular mechanisms in chronic age-related diseases.[34]

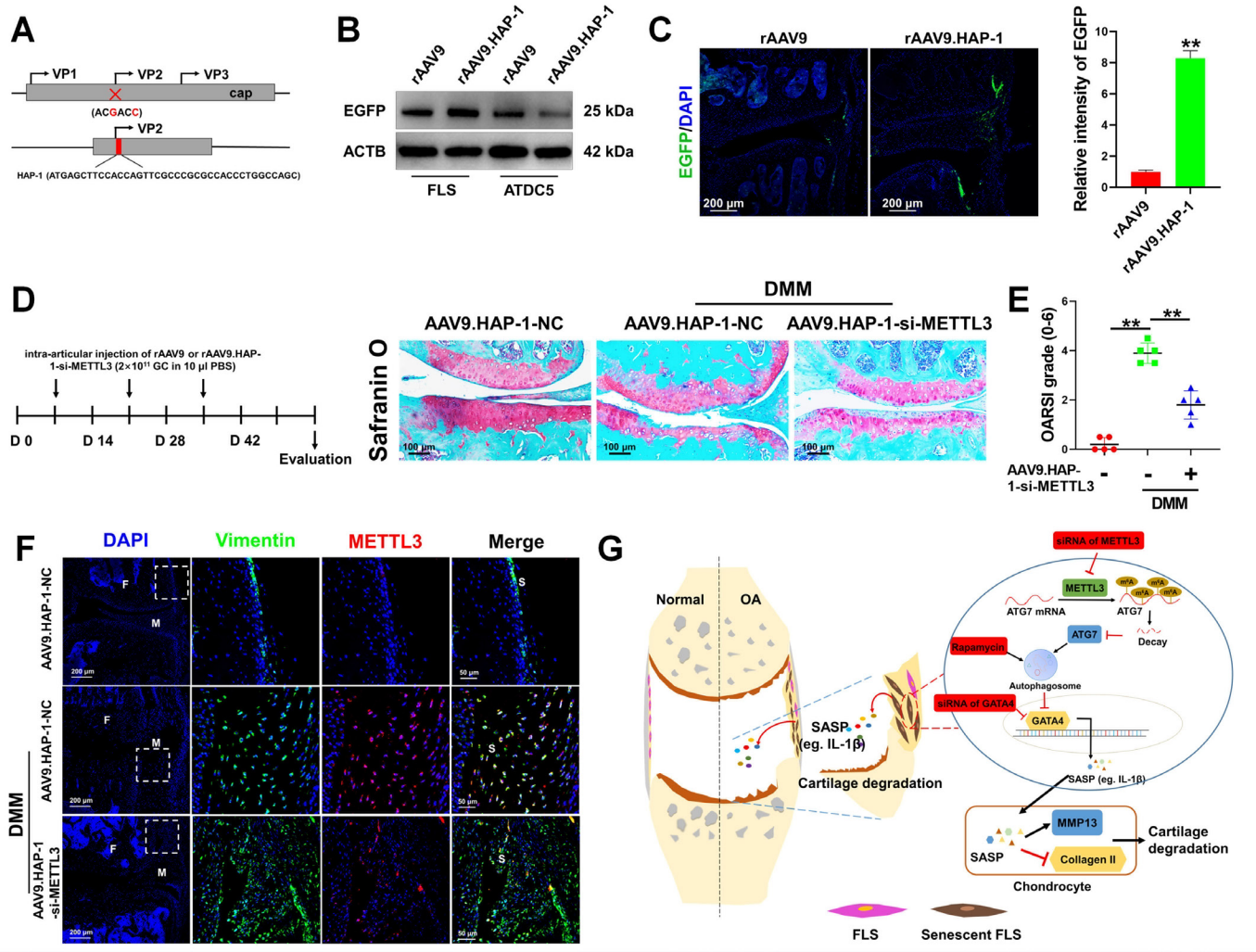

Figure 8 Synovium-targeted inhibition of METTL3 alleviates the pathological progression of DMM-induced OA. (A) Diagram of construct for rationally designed synovium-specific AAV capsids. The synovium-targeting peptide motif (HAP-1, red) was inserted into the AAV9 capsid at the N-terminus of AAV9-VP2. (B) Western blot analysis of EGFP expression level in FLS (passage 2) and ATDC5 cells infected with rAAV9 or rAAV9.HAP-1 at the concentration of 10^{11} GC/mL. (C) Confocal microscope analysis of the EGFP expression in the knee joints from mice after intra-articular injection with rAAV9 or rAAV9.HAP-1. n=3. **P<0.01. (D) The diagram for experimental design (left) and the representative photomicrographs of Safranin-O/fast green staining for knee joint sections from DMM mice after intra-articular injection with rAAV9.HAP-1-NC and rAAV9.HAP-1-si-METTL3, respectively. (E) The severity of OA-like phenotype was analysed by grading histological sections in medial femoral condyles and the medial tibial plateau using the OARSI score system. n=4 of each group. **P<0.01. (F) The representative images of double immunofluorescent staining of vimentin and METTL3 in the knee joint from DMM mice after intra-articular injection with rAAV9.HAP-1-NC or rAAV9.HAP-1-si-METTL3. (G) Schematic representation of mechanisms by which FLS senescence mediates OA development. All data were presented as the means±SEM. Paired t-test (C) and one-way analysis of variance with Dunnett's multiple comparisons (E) was used for statistical analysis. DMM, destabilisation of the medial meniscus; EGFP, enhanced green fluorescent protein; F, femur; FLS, fibroblast-like synoviocyte; IL, interleukin; M, meniscus; METTL3, methyltransferase-like 3; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International; S, synovium; SASP, senescence-associated secretory phenotype.

A common feature of these diseases is low-grade chronic systemic inflammation.[4 35] Emerging evidence demonstrated that increased production of proinflammatory and matrix-degrading molecules, also known as SASP, could be an important mechanism in OA, leading to a chronically inflamed microenvironment and complicating the implantation of stem cells to repair the joint injury.[36] Autophagy is a cellular homeostasis mechanism for the removal of dysfunctional organelles and macromolecules. Defective autophagy is involved in the pathogenesis of age-related diseases and promotes inflammation in multiple tissues.[37 38] During ageing, autophagy gradually decreases and induces senescence, which ultimately result in increased OA severity.[14] Augmentation of homeostasis mechanisms is discussed as a novel avenue to delay joint ageing and reduce OA risk. In our study, we found impaired autophagy in the OA synovium, and activation of autophagy effectively suppressed cellular senescence in FLSs. In addition, we found that GATA4, a novel senescence regulator,[8] was significantly elevated in OA-FLS. Upregulation of GATA4 dramatically induced the expression of SASP and markers of cellular senescence, and autophagy regulated FLS senescence in a GATA4-dependent manner. Our data suggest that autophagy regulates FLS senescence, and that modification of autophagy may provide a potential strategy for OA intervention.

Recent studies have shown that m^{6}A modification is widespread throughout the transcriptome, accounting for over 80%

of all RNA methylation modifications.[39] As one of the most common RNA modifications, m^{6}A modification plays critical roles in various physiological processes, including tumour invasion, cellular senescence and cell differentiation.[40–42] It has been reported that m^{6}A-modified mRNA transcripts are less stable due to YTHDF2-mediated mRNA decay,[31] and that the binding sites of YTHDF2 were usually enriched around stop codons and in 3′UTRs of mRNA.[22] In our study, we observed enhanced m^{6}A modification in OA-FLS, accompanied by increased expression of METTL3. Accumulating evidence has confirmed that increased METTL3 may result in enhanced m^{6}A levels,[43] and that elevated METTL3 could suppress autophagic flux by methylating the mRNA of transcription factor EB.[28] In senescent FLSs, we identified ATG7, which is required for the elongation of phagophores during autophagosome formation[30 44] and plays a key role in METTL3-mediated autophagy suppression. In addition, we demonstrated that METTL3-mediated m^{6}A modification of ATG7 is further regulated by YTHDF2. Using RIP-qPCR analysis, we validated the stronger YTHDF2 enrichment at ATG7 transcripts, demonstrating that ATG7 was the target gene of YTHDF2, but not YTHDF1. In vitro, loss of METTL3 in OA-FLS recovered autophagy and decreased the expression of GATA4. Targeted inhibition of METTL3 in the synovium via local intra-articular administration of rAAV9.HAP-1-si-METTL3 effectively decreased the number of senescent cells in the synovium and inhibited articular cartilage erosion.

In summary, the findings presented here expand our knowledge on the mechanisms, by which METTL3 plays a fundamental role in promoting cellular senescence and OA progression. METTL3 carries out these functions by regulating autophagy and affecting the stability of the ATG7 transcript in an m^{6}A-YTHDF2-dependent manner (figure 8G). Our study highlights the functional importance of the m^{6}A methylation machinery in autophagy, which provides insights into the underlying molecular mechanisms of METTL3 in regulating cellular senescence and the development of therapeutic strategies for the treatment of OA.

Contributors XC conducted the most assays and acquired and analysed the data. WG, XS and TS helped with animal housing and genotype identification. LZ, JD and YS participated in some experiments and collected human samples. QJ and BS conceived the project, designed the study, arranged the results and revised the manuscript. All authors approved the final version of the manuscript. BS accepted full responsibility for the finished work, had access to the data and controlled the decision to publish.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The animal use and the experimental protocols were reviewed and approved by the Animal Care Committee of Nanjing University in accordance with the Institutional Animal Care and Use Committee guidelines. Human study was approved by the ethical and protocol review committee of Nanjing Drum Tower Hospital.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Not applicable.

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CLINICAL SCIENCE

B-cell depletion with obinutuzumab for the treatment of proliferative lupus nephritis: a randomised, double-blind, placebo-controlled trial

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ABSTRACT

Objective Randomised trials of type I anti-CD20 antibodies rituximab and ocrelizumab failed to show benefit in proliferative lupus nephritis (LN). We compared obinutuzumab, a humanised type II anti-CD20 monoclonal antibody that induces potent B-cell depletion, with placebo for the treatment of LN in combination with standard therapies.

Methods Patients with LN receiving mycophenolate and corticosteroids were randomised to obinutuzumab 1000 mg or placebo on day 1 and weeks 2, 24 and 26, and followed through week 104. The primary endpoint was complete renal response (CRR) at week 52. Exploratory analyses through week 104 were conducted. The prespecified alpha level was 0.2.

Results A total of 125 patients were randomised and received blinded infusions. Achievement of CRR was greater with obinutuzumab at week 52 (primary endpoint, 22 (35%) vs 14 (23%) with placebo; percentage difference, 12% (95% CI −3.4% to 28%), p=0.115) and at week 104 (26 (41%) vs 14 (23%); percentage difference, 19% (95% CI 2.7% to 35%), p=0.026). Improvements in other renal response measures, serologies, estimated glomerular filtration rate and proteinuria were greater with obinutuzumab. Obinutuzumab was not associated with increases in serious adverse events, serious infections or deaths. Non-serious infusion-related reactions occurred more frequently with obinutuzumab.

Conclusions Improved renal responses through week 104 were observed in patients with LN who received obinutuzumab plus standard therapies compared with standard therapies alone. Obinutuzumab was well tolerated and no new safety signals were identified.

Trial registration number NCT02550652.

Key messages

What is already known about this subject?

- Although two randomised, placebo-controlled clinical trials of the type I anti-CD20 antibodies rituximab and ocrelizumab in patients with lupus nephritis failed to show a difference vs placebo in the primary endpoint of complete renal response, subsequent analyses suggested that the rapidity, depth and duration of peripheral B-cell depletion was associated with renal response.
- Obinutuzumab is a type II anti-CD20 antibody that results in greater B-cell depletion than rituximab; in a preclinical study, obinutuzumab was shown to be more effective than rituximab in a murine model of lupus nephritis.

What does this study add?

- In this randomised, placebo-controlled, phase 2 trial (NOBILITY), obinutuzumab was superior to placebo for the achievement of complete and overall renal responses at week 52 when added to mycophenolate and corticosteroids; improved renal responses with obinutuzumab compared with placebo continued through week 104.
- Obinutuzumab resulted in rapid and potent depletion of peripheral B cells without an increase in the incidence of serious adverse events, serious infections or death compared with placebo.

How might this impact on clinical practice or future developments?

- Compared with standard-of-care therapy alone, NOBILITY showed that obinutuzumab on a background of standard-of-care therapies improved renal responses through 104 weeks without increasing the frequency of serious adverse events. Based on the results from this study, the use of obinutuzumab in proliferative lupus nephritis is being further evaluated in a global phase 3 study (NCT04221477).

INTRODUCTION

Proliferative lupus nephritis (LN) is the most common severe organ-threatening manifestation of systemic lupus erythematosus (SLE). The goal of treatment is to preserve kidney function and avoid the need for kidney replacement therapy while minimising the toxicities of therapy.[1 2] The 15-year risk of patients with LN developing end-stage kidney disease (ESKD) is approximately 20%, with even greater risk occurring in class IV proliferative LN. This risk has not substantially lessened in the last 20 years despite the use of potent immunosuppressive therapies.[3 4]

B cells are recognised as key mediators of SLE pathogenesis.[5] However, randomised, placebo-controlled trials of the type I anti-CD20 antibodies rituximab and ocrelizumab failed to demonstrate

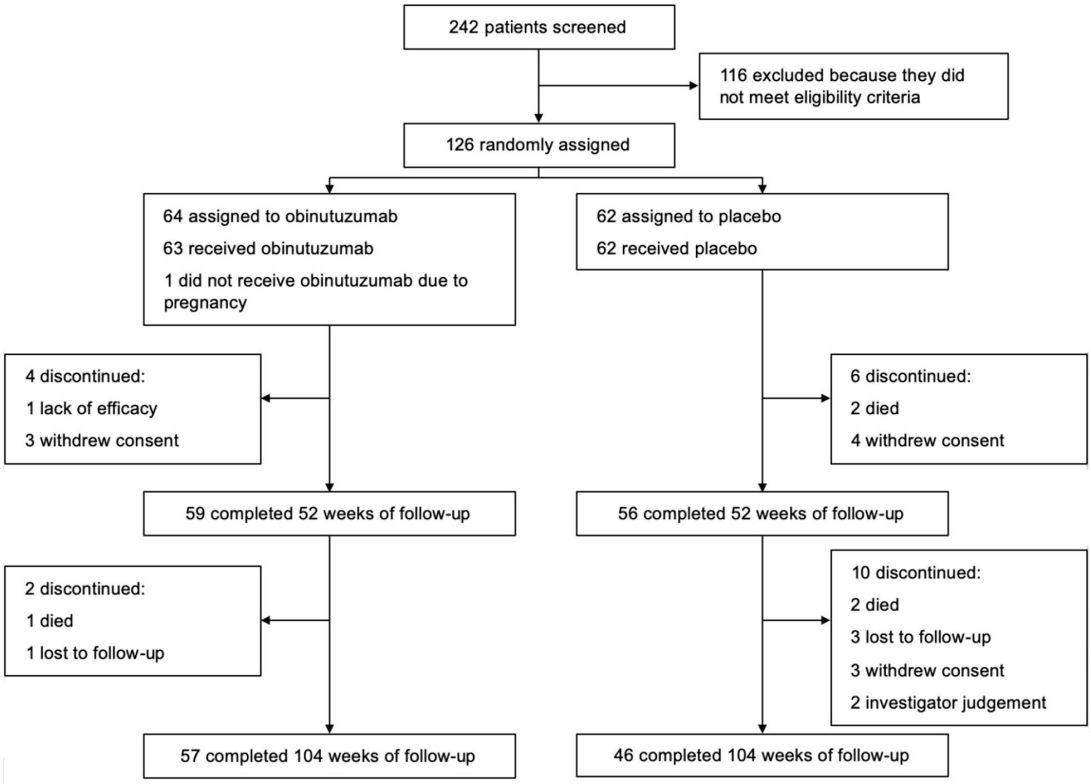

Figure 1 Patient flow diagram.

increases in rates of complete renal response (CRR) when added to standard-of-care immunosuppression.[6–8] Substantial variability in the degree of B-cell depletion has been observed following rituximab administration to patients with SLE, and the presence of residual B cells in peripheral blood after rituximab treatment has been associated with inferior clinical responses in SLE and LN.[9–12] Resistance to B-cell depletion by type I anti-CD20 antibodies in SLE may occur via Fcγ receptor IIB (FcγRIIB)–mediated internalisation of CD20, ineffective complement-dependent cytotoxicity, decreased engagement of effector cells due to natural killer cell defects or Fc receptor polymorphisms and acquired deficiencies in antibody-dependent cellular phagocytosis.[12–15]

Obinutuzumab is a humanised, type II anti-CD20 monoclonal antibody that has a distinct mode of binding to the CD20 antigen compared with type I anti-CD20 antibodies and is glycoengineered for greater affinity for the FcγRIII on effector cells. These properties promote greater antibody-dependent cellular cytotoxicity, superior direct B-cell killing, and, thus, less reliance on complement-dependent cytotoxicity than type I anti-CD20 antibodies.[16] Because obinutuzumab does not elicit CD20 redistribution to membrane-bound lipid rafts or activate FcγRIIB, it is associated with reduced CD20 internalisation compared with type I anti-CD20 antibodies.[14 17 18] Clinical superiority of obinutuzumab to rituximab for the treatment of chronic lymphocytic leukaemia and follicular lymphoma, when administered in combination with standard chemotherapy, has been demonstrated.[19 20] Obinutuzumab exhibited greater B-cell cytotoxicity and activation of natural killer cells than rituximab in SLE patient samples and was more effective than rituximab in the treatment of murine LN.[13 14 21]

The NOBILITY trial was conducted to test the hypothesis that enhanced B-cell depletion with obinutuzumab would increase the rate of CRR when added to background standard of care compared with standard of care alone. We report the results of a phase 2, multicentre, randomised, double-blind trial comparing obinutuzumab with placebo in patients with proliferative LN treated with mycophenolate and corticosteroids.

Table 1 Baseline characteristics and demographics

	Obinutuzumab (n=63)	Placebo (n=62)
Age—years	33.1±9.8	31.9±10.1
Female—no (%)	55 (87)	51 (82)
Region—no (%)		
Latin America and the Caribbean	38 (60)	47 (76)
Europe and Israel	18 (29)	7 (11)
USA	7 (11)	8 (13)
Hispanic or Latino ethnicity—no (%)	42 (67)	49 (79)
Race—no (%)		
White	28 (44)	26 (42)
American Indian or Alaska Native	11 (18)	17 (27)
Black or African American	6 (10)	5 (8)
Asian	3 (5)	2 (3)
Other or unknown	15 (24)	12 (20)
Prior history of lupus nephritis—no (%)	32 (51)	32 (52)
Class IV lupus nephritis—no (%)	40 (64)	35 (57)
Concomitant class V lupus nephritis—no (%)	20 (32)	17 (27)
Serum creatinine—mg/dL	0.87±0.34	0.80±0.33
eGFR—mL/min/1.73 m^2	102.0±30.6	102.1±32.9
UPCR—g/g	3.3±2.7	2.9±2.5
Anti-dsDNA Ab >30 IU/mL—no (%)	42 (67)	46 (74)
C3 <90 mg/dL—no (%)	43 (68)	37 (60)
C4 <16 mg/dL—no (%)	37 (59)	44 (71)

eGFR was calculated using the CKD-EPI creatinine equation.
Ab, antibody; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; ULN, upper limit of normal; UPCR, urine protein-to-creatinine ratio.

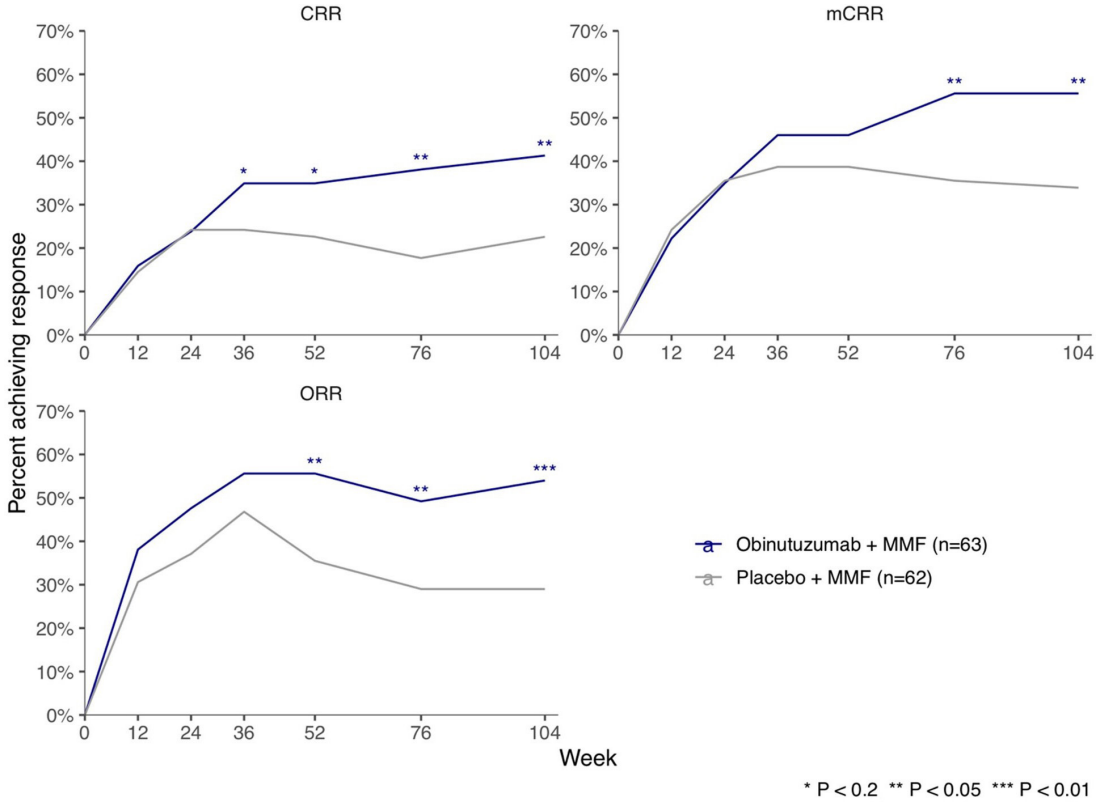

Figure 2 Renal responses over time. CRR, complete renal response; mCRR, modified CRR; MMF, mycophenolate mofetil; ORR, overall renal response.

METHODS

Study design

This multicentre, double-blind, phase 2, randomised, controlled trial was performed at 43 sites in North America, South America, Europe and Israel. This trial was executed in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. All patients provided informed consent. Patients and/or the public were not involved in the design, conduct, reporting, or dissemination plans of this research.

Patients

Eligible adults were aged 18–75 years, had SLE by American College of Rheumatology classification 1997 criteria,[22] kidney biopsy evidence of International Society of Nephrology/Renal Pathology Society 2003[23] class III or IV active or active/chronic LN within 6 months of screening (concomitant class V was permitted), urine protein-to-creatinine ratio (UPCR) >1 from a 24-hour urine collection, and estimated glomerular filtration rate (eGFR) of ≥30 mL/min/1.73 m^2. The full protocol is available in online supplemental file.

Randomisation and masking

Patients were randomly assigned (1:1) to receive either obinutuzumab 1000 mg or placebo infusions. Randomisation was performed using an interactive web response system and stratified by race (Afro-Caribbean/African American vs others) and region (USA vs non-USA). Randomisation codes were kept within the interactive web response system for patients and investigators to remain masked to treatment allocation. The sponsor was masked to treatment allocation up to the week 52 database lock.

Procedures

Obinutuzumab was administered as a blinded intravenous infusion of 1000 mg on day 1 and weeks 2, 24 and 26, after premedication with blinded methylprednisolone 80 mg intravenous to reduce the risk of infusion-related reactions. Patients randomly assigned to placebo received an intravenous placebo infusion on day 1 and weeks 2, 24 and 26 after infusion of placebo methylprednisolone. All patients received mycophenolate mofetil (MMF) (target dose 2–2.5 g/day or equivalent dose of mycophenolic acid). Protocol-mandated corticosteroid treatment included methylprednisolone (a total of 1000–3000 mg intravenous) and an oral corticosteroid regimen (initial prednisone dose: 0.5 mg/kg/day, maximum 60 mg/day, with taper to 7.5 mg/day by week 12). It was recommended that patients receive antimalarial medications, an ACE inhibitor or angiotensin receptor blocker, calcium and vitamin D at stable doses throughout the study. All patients were followed in a blinded fashion through week 104, and patients with persistent B-cell depletion were followed for safety and B-cell measurements thereafter.

Urinary protein excretion (measured by UPCR from a 24-hour urine collection and/or spot UPCR, preferably from a first morning void), serum creatinine, levels of autoantibodies and serum complement components were assessed at weeks 4, 12, 24, 36, 52, 76 and 104. Peripheral blood B-cells were measured at baseline and at weeks 2, 4, 12, 24, 52 and 104. Laboratory assessments were performed at a centralised laboratory. B cells were measured using a validated 6-colour, lyse/no-wash flow cytometry assay. Complement components were measured by immunonephelometry and anti-dsDNA titres by ELISA.

Outcome measures

The primary endpoint at week 52 was the proportion of patients who achieved CRR, a composite measure requiring UPCR <0.5,

Table 2 Primary and secondary endpoints at weeks 52 and 104

	Week 52				Week 104*			
	Obinutuzumab (n=63)	Placebo (n=62)	Difference (95% CI)	P value	Obinutuzumab (n=63)	Placebo (n=62)	Difference (95% CI)	P value
Primary endpoint								
CRR, n (%)	22 (35)	14 (23)	12 (–3.4 to 28)	0.115	26 (41)	14 (23)	19 (2.7 to 35)	0.026
Secondary endpoints								
mCRR, n (%)	29 (46)	24 (39)	7 (–10 to 25)	0.373	35 (56)	21 (34)	22 (5 to 39)	0.015
ORR (CRR or PRR), n (%)	35 (56)	22 (36)	20 (3.0 to 37)	0.025	34 (54)	18 (29)	25 (8.2 to 42)	0.005
Change in C3 from baseline, mean† (SE)	30 (3.4)	12 (3.5)	18 (8.0 to 27)	<0.001	29 (3.4)	11 (3.4)	19 (8.9 to 28)	<0.001
Change in C4 from baseline, mean† (SE)	9.7 (1.3)	0.8 (1.3)	8.8 (5.2 to 12)	<0.001	9.6 (1.3)	0.4 (1.3)	9.3 (5.7 to 13)	<0.001
Change in log anti-dsDNA titre from baseline, mean† (SE)	–0.91 (0.12)	–0.10 (0.12)	–0.81 (–1.1 to 0.48)	<0.001	–1.1 (0.13)	–0.05 (0.13)	–1.0 (–1.4 to 0.67)	<0.001
Renal response components								
UPCR <0.5, n (%)	33 (52)	24 (39)	14 (–3.6 to 31)	0.102	39 (62)	23 (37)	25 (7.8 to 42)	0.005
SCr ≤15% increase from baseline and ≤ULN	48 (76)	38 (61)	15 (–1.2 to 31)	0.080	45 (71)	32 (52)	20 (3.1 to 37)	0.019
Urinary RBCs <10/HPF without RBC casts	52 (83)	51 (82)	0.3 (–13 to 13)	0.987	49 (78)	41 (66)	12 (–4.0 to 27)	0.154
No rescue immunosuppression or early discontinuation	57 (91)	53 (86)	5 (–6.4 to 16)	0.414	51 (81)	38 (61)	20 (4.1 to 35)	0.012
CRR in prespecified subgroups								
Baseline proteinuria, n (%)								
UPCR <3 (n=73)	13 (38)	12 (31)	7.5 (–14 to 29)	0.468	16 (47)	12 (31)	16 (–5.9 to 39)	0.147
UPCR ≥3 (n=47)	8 (31)	2 (10)	21 (–0.5 to 43)	0.163	8 (31)	2 (10)	21 (–0.5 to 43)	0.098
Baseline biopsy class, n (%)								
Class III (n=31)	5 (36)	6 (35)	0.4 (–33 to 34)	0.952	3 (21)	7 (41)	–19 (–52 to 12)	0.338
Class IV (n=94)	17 (35)	8 (18)	17 (–0.5 to 34)	0.068	23 (47)	7 (16)	31 (14 to 49)	0.001
Baseline biopsy class, n (%)								
No class V (n=88)	17 (40)	9 (20)	20 (0.8 to 38)	0.054	17 (40)	10 (22)	17 (–1.7 to 36)	0.117
Class V (n=37)	5 (25)	5 (29)	–4.4 (–33 to 24)	0.825	9 (45)	4 (24)	22 (–8.2 to 51)	0.187
Post hoc endpoints								
UPCR <0.8, n (%)	41 (65)	31 (50)	15 (-2.1 to 32)	0.085	45 (71)	28 (45)	26 (9.6 to 43)	0.003

For all response analyses, non-response imputation was used after rescue immunosuppression or early discontinuation.
*Week 104 analyses were exploratory and not adjusted for multiplicity.
†Adjusted mean from analysis of covariance model adjusting baseline measurement and stratification factors race and region.
.CRR, complete renal response (which required UPCR <0; CRR, complete renal response; HPF, high-power field; mCRR, modified CRR; ORR, overall renal response; PRR, partial renal response; RBC, red blood cell; SCr, serum creatinine; UPCR, urine protein-to-creatinine ratio.

normal renal function (serum creatinine ≤ULN) without worsening of baseline serum creatinine by more than 15%, and inactive urinary sediment (<10 red blood cells (RBCs)/high-power field (HPF) without RBC casts). Patients who received rescue therapies such as cyclophosphamide, rituximab, tacrolimus or pulse-dose corticosteroids (equivalent to methylprednisolone 500 mg or greater) after baseline or who withdrew from the study prematurely were imputed as non-responders for all subsequent response endpoints.

Major secondary endpoints at week 52 were proportion of patients achieving a partial renal response (PRR), a composite measure requiring ≥50% reduction in UPCR from baseline to a value <1 (to <3 if baseline UPCR was ≥3), serum creatinine not increased >15% from baseline and urinary RBCs <10/HPF or ≤50% increase over the baseline value; proportion of patients achieving an overall renal response (ORR), which was met if CRR or PRR was achieved; proportion of patients achieving variations of the definition of CRR, including modified CRR (mCRR), a composite measure requiring UPCR <0.5 g/g and serum creatinine ≤ULN; changes in C3, C4 and anti-dsDNA antibody levels from baseline; and time to CRR and ORR. Additional prespecified endpoints included change in eGFR from baseline (as calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation) and achievement of renal responses at other time points. A post hoc endpoint, the proportion of patients achieving UPCR <0.8 g/g, was added based on the predictive value of this cut-off for long-term outcome.[24] Exploratory analyses were conducted to assess these measures at week 104.

Statistical analyses

Assuming a proportion of CRR responders at week 52 of 30% in the placebo group[6 7] and 50% in the obinutuzumab group (difference, 20%), 60 patients in each group were projected to yield 83% power to detect a significant difference using the Cochran-Mantel-Haenszel (CMH) test at a two-sided alpha of 0.2 for this proof-of-concept study. To control for type I error rate for the primary and secondary endpoints, hypothesis testing was conducted using a fixed sequence method, proceeding sequentially in a prespecified order starting from the primary endpoint and testing each endpoint after achieving statistical significance on the previous endpoint at an alpha of 0.2. Type I error rate was not controlled for exploratory analyses.

Efficacy analyses were done in a modified intention-to-treat population consisting of all randomised patients who had received ≥1 dose of study drug. Safety analyses were grouped according to the treatment received. Infusion-related reactions were defined as any adverse event that occurred during or within 24 hours after infusion of obinutuzumab or placebo and was judged to be related to the infusion. Descriptive statistics were used to evaluate safety.

Renal response endpoints and other categorical variables were evaluated by CMH test accounting for the stratification factors. Change from baseline endpoints were analysed by analysis of covariance model with baseline measurement and the stratification factors as covariates. All statistical analyses were performed using SAS, V.9.4. An independent data monitoring committee regularly reviewed unblinded interim data.

RESULTS

Patients

Patients were enrolled from November 2015 through December 2017. Final data collection was on 19 December 2019. Two hundred and forty-two patients were screened, of whom 125 were randomised and received placebo (n=62) or obinutuzumab (n=63) in addition to mycophenolate and corticosteroids in Latin America and the Caribbean (n=85), Europe and Israel (n=25) and the USA (n=15). The most common reason for screen failure was failure to meet the eligibility criteria. A total of 115 patients (92%) completed 52 weeks, and 103 patients (82%) completed 104 weeks of the protocol (figure 1).

Women comprised 85% of the study cohort, and the mean age was 33 years. Seventy-three per cent self-identified as Hispanic or Latino, and 43% were white. A total of 74% had class IV LN; the remainder had class III LN. Concomitant class V LN was present in 30%. Mean baseline values (±SD) were UPCR: 3.12±2.56; serum creatinine: 0.84±0.33 mg/dL; and eGFR: 102.0±31.7 mL/min/1.73 m^2. The patients' disease characteristics at baseline were similar between treatment groups (table 1).

Efficacy

A significantly greater proportion of patients in the obinutuzumab group achieved CRR than in the placebo group at week 52 (primary endpoint, 22 of 63 patients (35%) in the obinutuzumab group vs 14 of 62 patients (23%) in the placebo group; percentage difference, 12% (95% CI −3.4% to 28%), p=0.115) and week 104 (26 of 63 patients (41%) in the obinutuzumab group vs 14 of 62 patients (23%) in the placebo group; percentage difference, 19% (95% CI 2.7% to 35%), p=0.026) (table 2, figure 2). A significantly greater proportion of patients in the obinutuzumab group achieved CRR and ORR (CRR or PRR) at weeks 52, 76 and 104 and mCRR at weeks 76 and 104 (figure 2).

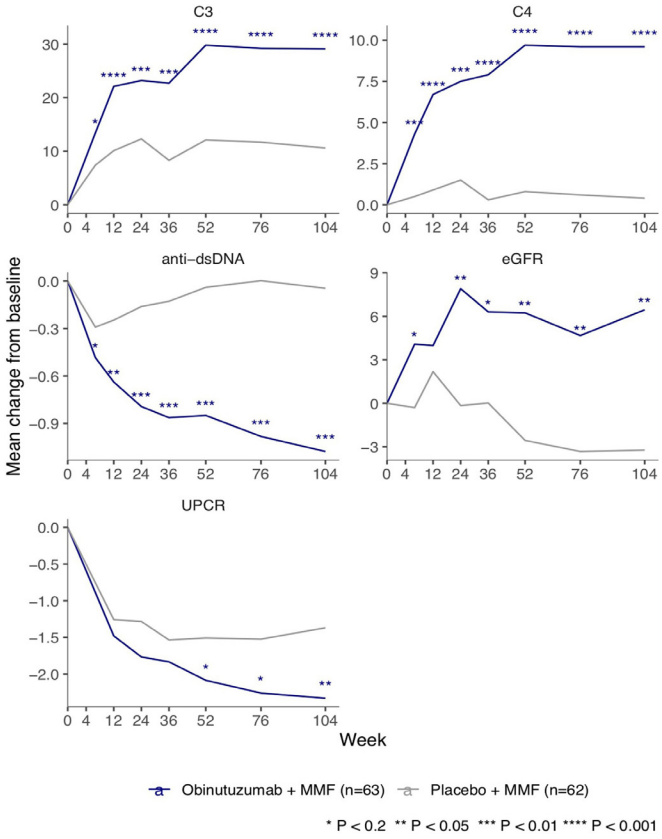

Figure 3 Change from baseline in laboratory parameters. Mean change from baseline was calculated with the last observation carried forward for missing data. If treatment failure occurred, the last measurement prior to treatment failure was used. eGFR, estimated glomerular filtration rate; MMF, mycophenolate mofetil; UPCR, urine protein-to-creatinine ratio.

In prespecified subgroup analyses, the benefit of obinutuzumab over placebo at 104 weeks was greatest among patients with baseline UPCR ≥3 and those with class IV (as compared with class III) LN on renal biopsy (table 2). While obinutuzumab was not associated with increased CRR among patients with concomitant class V LN at week 52, the treatment effects of obinutuzumab over placebo among patients with and without concomitant class V disease were similar at week 104. A post hoc analysis showed that, compared with placebo, obinutuzumab was associated with greater achievement of UPCR <0.8 at week 104 (45 of 63 patients (71%) in the obinutuzumab group vs 28 of 62 patients (45%) in the placebo group; percentage difference, 26% (95% CI 9.6% to 43%), p=0.003).

Compared with placebo, obinutuzumab resulted in greater improvements from baseline in C3, C4 and anti-dsDNA antibodies at weeks 4 through 104 and UPCR at weeks 52 through 104 (table 2, figure 3). Obinutuzumab also resulted in greater improvement in eGFR at week 4 and weeks 24 through 104 (adjusted mean difference, 9.7 mL/min/1.73 m^2 (95% CI 1.7 to 18), p=0.017). In the placebo group only, mean eGFR was decreased compared with baseline from week 24 through week 104 (figure 3).

By week 104, nine patients (14%) in the obinutuzumab group and 15 patients (24%) in the placebo group received one or more rescue therapies. Of these, six patients in the obinutuzumab group and 11 patients in the placebo group received rescue with cyclophosphamide or anti-CD20 therapy. The median initial (day 1) prednisone dose was 30 mg/day, and the median (IQR)

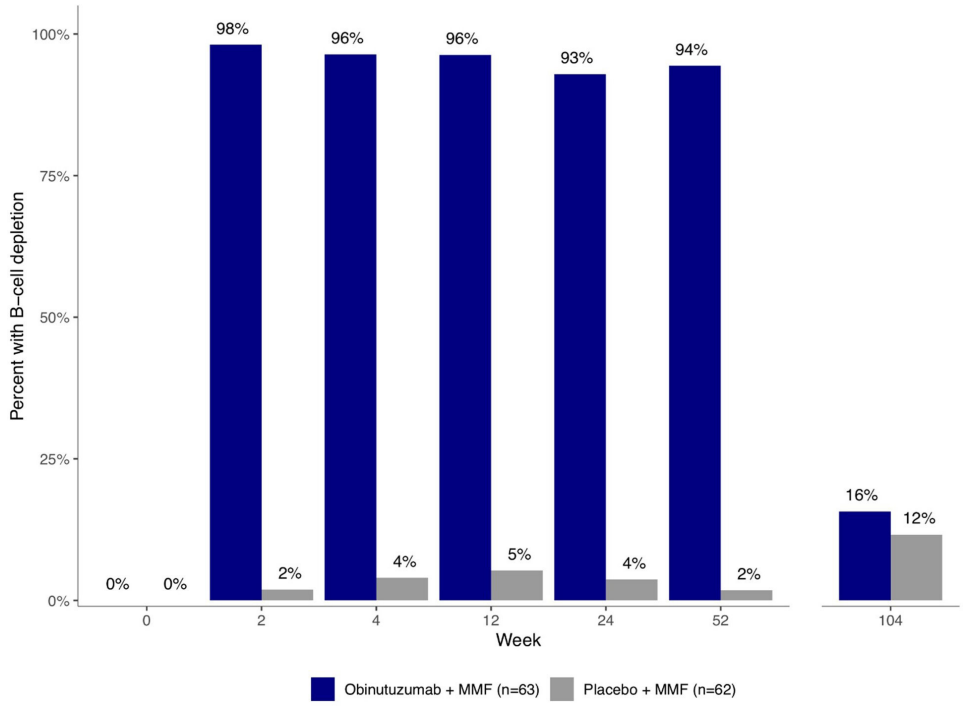

Figure 4 Proportions of patients with B-cell depletion over time. B-cell depletion is defined as an absolute CD19 count ≤5 cells/µL. MMF, mycophenolate mofetil.

cumulative corticosteroid exposure was 6561 (5938–7473) and 6672 (5785–7380) mg of prednisone equivalent in the obinutuzumab and placebo groups, respectively, inclusive of both oral and intravenous corticosteroid doses through week 104. Through week 104, the median MMF dose was 2.0 g/day in both groups. Thirty-eight patients (30%) required one or more MMF dose reductions due to adverse events, and nine patients (7%) received mycophenolic acid at some point during the trial.

Obinutuzumab resulted in rapid and sustained depletion of peripheral $CD19^+$ B cells to ≤5 cells/µL (figure 4). In the obinutuzumab group, 98% were depleted at week 2, after one infusion, and 94% were depleted at week 52. At the next measurement, week 104, similar rates of B-cell depletion were seen in the obinutuzumab and placebo groups (16% and 12%, respectively). Depletion of memory B cells, naïve B cells, and plasmablasts, and increases in serum BAFF, were also observed with obinutuzumab (online supplemental figure 1). Obinutuzumab was associated with a rapid and sustained decrease in IgM levels compared with placebo; at week 104, the proportions of patients with IgM below the lower limit of normal were 33% and 8% for obinutuzumab and placebo groups, respectively (online supplemental table 1). In contrast, the prevalence of low IgG decreased over time in both treatment groups (9% and 4% in the obinutuzumab and placebo groups, respectively, had IgG below the lower limit of normal at week 104). Titres of preformed antibodies against tetanus, rubella and mumps did not differ between treatment groups over time (data not shown).

Safety

One patient randomised to placebo inadvertently received obinutuzumab infusions during the first cycle and was included in the obinutuzumab group for safety analyses. Through week 104, 58 of 64 patients (91%) in the obinutuzumab group and 54 of 61 patients (89%) in the placebo group had at least one adverse event (table 3). Sixteen of 64 patients (25%) in the obinutuzumab group and 18 of 61 patients (30%) in the placebo group had at least one serious adverse event (table 3); 5 of 64 patients (8%) in the obinutuzumab group and 11 of 61 patients (18%) in the placebo group had at least one serious infection. The most frequent adverse events with obinutuzumab were urinary tract infections and bronchitis.

Infusion-related reactions, defined as any treatment-related adverse event that occurred within 24 hours of a blinded infusion, occurred in 10 of 64 patients (16%) in the obinutuzumab group and 6 of 61 patients (10%) in the placebo group. These events included headache, tachycardia, nausea and hypertension, and were most common with the first infusion. None were serious and all resolved with supportive care.

Five deaths occurred through week 104, one in the obinutuzumab group (gastrointestinal perforation) and four in the placebo group (gastrointestinal haemorrhage, refractory SLE, progressive multifocal leukoencephalopathy (PML), respiratory infection). The fatal case of PML occurred in a patient assigned to placebo who received cyclophosphamide rescue approximately 6 months prior to the diagnosis of PML.

DISCUSSION

Obinutuzumab was superior to placebo for the achievement of CRR and ORR in patients with proliferative LN when added to mycophenolate and corticosteroids. Greater improvements in anti-dsDNA antibodies, C3, C4, eGFR and proteinuria were also observed with obinutuzumab. Obinutuzumab resulted in rapid and potent depletion of peripheral $CD19^+$ B cells without an increase in the incidence of serious adverse events, serious infections or death compared with placebo. The treatment effect of obinutuzumab appeared to be greatest among patients with high levels of proteinuria at baseline and those with class IV LN. A similar treatment benefit was seen at week 104 among patients with and without concomitant class V disease.

We hypothesised that deeper and more durable depletion of B cells with obinutuzumab would result in superior clinical responses. NOBILITY used a similar design and patient population as the LUNAR trial, and comparison of $CD19^+$ B cell data suggests that

Table 3 Safety summary through week 104

	Obinutuzumab n=64	Placebo n=61
Any adverse event	58 (91)	54 (89)
Deaths	1 (2)	4 (7)
Serious adverse events	16 (25)	18 (30)
Serious infection adverse events	5 (8)	11 (18)
Infection adverse event	48 (75)	38 (62)
Most common adverse events*		
Urinary tract infection	15 (23)	13 (21)
Bronchitis	12 (19)	5 (8)
Herpes zoster	9 (15)	6 (10)
Abdominal pain	7 (11)	3 (5)
Infusion-related reaction	7 (11)	6 (10)
Nausea	6 (9)	3 (5)
Upper respiratory tract infection	6 (9)	5 (8)
Hypertension	6 (9)	3 (5)
Anaemia	5 (8)	4 (7)
Nasopharyngitis	5 (8)	6 (10)
Pharyngitis	5 (8)	4 (7)
Arthralgia	5 (8)	4 (7)
Headache	5 (8)	4 (7)
Conjunctivitis	4 (6)	2 (3)
Influenza	4 (6)	2 (3)
Neutropaenia	3 (5)	3 (5)
Diarrhoea	3 (5)	5 (8)
Peripheral oedema	3 (5)	3 (5)
Gastroenteritis	3 (5)	6 (10)
Sinusitis	3 (5)	0
Insomnia	3 (5)	4 (7)
Frequent urination	3 (5)	0
Cough	3 (5)	1 (2)
Infusion-related reaction†	10 (16)	6 (10)
Serious infusion related reaction	0	0
Progressive multifocal leukoencephalopathy	0	1 (2)

Data are n (%) of patients. One patient randomised to placebo inadvertently received obinutuzumab during the first cycle. This patient is included in the obinutuzumab group for safety analyses.
*Events that occurred in at least 5% of patients in the obinutuzumab group.
†Includes all treatment-related adverse events that occurred in the 24 hours from the start of blinded obinutuzumab or placebo infusions.

obinutuzumab results in more rapid, deep, and durable peripheral B-cell depletion than rituximab (online supplemental table 2).[6] The results from NOBILITY support prior reports correlating the degree and duration of B-cell depletion to clinical responses in LN.[9–11] Though all four doses of obinutuzumab were completed by 6 months, there was increasing clinical benefit through 24 months, implying that prolonged time may be required for healing of the kidney and achievement of CRR. Observational data from other studies indicate that short-term responses are predictive of improved long-term kidney outcomes, and, consistent with this, obinutuzumab was associated with greater preservation of eGFR over 2 years.[24 25] Taken together, these observations suggest the addition of obinutuzumab to standard therapy may more effectively prevent damage accrual and thus be more likely to preserve kidney function.

B-cell depletion with obinutuzumab was not associated with increases in serious adverse events at 2 years. Obinutuzumab was associated with an increased prevalence of low IgM, but not low IgG, compared with baseline, and was not associated with reductions in concentrations of pre-existing protective antibodies, a pattern consistent with the preservation of CD20-negative long-lived plasma cells. Similar to a previous study of obinutuzumab in patients with ESKD prior to kidney transplantation,[26] there were no severe infusion-related reactions or cases of severe thrombocytopaenia or neutropaenia, the most common severe toxicities seen with obinutuzumab in CLL and NHL (Gazyva US Prescribing Information; Gazyvaro EMA Summary of Product Characteristics). In CLL and NHL, patients with high levels of circulating malignant B cells appear to be at greatest risk for infusion-related reactions, which occur as a result of rapid lysis of B cells with release of proinflammatory cytokines.[27] Pretreatment quantitative and/or qualitative differences in circulating B cells therefore provide a potential mechanistic basis for the apparent lower incidence and severity of infusion-related reactions and cytopeanias with obinutuzumab in non-malignant conditions. In addition, high-dose background corticosteroids may have reduced the frequency and severity of infusion-related reactions as suggested by a non-randomised study in patients with CLL comparing prolonged corticosteroid premedication or standard premedication prior to the first obinutuzumab infusion.[28]

Approximately two-thirds of patients in this study were enrolled from Latin American countries, and similar to other recent LN studies, only a small proportion of our study population was of African ancestry.[29 30] This proof-of-concept study does not permit conclusions to be drawn regarding differences in treatment effect by region or ancestry. The use of blinded preinfusion methylprednisolone (active in the obinutuzumab group, placebo in the placebo group) prior to infusions at baseline and weeks 2, 24 and 26 could have biased towards a clinical benefit of obinutuzumab, although the durability of the observed treatment effect (through week 104) and the similarity of cumulative corticosteroid exposure between treatment groups argue against a substantial effect from this difference. Finally, this study had a limited sample size, a prespecified alpha level of 0.2, and no typeI error control for analyses after week 52; hence, these results require confirmation in a larger study.

Results from the present study indicate that B cells play a key role in LN pathogenesis and demonstrate that obinutuzumab contributes to improved clinical responses without increasing the frequency of serious safety events. Despite widespread use of immunosuppressive therapies for LN, the risk of ESKD has not been substantially reduced in recent decades.[4] This underscores the critical need for more efficacious and safer therapies for patients with proliferative LN. The use of obinutuzumab in proliferative LN is being further evaluated in a global phase 3 study (NCT04221477).

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TRANSLATIONAL SCIENCE

Integrative analysis of lung molecular signatures reveals key drivers of systemic sclerosis-associated interstitial lung disease

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ABSTRACT

Objectives Interstitial lung disease is a significant comorbidity and the leading cause of mortality in patients with systemic sclerosis. Transcriptomic data of systemic sclerosis-associated interstitial lung disease (SSc-ILD) were analysed to evaluate the salient molecular and cellular signatures in comparison with those in related pulmonary diseases and to identify the key driver genes and target molecules in the disease module.

Methods A transcriptomic dataset of lung tissues from patients with SSc-ILD (n=52), idiopathic pulmonary fibrosis (IPF) (n=549), non-specific interstitial pneumonia (n=49) and pulmonary arterial hypertension (n=81) and from normal healthy controls (n=331) was subjected to filtration of differentially expressed genes, functional enrichment analysis, network-based key driver analysis and kernel-based diffusion scoring. The association of enriched pathways with clinical parameters was evaluated in patients with SSc-ILD.

Results SSc-ILD shared key pathogenic pathways with other fibrosing pulmonary diseases but was distinguishable in some pathological processes. SSc-ILD showed general similarity with IPF in molecular and cellular signatures but stronger signals for myofibroblasts, which in SSc-ILD were in a senescent and apoptosis-resistant state. The p53 signalling pathway was the most enriched signature in lung tissues and lung fibroblasts of SSc-ILD, and was significantly correlated with carbon monoxide diffusing capacity of lung, cellular senescence and apoptosis. *EEF2*, *EFF2K*, *PHKG2*, *VCAM1*, *PRKACB*, *ITGA4*, *CDK1*, *CDK2*, *FN1* and *HDAC1* were key regulators with high diffusion scores in the disease module.

Conclusions Integrative transcriptomic analysis of lung tissues revealed key signatures of fibrosis in SSc-ILD. A network-based Bayesian approach provides deep insights into key regulatory genes and molecular targets applicable to treating SSc-ILD.

Key messages

What is already known about this subject?

- Interstitial lung disease is a significant comorbidity and the leading cause of mortality in patients with systemic sclerosis, but effective treatment remains unaccomplished.
- Integrative systems analysis could provide a novel insight into the mechanistic features and therapeutic targets in complex diseases.

What does this study add?

- The p53 signalling pathway was the most enriched pathway in lung tissues and lung fibroblasts of systemic sclerosis-associated interstitial lung disease (SSc-ILD) and significantly correlated with carbon monoxide diffusing capacity of the lung, cellular senescence and apoptosis.
- Key driver genes playing critical roles in lung fibrosis were discovered and had high capacity to control the disease module of SSc-ILD but were not direct targets of current treatment modalities.

How might this impact on clinical practice or future developments?

- Identification of the key pathways and druggable molecules could be leveraged to provide novel insights into promising drug discovery for SSc-ILD.

INTRODUCTION

Systemic sclerosis (SSc) is a chronic autoimmune disease that is characterised by the distinctive pathogenic combination of microvascular damage, dysregulated autoimmunity, and progressive fibrosis of the skin and multiple internal organs.[1 2] Lung fibrosis, also known as interstitial lung disease (ILD), occurs in over half of patients with SSc and is the leading cause of death responsible for one-third of SSc-related mortality.[3 4]

Systemic sclerosis-associated interstitial lung disease (SSc-ILD) shows great diversity and heterogeneity regarding the extent and types of lung parenchymal abnormalities, as well as the clinical progression rate and outcome. The mere presence of ILD at the diagnosis of SSc appears to affect outcome, and some patients experience gradual progressive respiratory failure or rapid deterioration of respiratory function through acute exacerbations.[4] However, not all patients with ILD progressed, and a significant proportion survived for over 10 years.[4] Although SSc-ILD shares many pathogenic features with idiopathic pulmonary fibrosis (IPF), the outcome of SSc-ILD is better than that of IPF and dependent on other clinical factors (male sex, active smoking and older age at presentation) and the presence of extrapulmonary manifestations (arthritis, digital ulcers, pulmonary hypertension, progressive skin fibrosis, renal disease and myocardial fibrosis).[3 5 6] Moreover, a dysregulated immunological response

is more strongly implicated in SSc-ILD than in IPF, and immunosuppressive therapy, such as cyclophosphamide, azathioprine or mycophenolate mofetil, was shown to be somewhat beneficial for slowing the progression in SSc-ILD but not in IPF.[3 7]

In SSc-ILD, many therapeutic approaches against immune or inflammatory responses have been attempted, but they failed to halt the disease or had only a marginal effect.[5 6 8] Although some drugs have a therapeutic benefit in a subset of patients with SSc-ILD, the treatment effect has been attenuated in 2 years.[5 6 8] In a recent study, the antifibrotic agent nintedanib has been shown to be effective at reducing the rate of decline in forced vital capacity (FVC), especially in combination with mycophenolate.[9 10] However, patient-reported outcomes have not improved, and the long-term effect and toxicity-related tolerability have remained unresolved.[9] These unsatisfactory results may be attributable partly to the suboptimal targeting by drugs, in addition to the heterogeneity of SSc-ILD.

Integrative systems analysis has yielded comprehensive disease-specific functional networks that model the perturbed interactions of genes and molecules in a disease module[11 12] and provide novel insights into the prioritisation of targetable disease-associated genes and drug repurposing.[13 14] These approaches have been successfully applied to the molecular stratification and identification of key drivers in some pulmonary or fibrotic diseases.[12 15–18] SSc-ILD has clinical and mechanistic features in common with other pulmonary diseases such as IPF, non-specific interstitial pneumonia (NSIP) and pulmonary arterial hypertension (PAH), but is a distinct identity with a different clinical course and treatment response. In the present study, we collected transcriptomic datasets of lung tissues from patients with SSc-ILD, IPF, NSIP or PAH from public data repositories and made a compendium of these diseases. We delineated the cellular and molecular characteristics of SSc-ILD compared with those of IPF, NSIP and PAH and investigated their association with pulmonary functional parameters. Finally, we applied an integrative network-based approach and Bayesian inference to identify key driver genes (KDGs) and evaluated the impact of current and investigational drugs in the context of the disease module.

METHODS

Overview of data processing and analysis

We searched for publications on the lung gene signatures of patients with SSc-ILD, IPF, NSIP and PAH in Google Scholar and PubMed and obtained relevant datasets that were available from publicly accessible academic repositories. We obtained 18 transcriptomic datasets of lung tissues from patients with SSc-ILD (n=52), IPF (n=549), NSIP (n=49) and PAH (n=81), as well as normal healthy controls (n=331) (online supplemental tables S1 and S2). After normalising the vectors for the matrix using quantile normalisation and correcting the batch effect, we performed differential expression analysis, functional and gene set enrichment analysis (GSEA), and protein–protein interaction (PPI) network analysis. To deconvolute the enrichment of a specific pathway or cell subset in the tissue, we used a single-sample version of GSEA and a digital sorting algorithm. Finally, to determine the key regulatory genes and their impact in the disease module, we employed Bayesian network-based key driver analysis (KDA) and kernel-based diffusion scoring. The methods are described in detail in the online supplemental methods.

RESULTS

Differentially expressed genes (DEGs) and their network and enriched pathways

Upregulated DEGs were identified from the gene expression profiles of patients with SSc-ILD, IPF, NSIP or PAH compared with normal healthy controls (online supplemental file 1). A total of 990 DEGs were acquired in SSc-ILD, and 3187, 1042 and 775 were obtained in IPF, NSIP and PAH, respectively. The four disease groups shared 395 DEGs (figure 1A). The PPI networks constructed from the SSc-ILD DEGs identified 1473 interactions and 579 genes had links to more than one gene (figure 1B). The network included eight diagnostic or prognostic biomarkers (*C3*, *CCL18*, *CHI3L1*, *GDF15*, *MMP7*, *MMP13*, *TIMP1* and *VCAM1*).[3] The largest connected component (LCC), also known as the giant component, is the connected component of a network that contains a significant proportion of all nodes in the network.[19 20] The LCC is typically the most complex part of a network and represents the core that sustains the whole network. The LCC consisted of 558 genes in the SSc-ILD PPI network. Centrality analysis detected 55 hub molecules, and *VCAM1* was the only biomarker with a hub position (figure 1B).

We performed functional enrichment analysis for the SSc-ILD DEGs and obtained 171 Gene Ontology biological process terms. Twenty-three of these terms were shared by the four disease groups and were mainly related to antigen processing and presentation, extracellular matrix (ECM) organisation, apoptosis, and mitogen-activated protein kinase (MAPK) and extracellular-signal-regulated kinase (ERK) cascades (figure 1C and online supplemental file 2). Key enriched Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways were also identified, namely, p53 signalling pathway ($p=3.76\times10^{-7}$), complement and coagulation cascades ($p=6.49\times10^{-4}$), cellular senescence (p=0.0328), interleukin (IL)-17 signalling pathway (p=0.0320) and PI3K–Akt signalling pathway (p=0.0252) (figure 1D and online supplemental file 2).

The p53 signalling pathway, which was the most enriched and significant pathway in the DEG-driven functional enrichment analysis ($p=3.76\times10^{-7}$, OR=6.998), was confirmed by GSEA using KEGG gene sets (normalised enrichment score=1.6250, nominal p value=0.0140, false discovery rate (FDR) q-value=0.1007) (figure 1E and online supplemental file 3). The p53 signalling pathway contains leading-edge genes such as *IGF1*, *CCNB1*, *CCNB2*, *CDK1*, *CCND2* and *CHEK2*.

Cell subset and pathway-driven characterisation of SSc-ILD

To better understand the differential enrichment of biological pathways, we curated the pathways involved in lung fibrosis using information obtained from the literature, performed GSEA and compared the enrichment scores (figure 2A). Collagen formation, signalling by PDGF, p53 signalling pathway, ECM organisation and ECM–receptor interaction were markedly activated among the SSc-ILD, IPF, NSIP and PAH groups. The oestrogen signalling pathway, transforming growth factor beta (TGF-β) signalling pathway and Wnt signalling pathway were also activated in SSc-ILD, IPF and PAH, but not in NSIP. The PI3K–Akt signalling pathway was more enriched in IPF and PAH. Together, these findings show that SSc-ILD was similar to IPF, but the PI3K–Akt and IL-17 signalling pathways were more prominent in IPF.

Next, we compared the cell subset signatures across the four disease groups (figure 2B). Most cell subpopulations, with the exception of ciliated cells, were present in different proportions in these groups. Type two alveolar epithelial cells, basal cells,

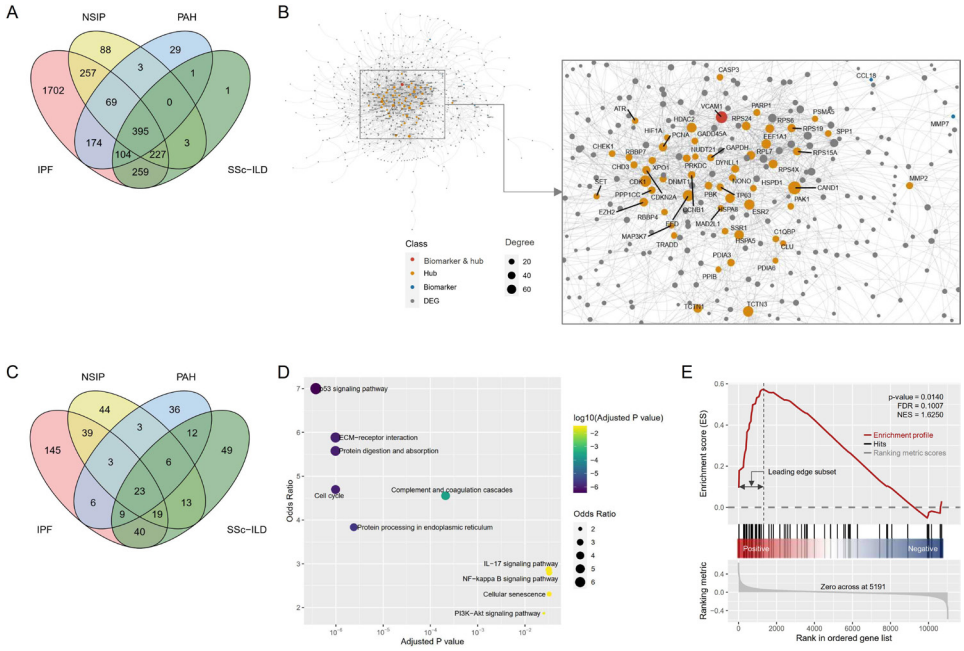

Figure 1 DEGs and the enriched BPs. (A) Venn diagram illustrating the numbers of shared and distinct DEGs among the four disease groups (IPF, NSIP, PAH and SSc-ILD). (B) Protein–protein interaction network of the SSc-ILD DEGs, which consisted of 990 nodes and 1473 interactions. The SSc-ILD DEGs were obtained by comparison with normal control samples and the network was constructed based on the human interactome database (http://www.interactome-atlas.org/). Node size was proportional to the graph degree and nodes were coloured by the category (biomarker, hub and simple DEG). The magnified core of the network is displayed in the box on the right. (C) Venn diagram illustrating the numbers of shared and distinct GO BP terms among the four disease groups (IPF, NSIP, PAH and SSc-ILD). (D) Functional enrichment analysis of the KEGG pathways associated with the SSc-ILD DEGs. The result was plotted in two ranking metrics: adjusted p value (x-axis) by OR (y-axis). Point size and colour represent the OR and adjusted p values, respectively. (E) GSEA plot of the p53 signalling pathway. Details are described in the GSEA user guide (https://www.gsea-msigdb.org/gsea). BP, biological process; DEG, differentially expressed gene; ECM, extracellular matrix; FDR, false discovery rate; GO, Gene Ontology; GSEA, gene set enrichment analysis; IL, interleukin; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; KEGG, Kyoto Encyclopaedia of Genes and Genomes; NES, Normalised Enrichment Score; NSIP, non-specific interstitial pneumonia; PAH, pulmonary arterial hypertension; SSc-ILD, systemic sclerosis-associated interstitial lung disease.

dendritic cells, macrophages, monocytes, mast cells and T/NKT cells were more enriched in SSc-ILD and IPF, whereas type 1 alveolar epithelial cells, B cells, club cells and plasma cells were more enriched in NSIP and PAH. Fibroblasts and endothelial cells were most enriched in PAH. Furthermore, the cell composition of SSc-ILD was similar to that of IPF, but the signatures of myofibroblasts, an active form of fibroblast, were stronger in SSc-ILD than in IPF, whereas the signatures of fibrotic macrophages did not differ between the two groups (figure 2C).

Molecular characterisation of lung fibroblasts of SSc

To confirm the enriched molecular features in lung fibroblasts from patients with SSc-ILD, we imported a microarray dataset of fibroblasts isolated from lung tissues of 8 patients with SSc-ILD and 10 normal healthy controls (GSE40839).[21] Among the 1030 DEGs identified in the SSc-ILD fibroblasts, the p53 signalling pathway was the most significantly enriched process ($p=3.239\times10^{-3}$, OR=6.010), consistent with the result from lung tissues of SSc-ILD (online supplemental file 4). Seven leading-edge genes (*IGF1*, *CCNB1*, *CCNB2*, *CDK1*, *RRM2*, *CHEK1* and *IGFBP3*) were common to the SSc-ILD lung tissues and fibroblasts (online supplemental figure S3). The expression of antiapoptotic genes did not differ between the SSc-ILD and control fibroblasts, but the expression of the proapoptotic genes *BID* and *PMAIP1* (also known as *NOXA*) was significantly suppressed in the SSc-ILD fibroblasts, indicating a lower propensity for apoptosis (figure 3A). Furthermore, two cellular senescence markers, *BHLHE40* (also known as *DEC1*) and *PPP1CA*, and three representative myofibroblasts markers, *ACTA2* (also known as *α-SMA*), *CDH2* (N-cadherin) and *FN1* (fibronectin), were also significantly upregulated in the SSc-ILD fibroblasts compared with the controls (figure 3B,C). These findings demonstrate that lung fibroblasts from patients with SSc-ILD had profibrotic features by avoiding apoptosis and becoming senescent.[22]

Correlation of the enriched pathways with pulmonary functional parameters

Decline in FVC and diffusing capacity of the lung for carbon monoxide (D_{LCO}) are the two key measures to predict the progression in patients with SSc-ILD.[23] We examined the enriched pathways that correlated with pulmonary functional indices (online supplemental figure S4). D_{LCO} had an inverse correlation with the p53 signalling pathway, which was the most significant ($\gamma=-0.7441$, $p=2.588\times10^{-4}$) (figure 4A), and positive correlations with the Wnt signalling pathway ($\gamma=0.6604$, $p=0.0020$), calcium signalling pathway ($\gamma=0.6430$, $p=0.0029$) and Fc-epsilon RI signalling pathway ($\gamma=0.6360$, $p=0.0034$). FVC was negatively correlated with collagen formation ($\gamma=-0.5594$, $p=0.0055$), IL-17 signalling pathway ($\gamma=-0.4910$, $p=0.0173$), oestrogen signalling pathway ($\gamma=-0.4469$, $p=0.0324$) and p53 signalling pathway ($\gamma=-0.4143$, $p=0.0493$) (figure 4A), and positively correlated with bone morphogenetic protein (BMP) signalling ($\gamma=0.5215$, $p=0.0107$).

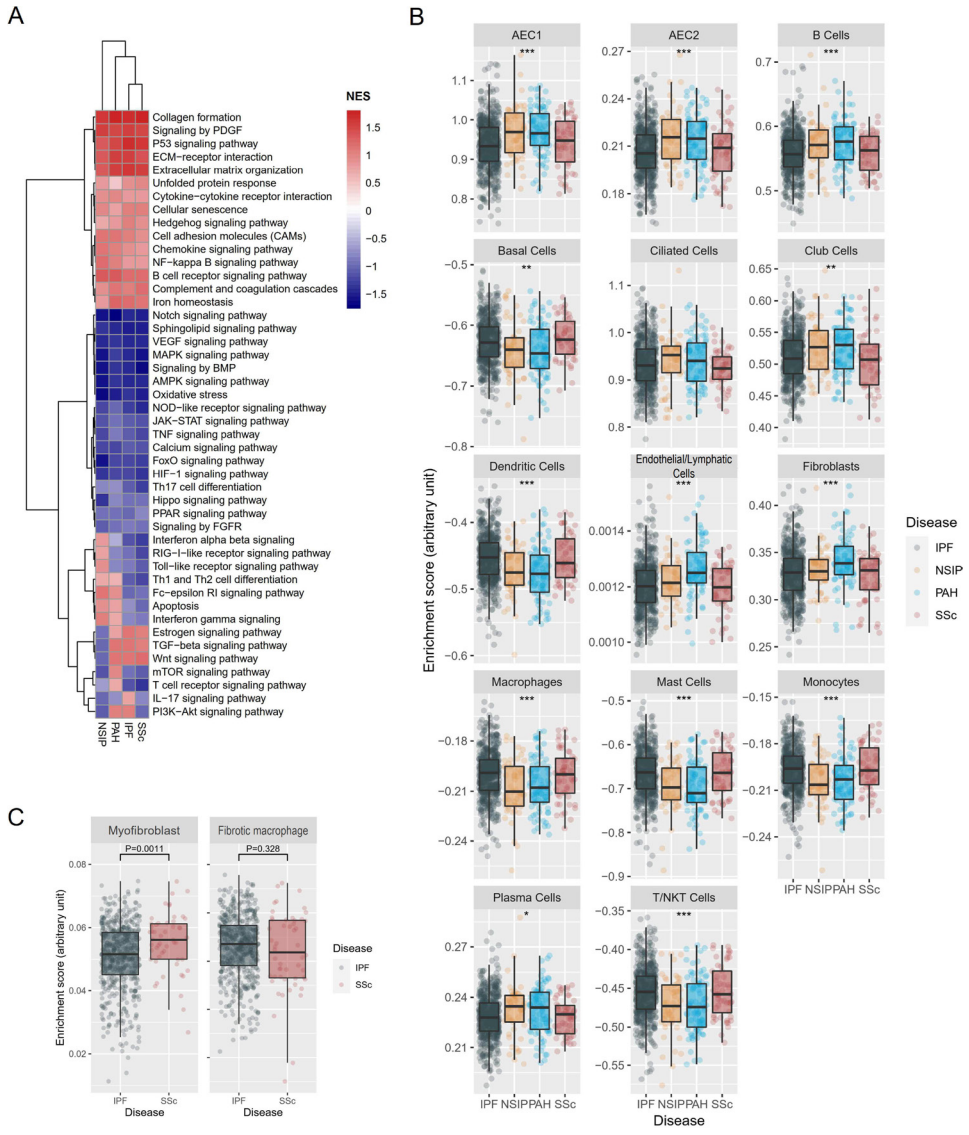

Figure 2 Comparison of cellular and molecular signatures across four disease groups. (A) NES of the disease-related pathways. The dendrogram was produced by agglomerative hierarchical clustering using Euclidean distance and Ward's method. (B) Enrichment scores of lung cell subsets. (C) Enrichment scores of myofibroblasts and fibrogenic macrophages in IPF and SSc-ILD. *P<0.05, **P<0.01, ***P<0.001. ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; NES, NES, Normalised Enrichment Score; NSIP, non-specific interstitial pneumonia; PAH, pulmonary arterial hypertension; SSc, systemic sclerosis; SSc-ILD, systemic sclerosis-associated interstitial lung disease.

Cellular senescence and resistance of myofibroblasts to apoptosis are the pathological hallmarks of progressive pulmonary fibrosis.[22 24] The enrichment score of the p53 signalling pathway was positively correlated with those of cellular senescence and apoptosis ($\gamma=0.6923$, $p=1.306\times10^{-8}$ and $\gamma=0.5896$, $p=4.239\times10^{-6}$, respectively) (figure 4B).

Identification of key drivers of the disease module

Elucidating the connectivity structure within the disease module can lead to the identification of KDGs that are predicted to perturb the regulatory state of the module and would be particularly suitable to prioritise as causative of disease development and progression.[25–28] We constructed a Bayesian network by projecting the DEGs onto the human interactome and employed KDA, an algorithm that mathematically identifies causal modulators of the regulatory state of functionally relevant gene groups to predict genes that modulate the regulatory state of the SSc-ILD core module. We identified 71 KDGs, of which 12 were DEGs (figure 5A,B). *FN1* (FDR=3.15×10^{-7}, fold change=2.26) and *CDK2* (FDR=3.96×10^{-7}, fold change=1.96) were the top-ranked KDGs, followed by *PAN2*, *MMP2* and *JUN*. The diffusion kernel gives a ranking of a gene using the sum of a global distance measure and diffusion rate from all seed genes of a disease.[29] To further determine the potential impact of KDGs on the core disease module, we calculated the network-based diffusion score for the KDGs with membership of the pulmonary fibrosis-associated pathways using a network diffusion algorithm[29] (figure 5C). Eukaryotic translation elongation factor 2 (*EEF2*) (diffusion score=19.23) was top ranked by diffusion score, followed by *VCAM1* (18.56), *PHKG2* (18.37), *EEF2K* (17.93), *ITGA4* (16.67) and *PRKACB* (16.06). *CDK1* (13.19), *CDK2* (13.08), *CDKN1A* (13.67) and *FN1* (14.13) were also ranked with high priority.

We collected information on the target molecules of eight current treatment drugs (prednisolone, cyclophosphamide, azathioprine, mycophenolate mofetil, rituximab, tocilizumab, nintedanib and pirfenidone) and five investigational drugs (abituzumab, bortezomib, pomalidomide, romikimab and UCN-01)

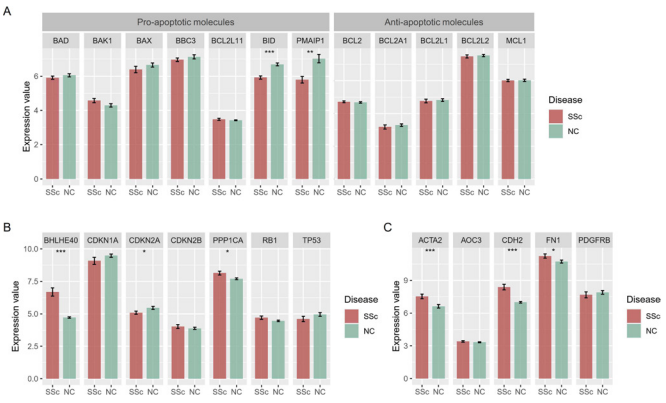

Figure 3 Molecular signatures of lung fibroblasts in SSc-ILD. (A) Expression levels of apoptotic genes between SSc-ILD and NCs. Synonyms: BCL2L11=BIM, BBC3=PUMA, PMAIP1=NOXA, BCL2L1=BCL XL, BCL2L2=BCL W, BCL2A1=BFL-1. (B) Expression levels of cellular senescence genes between SSc-ILD and NC. Synonyms: CDKN2A=p16, CDKN2B=p15, CDKN1A=p21, RB1=pRB, BHLHE40=DEC1. (C) Expression levels of myofibroblast markers between SSc-ILD and NC. Synonyms: ACTA2=α-SMA, CDH2=N-cadherin. *P<0.01, **P<0.01, ***P<0.001. ILD, interstitial lung disease; NC, normal healthy control; SSc, systemic sclerosis; SSc-ILD, systemic sclerosis-associated interstitial lung disease.

from publicly available databases and the literature,[6 30–32] and compared their diffusion scores (figure 5D). None of the current treatment drugs directly disturbed the KDGs. Target molecules of bortezomib, nintedanib and prednisone scored better than the target molecules of the other drugs but were not so comparable with KDGs. UCN-01 (7-hydroxystaurosporine), an ATP competitive inhibitor, targeted the cyclin-dependent kinases (CDKs) with high diffusion scores, and the KDGs *CDK1* and *CDK2* are key elements of the p53 signalling pathway and cellular senescence. Integrin subunit α4 (*ITGA4*) is one of the KDGs, but abituzumab was shown to block integrin subunit αV (ITGAV).

To confirm the significance of targetable molecules at the cellular level, we performed KDA using the SSc-ILD fibroblast-driven DEGs and identified 101 KDGs (online supplemental figure S5), which were subjected to network-based diffusion scoring. Members of the tubulin family, *PHKG2*, *VCAM1* and *PGK1*, were highly ranked, and *CDK2*, *FN1* and *ITGA4* were also ranked highly (online supplemental figure S6). Among the targeted molecules, 22 with high diffusion scores in both lung tissue and fibroblasts were shared KDGs, including *CDK2*, *VCAM1*, *FN1* and *ITGA4* (online supplemental figure S7).

DISCUSSION

In the present study, we built a comprehensive transcriptomic compendium of SSc-ILD lung tissue and carried out integrative analysis to better understand the cellular and molecular expression patterns of SSc-ILD compared with those in related pulmonary diseases such as IPF, NSIP and PAH. The p53 signalling pathway was not only the most significantly enriched pathway but also closely correlated with the pulmonary functional indices and intimately associated with profibrotic phenotypes such as cellular senescence and apoptosis resistance, at both tissue and cellular levels. Finally, we suggested the key drivers and molecules that may serve as promising targets for therapeutic intervention based on network-based Bayesian inference.

Biological processes in the human body are orchestrated by cooperative interactions of multiple genes, proteins and chemical compounds. Complex disease trajectories are rarely a result of deviations in a single gene or molecule, but by integrated cellular processes of perturbed pathways or disease modules formed by abnormally activated genes and their linked neighbours.[11] Even a single disease class can have a different phenotype or clinical outcome, depending on the cellular and molecular backgrounds.[33–35] SSc-ILD shares some clinical features and imaging findings with IPF, NSIP and PAH but responds differently even to the same treatment. A small but definite benefit from immunosuppressive therapy was identified in SSc-ILD but not in IPF.[5] The response of patients with SSc-associated PAH was less efficacious than that of patients with other forms of PAH.[5] We identified common and distinct pathways involved in the four disease groups. The composition of key cell populations also differed among the diseases. SSc-ILD had high similarity to IPF but low activity of the IL-17 and PI3K–Akt signalling pathways. However, the myofibroblast signature in SSc-ILD was stronger than that in IPF, which might be attributable, at least in part, to the systemic activation of fibroblasts under autoimmunity in SSc.

Myofibroblasts are active fibrosing cells that are ultimately responsible for the excessive synthesis, deposition and remodelling of ECM proteins in fibrosis.[22] We confirmed that SSc-ILD fibroblasts were profibrotic and senescent, in addition to having a low apoptotic state. However, growing evidence suggests that myofibroblasts are actually primed for apoptosis because of the concomitant activation of the cell death signalling pathway,[22] which is in line with activation of the p53 signalling pathway as our results showed. We found that the p53 signalling pathway was the most significantly enriched pathway in SSc-ILD, and its enrichment score was closely correlated with cellular senescence and apoptosis in lung tissue of patients with SSc-ILD. p53 and its linked neighbours are the key mediators in both cellular

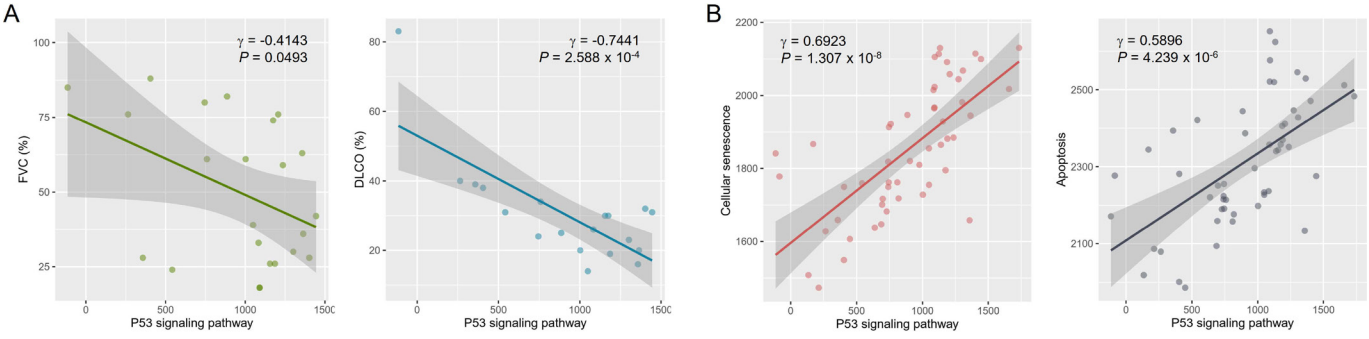

Figure 4 (A) Correlation of p53 signalling pathway with FVC and D_{LCO}. (B) Correlation of p53 signalling pathway with cellular senescence and apoptosis. Correlation analysis between two variables was carried out using Pearson's method. D_{LCO}, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity.

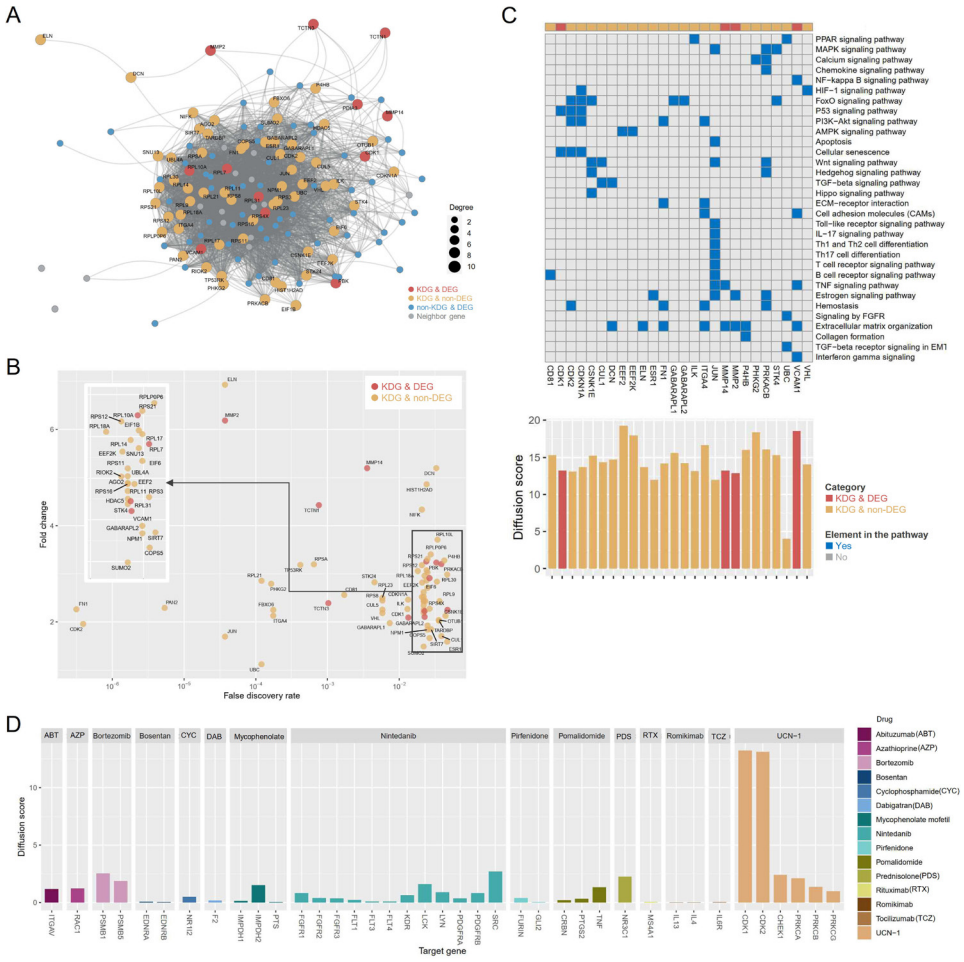

Figure 5 KDA and kernel-based diffusion scoring. (A) Probabilistic causal gene network projection and KDA for the identification of causal regulators in the SSc-ILD disease module. KDGs and their neighbours are distinguished by colours, as indicated at the lower right corner. (B) KDA result plotted as false discovery rate (x-axis) by fold change (y-axis). Because the right lower area is too dense to be fully annotated with labels, an enlarged and annotated subset is presented separately (left inset box). (C) KDGs that are involved in SSc-ILD-associated pathways and their diffusion scores. Whether each KDG was an element of the SSc-ILD-associated pathways is marked in the upper panel. Kernel-based diffusion scores of genes targeting the SSc-ILD DEGs were calculated using a z-scaled Monte Carlo method. Diffusion scores were plotted in pairs with the KDGs in the lower panel. A high diffusion score indicates that the gene has strong potential to perturb the disease module. (D) Current and investigational drugs for SSc-ILD and their molecular targets and diffusion scores. The diffusion score was calculated using a z-scaled Monte Carlo method based on the DEG-driven disease module. ABT, abituzumab; AZP, azathioprine; CYC, cyclophosphamide; DAB, dabigatran; DEG, differentially expressed gene; ILD, interstitial lung disease; KDA, key driver analysis; KDG, key driver gene; PDS, prednisolone; RTX, rituximab; SSc-ILD, systemic sclerosis-associated interstitial lung disease; TCZ, tocilizumab.

apoptosis and senescence.[36 37] In IPF, alveolar epithelial cells with propensities for proliferation, apoptosis and senescence are key drivers of lung fibrosis.[38] However, the role of alveolar epithelial cells in SSc-ILD remains less well defined.

Low FVC and D_{LCO} are risk factors for the development, progression and mortality of SSc-ILD.[6 23] Early SSc-ILD may have preserved lung volume, despite clear evidence of structural lung disease on high-resolution CT, but a decrease in D_{LCO}.[23] Given that our results showed that the p53 signalling pathway had a much closer correlation with D_{LCO} than FVC, the activity of the p53 signalling pathway could be an intriguing biomarker that bridges the mechanistic features and clinical progression in SSc-ILD. However, the PI3K–Akt signalling pathway has been shown to be the most significant pathway in skin fibrosis of SSc.[15] Fibrosis is the final, common pathological outcome among the four diseases, but there may be tissue-specific differences in the characteristic mechanism of fibrosis even in a single disease entity, indicating the need for context-dependent approaches.

In patients with pulmonary fibrosis, fibrotic destruction of the lung parenchyma leads to hypoxic vasoconstriction and loss of vascular bed density, thus creating a fertile ground for pulmonary hypertension (PH) development.[39] When ILD is present, PH should be classified in group 3 according to the current classification guideline.[40] In a microarray analysis study of distal vasculature, there were no significant differences in pulmonary arteriolar gene expression between patients wtih IPF with and without coexistent PH.[41] In another study that compared lung tissue gene expression profiles between ILD samples with and without PH, distinct gene signatures were found.[42] However, only four gene sets were significantly enriched in the PH group compared with the non-PH group. These genes sets were ‘establishment and/or maintenance of chromatin architecture’, ‘chromatin modification’, ‘chromosome organisation and biogenesis’ and ‘microtubule organising centre part’. In the GSE48149 dataset, patients with SSc were annotated with ILD only (n=13) or ILD combined with PH (n=10). We compared the key

signalling pathways between the two subgroups (online supplemental figure S8A). Apoptosis and ECM organisation were more enriched in the ILD group, whereas oxidative stress and signalling by BMP were more enriched in the ILD-PH group. However, the p53, IL-17, PI3K–Akt and TGF-β signalling pathways were comparable between these two groups. There was also no difference in gene expression of KDGs between the ILD and ILD-PH subgroups (online supplemental figure S8B). The presence of PH would not hamper the ability to identify key pathways and molecules driving the pulmonary fibrosis.

Current therapeutic options to halt or reverse the progression of SSc-ILD are unsatisfactory and their marginal effects, intolerable dose and/or adverse effects also obstruct the steady management of SSc-ILD.[5 6] The failure of optimal targeting to rectify the SSc-ILD disease module is one of the reasons for its limited and heterogeneous efficacy. To focus on this issue, we constructed networks of DEGs and probabilistic causative genes to model molecular interactions and causal gene relationships; we also identified key drivers of the SSc-ILD disease module by applying Bayesian network-based KDA. To estimate the ripple effect of KDGs on the network of the disease module, we calculated the diffusion score. Critical roles of KDGs with high diffusion scores in fibrosis were verified. EEF2 and EEF2K were identified as the top-ranked regulators. EEF2K physiologically suppresses EEF2, and the inhibition of EEF2K was proven to induce proliferation and differentiation and to reduce apoptosis through the p38 MAPK signalling pathway in human lung fibroblasts.[43] Cellular VCAM-1 depletion inhibits fibroblast proliferation, and VCAM-1 was shown to be highly abundant in fibrosing lung disease, where it was detected in fibrotic foci and blood vessels.[44] FN1 mediates cell–matrix adhesion and is essential in driving myofibroblast differentiation.[45] Inhibition of FN1 deposition attenuated fibrosis in hepatic and cardiac fibrosis models.[46 47] ITGA4 binds FN1 and VCAM-1, thereby mediating cell-to-cell adhesion, which is crucial to fibroblast function.[48 49]

CDKs and histone deacetylases (HDACs) are interesting targets because they are ranked by high priorities, and drugs against them are currently in use or under clinical trials as anticancer agents. CDKN1A (also known as p21) is a physiological CDK antagonist under the control of p53. In the bleomycin-induced pulmonary fibrosis model, the forced expression of CDKN1A was shown to induce both antiapoptotic and antifibrotic effects.[50 51] HDAC inhibitors cause cell cycle arrest by inducing CDKN1A or inhibiting CDKs and effectively suppress the profibrotic phenotype of fibroblasts in IPF, showing better efficacy in this regard than pirfenidone.[52 53] These approaches are promising because their mechanisms of action differ from those of the current immunosuppressive or antifibrotic drugs, but significant additional research is needed to translate the strategies targeting these molecules into clinical applications, given their low specificity and high toxicity.

To evaluate the leverage of treatment drugs in the disease module, we compared the diffusion scores of their target molecules. None of the current treatment drugs targeted the KDGs, and diffusion scores of their target molecules were also less than those of the KDGs, indicating that the current treatment drugs do not effectively perturb the disease module of SSc-ILD. Notably, an investigational drug, UCN-01 (7-hydroxystaurosporine), showed a good diffusion score for its targets. UCN-01 targets CDK1, CDK2 and CHEK1, the main components of the p53 signalling pathway and cellular senescence, and reactivates FoxO3 to control inappropriate proliferation and differentiation.[30 54] In particular, UCN-01 showed great promise in a preclinical model of lung fibrosis by reverting the myofibroblast phenotype in vitro and blocking bleomycin-induced lung fibrosis in vivo.[30] The p53 signalling pathway was also enriched in the lung tissue of patients with PAH. Inactivation of p53 aggravated pulmonary hypertension by inducing pulmonary vascular remodeling[55 56] and treatment with CDK inhibitors had a therapeutic effect by suppressing vascular remodelling in PAH models.[57] It is conceivable that the modulation of CDKs may be beneficial to suppress the development of pulmonary hypertension, in addition to treatment of ILD.

This study had several limitations. First, the combination of multiple datasets inevitably caused the loss of genes that overlapped only among some datasets, and the correction of the batch effect was not ideal. Second, the immunological domain of SSc-ILD was not covered. This is probably because lung tissues were sampled at the fibrosing or fibrosed stages, whereas the major immune responses dominated in the early developmental stage of SSc-ILD. Third, we did not address the association with clinical factors such as radiographical pattern or fibrosis score because of the lack of this information. Fourth, minority signatures by specific cell subsets might have been diluted because the gene expression signature was at the tissue level. However, we validated key molecular signatures in lung fibroblasts. Fifth, the three datasets of SSc-ILD did not provide the background medications of the individual patients. According to the surgical lung biopsy protocol,[58] fresh lung area best representing the disease should be biopsied for reliable results. Lung tissue under ongoing fibrosis reflects the current pathological status rather than the response to treatment. We found that the p53 signalling pathway was the most significant ($p=3.76\times10^{-7}$) and enriched (enrichment score=143.2) pathway among the other signalling pathways (online supplemental files 2 and 3). Although some molecular signatures could be susceptible to the effect of current or past treatments and potentially biased, it is considered that they would not be enough to overturn our finding of the overwhelming significance of the p53 signalling pathway. It is presumed that the patients in the GSE48149 dataset would be under supportive therapy without active immunosuppressive therapy until lung transplantation because the lung tissues were obtained from the lung explanted during lung transplantation. It was stated that the tissues in datasets GSE76808 and GSE81292 were obtained by surgical lung biopsy immediately before immunosuppressive therapy (cyclophosphamide treatment). Therefore, it is reasonable to assume that the lung tissue samples used in the study were far from ILD-targeted immunotherapy at the point of lung tissue sampling.

ILD is a major challenge with a high unmet need in the management of SSc. Our network-based integrative approach described the cellular and molecular characteristics of SSc-ILD compared with those of related pulmonary diseases and revealed their significance with regard to pulmonary functional indices. The identification of KDGs and target molecules in the defined disease module not only explained the limitation of current pharmacotherapy but also can be leveraged to provide insights into the discovery of promising drugs for SSc-ILD.

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CLINICAL SCIENCE

Treatment efficacy and safety of tofacitinib versus methotrexate in Takayasu arteritis: a prospective observational study

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ABSTRACT

Objective To compare the treatment efficacy and safety of tofacitinib (TOF) versus methotrexate (MTX) in Takayasu arteritis (TAK).

Methods Fifty-three patients with active disease from an ongoing prospective TAK cohort in China were included in this study. Twenty-seven patients were treated with glucocorticoids (GCs) and TOF, and 26 patients were treated with GCs with MTX. The observation period was 12 months. Complete remission (CR), inflammatory parameter changes, GCs tapering and safety were assessed at the 6th, 9th and 12th month. Vascular lesions were evaluated at the 6th and 12th month, and relapse was analysed during 12 months.

Results The CR rate was higher in the TOF group than in the MTX group (6 months: 85.19% vs 61.54%, p=0.07; 12 months: 88.46% vs 56.52%, p=0.02). During 12 months' treatment, patients in the TOF group achieved a relatively lower relapse rate (11.54% vs 34.78%, p=0.052) and a longer median relapse-free duration (11.65±0.98 vs 10.48±2.31 months, p=0.03). Average GCs dose at the 3rd, 6th and 12th month was lower in the TOF group than that in the MTX group (p<0.05). A difference was not observed in disease improvement or disease progression on imaging between the two groups (p>0.05). Prevalence of side effects was low in both groups (3.70% vs 15.38%, p=0.19).

Conclusion TOF was superior to MTX for CR induction, a tendency to prevent relapse and tapering of the GCs dose in TAK treatment. A good safety profile for TOF was also documented in patients with TAK.

Key messages

What is already known about this subject?

► Tofacitinib is a janus kinase inhibitor and has been reported to be efficacious in patients who are refractory to treatment for Takayasu arteritis.

What does this study add?

► We compared the treatment effect of tofacitinib and traditional immunosuppressant methotrexate in naive and refractory patients. The results demonstrate that tofacitinib was superior to methotrexate in inducing clinical remission, preventing disease relapse and tapering the glucocorticoids dose.

How might this impact on clinical practice or future developments?

► This study has provided more evidence for the application of tofacitinib in the treatment of Takayasu arteritis and shed light on a novel treatment target in Takayasu arteritis.

INTRODUCTION

Takayasu arteritis (TAK) is a chronic granulomatous vasculitis of large vessels. It predominantly involves the aorta and its main branches and preferentially occurs in young (<40 years) women.[1] The involved arteries can become stenotic or occluded, which leads to insufficient blood perfusion (or even ischaemia) of the corresponding tissue or organ.[2] Glucocorticoids (GCs) and immunosuppressants such as cyclophosphamide, methotrexate (MTX), leflunomide, azathioprine and mycophenolate mofetil are recommended as initial treatment for patients with TAK,[3] and about 60%–80% patients can achieve clinical remission.[4 5] However, a high prevalence of relapse (>50%) and disease progression on imaging demonstrate the need for finding more efficacious options.[6]

Tofacitinib (TOF) preferentially inhibits janus kinase (JAK) 1 or JAK3. Increasing evidence has shown its treatment effect in multiple autoimmune diseases or inflammatory diseases.[7] JAK/signal transducer and activator of transcription (STAT) is involved in the signalling pathways of multiple cytokines, such as interleukin (IL) 6 and interferon (IFN)-γ.[8] Previously, we showed that JAK/STAT was critical in vascular fibrosis mediated by IL-6 in TAK.[9] It has also been reported that JAK inhibitors can downregulate subsets of T helper (Th) 1 and Th17 cells and upregulate T-regulatory cells in patients with TAK.[10] Animal models of large-vessel vasculitis have also shown that JAK inhibitors can suppress tissue-resident T-memory cells and inhibit microvascular angiogenesis.[11] Several case reports have demonstrated TOF to be efficacious in refractory patients with TAK,[12–15] but good-quality evidence for its application in TAK is lacking.

MTX is a conventional disease-modifying antirheumatic drug (cDMARD), which has been widely used in multiple types of rheumatic disease. Its treatment effect in TAK has been demonstrated in an open-label prospective study.[16] MTX has also been suggested for the initial treatment of TAK

according to European League against Rheumatism (EULAR) recommendations.[3]

Here, we investigated the efficacy of TOF compared with that of the conventional immunosuppressant MTX against TAK.

MATERIALS AND METHODS

Patients

This study was based on an ongoing prospective observational TAK cohort: East China Takayasu Arteritis. This cohort was established in 2009 by the department of rheumatology within Zhongshan Hospital (which is affiliated to Fudan University). Patients in this cohort were (1) diagnosed according to 1990 American College of Rheumatology (ACR) classification criteria[17] and (2) followed-up based on a predesigned plan and assessed by a team including professional rheumatologists and radiologists. Their clinical data at each visit were collected by a specific person and recorded into a uniform database. The protocol of this cohort was approved (B-2016-168(2)R) by the ethics committees of Zhongshan Hospital (Shanghai, China). It conformed to the ethical guidelines set in the Declaration of Helsinki (1965) and its later amendments. Written informed consent was obtained from all patients. Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

In this cohort, patients treated with GCs combined with TOF or MTX were recruited if they met specific inclusion and exclusion criteria. The inclusion criteria were patients who (1) satisfied the classification criteria set by ACR in 1990, (2) had active disease (National Institutes of Health (NIH) criteria ≥2 points) when they started using MTX or TOF[18] and (3) had complete data at baseline and during the follow-up. The exclusion criteria were (1) concurrent tumours, infections or other autoimmune disease and (2) combined treatment with other immunosuppressants or biological agents.

After screening, 53 patients were recruited in this study: 27 patients treated with GCs and TOF (TOF group) and 26 patients treated with GCs and MTX (MTX group). Among these patients, 19 patients in the TOF group and seven patients in MTX group were refractory to treatment for TAK, which indicated a poor response to ≥2 types of conventional immunosuppressants in previous treatment. The remaining eight patients in TOF group and 19 patients in the MTX group were treatment-naive.

Study design

Visit and assessment plans in this cohort are demonstrated in figure 1. In this study, the observation period was 12 months. The treatment process included an induction period (0–6 months) and maintenance period (6–12 months). Patients were followed up every month in the induction period and every 3 months in the maintenance period. For naive patients in both groups, GCs (Xin Yi Pharmaceuticals, Shanghai, China) were started initially at 0.8 mg/kg/day. For refractory patients, the initial GCs were continued on the same dose at the time of disease relapse. During the induction period, the initial GC dose was maintained for 4 weeks and then tapered by 5 mg every 2 weeks. When the GC dose reached 20 mg/day, it was tapered by 5 mg every month and gradually to 0.1–0.2 mg/kg/day for maintenance. During this process, GCs tapering was under the condition NIH points <2. TOF (Pfizer, New York, USA) was given 5 mg twice daily, and MTX (Xin Yi Pharmaceuticals) was given orally with a fixed dose of 10–15 mg per week. The dose of TOF or MTX could be reduced (or even withdrawn) if patients developed side effects such as increase in the level of liver enzymes, kidney injury (eg, blood creatinine level >177 μmol/L), menstrual disorders and bone marrow suppression.

At each visit, symptoms/signs, laboratory tests and imaging results were recorded. At baseline, 6 months and 12 months, whole-body enhanced magnetic resonance angiography (MRA) was undertaken.[19] Among them, two patients in the TOF group underwent CT angiography as an alternative due to MRA contradictions (one case had an allergic reaction to contrast, and one patient had an incompatible implant). During a severe pandemic of COVID-19 in China (1 February to 1 May 2020), an electronic questionnaire was also designed to allow patients to report discomforts.

Outcome assessment

Complete remission (CR) and partial remission (PR) were evaluated at the 6th, 9th and 12th month. Relapse was assessed during the 12 months of treatment. CR was defined to satisfy four criteria: (1) no new/worsened systemic symptoms, (2) no new/worsened vascular symptoms or signs, (3) erythrocyte sedimentation rate (ESR) was normal (≤40 mm/hour) and (4) GC dose ≤15 mg/day.[20] PR was defined as 2 combined with any two of 1, 3 or 4. Relapse was denoted as reactivation of disease activity (NIH criteria ≥2 points) for patients who had achieved CR or PR.

In addition, the treatment effect was evaluated from the following five aspects: (1) disease activity, NIH score ≥2 points; (2) decrease in inflammatory parameters (ESR and C reactive protein (CRP) levels); (3) changes on vascular imaging (progression, improvement or stable disease), as described previously[20]; (4) tapering of the GCs dose (the average GCs dose at different time points and the reduction of it from baseline); and (5) side effects and safety (any intolerant symptoms or signs such as rashes, appetite upset, hair loss, abnormal menstruation and liver/kidney injury). Besides imaging changes, which were evaluated at the 6th and 12th month, the other aspects were assessed at the 6th, 9th and 12th month.

Statistical analyses

Categorical variables are presented as frequencies and percentages. Continuous variables with a normal distribution are presented as the mean±SD. Continuous variables with a skewed distribution are presented as median and IQR. Comparisons between two groups were performed using Student's t-test, Mann-Whitney test or χ^2 test, as appropriate. Paired *t*-tests were applied to analyse continuous variables at different post-treatment time points from baseline. Relapse-free survival was analysed using Kaplan-Meier curves. The log-rank test was used to analyse the difference between two treatment groups. Baseline ESR levels, treatment group, systemic symptoms and treatment-naive or treatment-refractory characteristics were included in the binary logistic regression analysis with the method 'Enter' to explore the risk factors for CR at the 12th month. P<0.05 (two-sided) was deemed significant. SPSS V.20.0 was used for statistical analyses.

RESULTS

Patient characteristics

The demographic and clinical characteristics of patients are shown in table 1. The average age of the study cohort was 32.28±12.41 years, which comprised 45 women and eight men. Their median disease duration was 25.00 (IQR: 10.00 and 59.00) months. Between these two treatment groups, differences were not observed for age, sex ratio, disease duration or disease

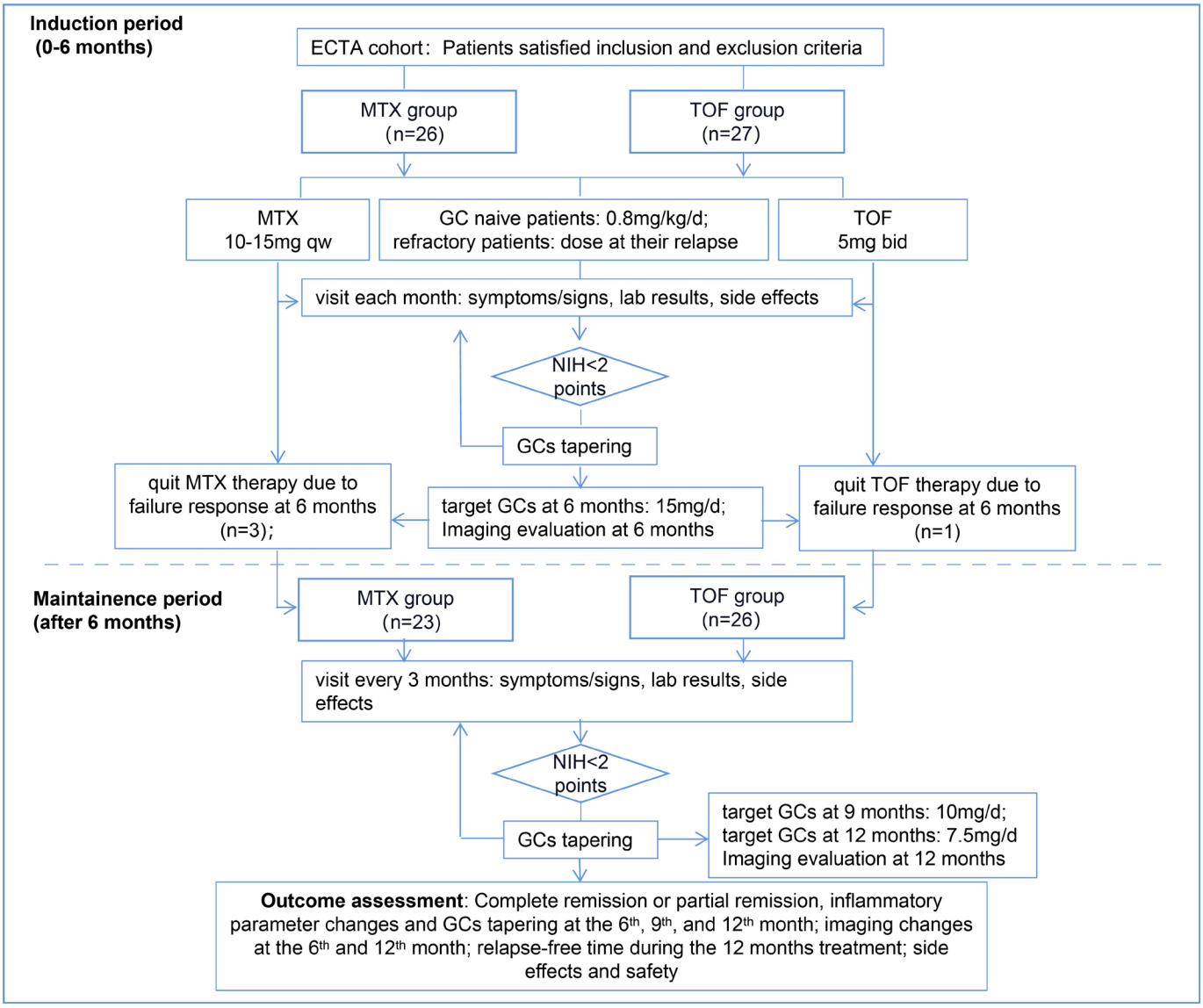

Figure 1 Study design. patients treated with glucocorticoids (GCs) and methotrexate (MTX) or tofacitinib (TOF) were enrolled from the prospective East China Takayasu Arteritis (ECTA) cohort. All patients were observed for 12 months: an induction period (0–6 months) and maintenance period (6–12 months). Patients were followed up monthly in the induction period and every 3 months in the maintenance period. In the TOF group, patients were treated with GCs combined with TOF (5 mg, twice daily). In the MTX group, patients were treated with GCs combined with MTX (orally, 10–15 mg per week). For both groups, GCs were started initially at 0.8 mg/kg/day for naive patients, while it was kept similar as the dose at relapse for refractory patients. Symptoms, signs, laboratory results and side effects were documented at each visit, and vascular imaging was undertaken every 6 months. Complete remission or partial remission, inflammatory parameter changes and tapering of the GCs dose at the 6th, 9th and 12th month were evaluated. Imaging changes at the 6th and 12th month were assessed. Relapse-free time, side effects and safety during the 12 months of treatment were analysed. NIH, National Institutes of Health.

activity (p>0.05) (table 1). The percentage of refractory patients was relatively higher in the TOF group in comparison with the MTX group (p=0.002) (table 1). The average dose of MTX at the end of the 12th month was 11.58±2.16 mg/week. Clinical characteristics for naive and refractory patients, respectively, in both groups are demonstrated in online supplemental table 1. For refractory patients, their previous treatment is also listed in online supplemental table 1.

Treatment efficacy

Remission

At the 6th month, the CR rate tended to be higher in the TOF group than that in the MTX group (23/27, 85.19%, vs 16/26, 61.54%; p=0.07; table 2). At the 6th month, one patient in the TOF group and three patients in the MTX group changed to other medications due to a poor response to treatment. Thus, 26 and 23 patients remained in the TOF group and MTX group, respectively, after 6 months of treatment. The CR rate was higher in the TOF group than that in the MTX group (23/26, 88.46%, vs 13/23, 56.52%; p=0.02) (table 2) at the 12th month. PR was also evaluated at different time points, but a difference was not observed between the TOF group and MTX group (p>0.05) (table 2).

We wished to ascertain the difference in the treatment effect between these two groups for naive and refractory patients. Hence, post-treatment characteristics were also evaluated separately for naive and refractory patients in both groups (online supplemental table 2). The CR rate for naive

Table 1 Characteristics of the study cohort

Parameters	Total (n=53)	GCs+TOF (n=27)	GCs+MTX (n=26)	P
General information				
Woman/man (n)	45:8	22:5	23:03	0.71
Age (mean±SD, years)	32.28±12.41	31.11±9.58	33.50±14.89	0.49
Disease duration (median, IQR, months)	25.00 (10.00, 59.00)	31.00 (10.00, 72.00)	12.00 (2.25, 45.00)	0.17
Naive/refractory patients (n)	27:26	8:19	19:7	**0.002**
Imaging types, n (%)				
I	19 (0.36)	7 (25.93)	12 (46.15)	0.38
II	10 (0.19)	6 (22.22)	4 (15.38)	
III	4 (0.08)	3 (11.11)	1 (3.85)	
IV	2 (0.04)	2 (7.40)	0 (0)	
V	18 (0.34)	9 (33.33)	9 (34.62)	
Symptoms and signs, n (%)				
Systemic symptoms	22 (0.42)	10 (0.37)	12 (0.46)	0.58
Fever	10 (0.19)	5 (0.19)	5 (0.19)	0.74
Weakness	17 (0.32)	11 (0.41)	6 (0.23)	0.38
Ischaemia symptoms or signs	44 (0.83)	24 (0.89)	20 (0.77)	0.46
Headache/dizziness	25 (0.47)	15 (0.56)	10 (0.38)	0.57
Chest pain/distress	16 (0.30)	12 (0.44)	4 (0.15)	0.08
Vascular murmur	29 (0.55)	17 (0.63)	12 (0.46)	0.57
Neck pain	10 (0.19)	5 (0.19)	5 (0.19)	0.74
Laboratory results (median, IQR)				
ESR (mm/H)	21.00 (7.00, 42.00)	20.00 (2.00, 42.00)	29.50 (16.00, 44.00)	0.13
CRP (mg/L)	10.70 (1.70, 32.95)	6.30 (1.30, 32.60)	17.55 (3.70, 43.60)	0.32
Initial treatment				
Initial oral GCs dose (median, IQR, mg/d)	20.00 (14.00, 30.00)	15.00 (12.00, 30.00)	30.00 (15.00, 40.00)	0.13
TOF (mg/d)/MTX (mean±SD, mg/w)	/	10.00	10.77±2.84	/

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GCs, glucocorticoids; MTX, methotrexate; TOF, tofacitinib.

patients at 12 months was higher in the TOF group (8/8, 100%, vs 9/19, 47.37%; p=0.03) (online supplemental table 2), but a difference in the CR rate was not observed in refractory patients between these two groups (p>0.05) (online supplemental table 2).

Relapse

The number of patients with NIH criteria <2 was significantly higher in the TOF group compared with that in the MTX group (26/27, 96.30%, vs 17/26, 65.38%; p=0.005) at the 6th month, whereas no difference was observed in the percentage of patients with NIH criteria <2 at the 12th month (23/26, 88.46%, vs 15/23, 65.22%; p=0.052).

During 12 months of treatment, three (11.54%) patients suffered relapse in the TOF group, which was relatively less than that in the MTX group (eight, 34.78%; p=0.052). However, the average relapse-free duration was longer in the TOF group than that in the MTX group (11.65±0.98 vs 10.48±2.31 months, p=0.03). The HR for the time to relapse was 0.28 (95% CI 0.08 to 0.95, p=0.04) (figure 2A). When analysing relapses in naive and refractory patients separately, it was interesting to find that all relapses in the MTX group occurred in naive patients (eight, 42.11%), whereas all relapses in the TOF group occurred in refractory patients (three, 15.79%) (online supplemental table 2).

Table 2 Evaluation of treatment efficacy

	GCs+TOF		GCs+MTX		
	N	N (%)	N	N (%)	P
CR					
6 months	27	23 (85.19)	26	16 (61.54)	0.07
9 months	26	22 (84.61)	23	14 (60.87)	0.10
12 months	26	23 (88.46)	23	13 (56.52)	**0.02**
PR					
6 months	27	3 (11.11)	26	1 (3.85)	0.61
9 months	26	2 (7.69)	23	3 (13.04)	0.65
12 months	26	0 (0)	23	2 (13.04)	0.22

CR, complete remission; GCs, glucocorticoids; MTX, methotrexate; PR, partial remission; TOF, tofacitinib.

Changes of inflammatory parameters

A significant reduction in the ESR was observed at the 3rd month and 6th months in the TOF group compared with that at baseline (baseline vs the 3rd month: p=0.03; baseline vs the 6th month: p=0.04) (figure 2D). A significant change in the ESR was not observed in the MTX group after treatment (p>0.05 for all) (figure 2D). In addition, the ESR was significantly lower in the TOF group compared with that in the MTX group at the 3rd and 9th month (the 3rd month: p=0.03; the 9th month: p<0.001) (figure 2D).

A decrease in the CRP level was observed in the TOF group at the 3rd, 6th, 9th and 12th month compared with that at baseline (p=0.006, p=0.008, p=0.02 and p=0.04, respectively) (figure 2E). However, a significant change in the CRP level was not observed in the MTX group (p>0.05 for all) (figure 2E). Compared with the MTX group, the CRP level was also significantly lower in the TOF group at the 9th month and 12th month (the 9th month: p=0.003; the 12th month: p=0.03) (figure 2E).

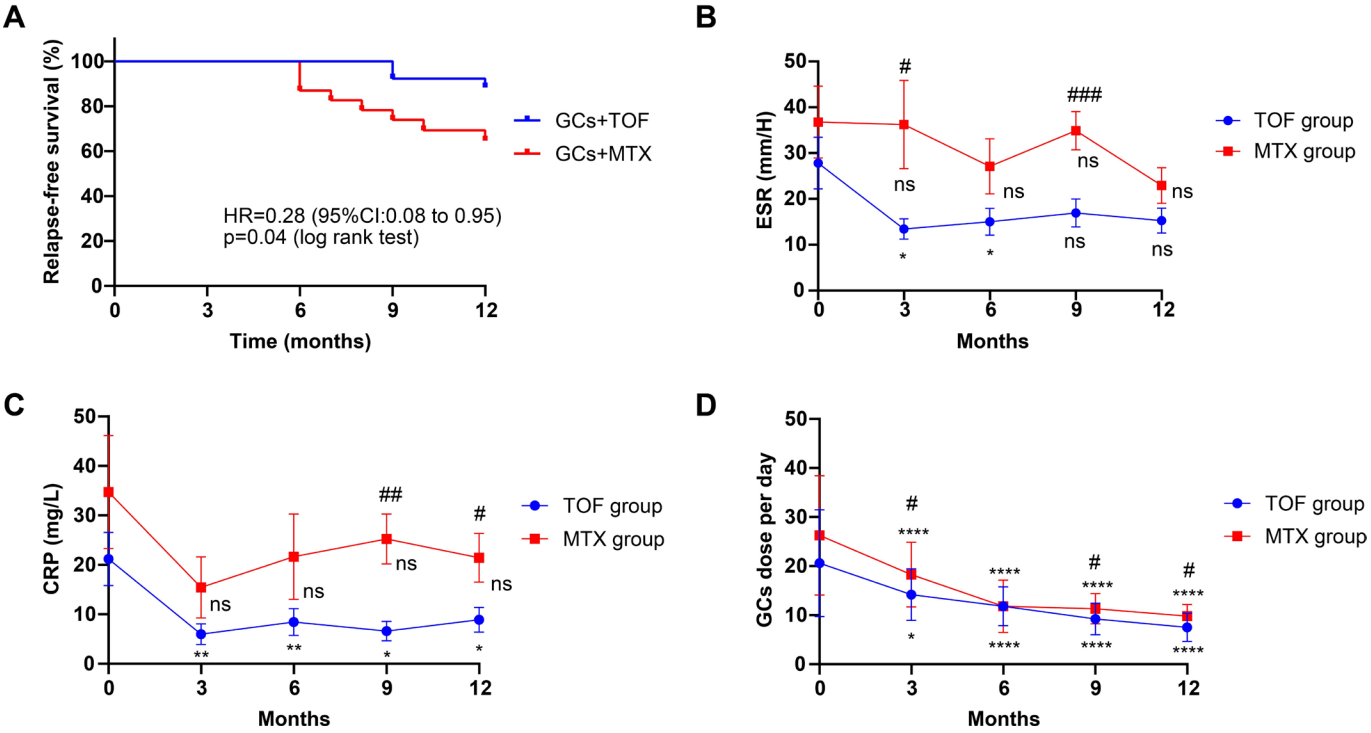

Figure 2 Evaluation of multiple other aspects post treatment. (A) Relapse-free survival analysis in tofacitinib (TOF) and methotrexate (MTX) groups; (B) changes in the erythrocyte sedimentation rate (ESR) level in both groups at 0, 3, 6, 9 and 12 months; (C) changes in the C reactive protein (CRP) level in both groups at 0, 3, 6, 9 and 12 months; (D) changes in the glucocorticoids (GCs) dose in both groups at 0, 3, 6, 9 and 12 months. ns: not significant; *comparison between different post-treatment time points and baseline; #comparison between the TOF group and MTX group at different time points; */#p<0.05; **/##p<0.01; ***/###p<0.001.

Tapering of the GCs dose

The GCs dose was reduced significantly in both groups. A significant decrease was observed in both groups at the 3rd, 6th, 9th and 12th month compared with their corresponding baseline levels (all p<0.05) (figure 2F). Although a significant difference was not observed in the reduction of the GCs dose from baseline to the 12th month between these two groups (TOF vs MTX group: 10.00 (IQR: 5.00 and 25.00) vs 20.00 (IQR: 2.50 and 25.00) mg, p=0.70), the median GCs dose per day was lower in the TOF group than that in the MTX group at the 3rd (15.00 (IQR: 10.00 and 20.00) vs 20.00 (IQR: 13.13 and 23.75) mg/day, p=0.04), 9th (10.00 (IQR: 7.50 and 10.00) vs 11.25 (IQR: 10.00 and 15.00) mg/day, p=0.01) and 12th month (5.00 (IQR: 5.00 and 10.00) vs 10.00 (IQR: 7.50 and 10.00) mg/day, p=0.01) (figure 2F). Furthermore, the percentage of patients taking GCs ≤10 mg/day at the 9th month and patients taking GCs ≤7.5 mg/day at the 12th month was higher in the TOF group than that in the MTX group (the 9th month: 20/27, 74.07%, vs 10/26, 38.46%; p=0.01; the 12th month: 16/26, 61.54%, vs 5/23, 21.74%; p=0.009).

When analysing tapering of the GCs dose in naive or refractory patients separately, we found that the decrease of the GCs dose in both groups was predominantly driven by naive patients. A significant decrease of the GCs dose in naive patients was observed in the TOF and MTX groups at the 3rd, 6th, 9th and 12th month compared with their corresponding baseline GCs dose (all p<0.05) (online supplemental table 2).

Imaging changes

Imaging evaluation from baseline to the 6th month and from the 6th month to the 12th month is shown in table 3. Overall, a significant difference was not observed between the two groups (all p>0.05) (table 3). However, when analysing naive or refractory patients separately, a significant difference in imaging changes throughout the 12 months was demonstrated in naive patients between TOF and MTX groups (p=0.004) (online supplemental table 2). Representative images before and after treatment are demonstrated in online supplemental figure 1.

Safety

During 12 months of treatment, shingles developed in one (3.70%) patient in the TOF group, which was treated by acyclovir. At the same time, TOF was stopped for ~2 weeks until the rashes became scabbed. Other side effects such as gastrointestinal upset, liver/kidney injury, hyperglycaemia, hyperlipaemia and vascular thrombosis were not observed. In the MTX group, an increase in the levels of liver enzymes

Table 3 Imaging evaluation after treatment

	GCs+TOF group		GCs+MTX group		
	N	N (%)	N	N (%)	P
6 months	27				
Stable lesions		19 (70.37%)	26	24 (92.31%)	0.11
Imaging improvement		6 (22.22%)		1 (3.85%)	
Imaging progression		2 (7.41%)		1 (3.85%)	
12 months	26				
Stable lesions		23 (88.46%)	23	21 (91.30%)	0.89
Imaging improvement		2 (7.69%)		1 (4.35%)	
Imaging progression		1 (3.85%)		1 (4.35%)	

6 months: changes from baseline to the 6th month; 12 months: changes from the 6th month to the 12th month.
GCs, glucocorticoids; MTX, methotrexate; TOF, tofacitinib.

Table 4 Multivariate adjusted OR (95% CI) of potential risk factors associated with CR at 12 months

	B	OR	95% CI for OR		P
			Lower	Upper	
Baseline ESR (mm/hour)	−0.04	0.96	0.93	0.99	**0.03**
TOF treatment	2.92	18.51	1.04	328.87	**0.047**
Systemic symptoms	3.01	20.28	1.34	305.91	**0.03**
Naive patients	2.02	7.54	0.39	146.41	0.18

CR, complete remission; ESR, erythrocyte sedimentation rate; TOF, tofacitinib.

(higher than two-times the upper limit of normal) was observed in three (11.54%) patients, which were all treated by glutathione without discontinuing MTX. One (3.85%) patient had weakness and upset appetite at the initial dose of MTX that disappeared spontaneously without MTX withdrawal. Overall, no significant difference was observed in the prevalence of side effects between these two groups (1/27, 3.70%, vs 4/26, 15.38%; p=0.19).

Associated factors with CR at the 12th month

Multiple parameters including sex, age, disease duration, disease activity at baseline, baseline ESR levels, baseline CRP levels, imaging types, naive or refractory characteristics, treatment and initial GCs dose were compared between patients who achieved CR at the 12th month and those who did not (online supplemental table 3). Based on the results, three parameters with p value<0.1 (baseline ESR levels, treatment group and systemic symptoms) were chosen for the logistic regression analysis. In addition, to ascertain if naive or refractory characteristics affected the CR at the 12th month, this parameter was also included. Results indicated that lower baseline ESR levels, TOF treatment and the presence of systemic symptoms were associated with higher CR rates at the 12th month (all p<0.05) (table 4).

DISCUSSION

This is the first clinical study to compare the treatment effect of TOF and MTX in TAK. Disease remission, disease changes on imaging, GCs doses, inflammatory parameters and safety were assessed during 12 months of treatment. We demonstrated that TOF had advantages with regard to CR induction, a tendency to prevent relapse (p=0.052) and tapering of the GCs dose.

The CR rate at the 6th month (85.19% vs 61.54%) and 12th month (88.46% vs 65.22%) was relatively higher in the TOF group than that in the MTX group. One meta-analysis of studies on TAK demonstrated the pooled CR rate to be 58% for patients treated with immunosuppressants and 64% for patients treated with biological agents.[5] Among the latter, anti-tumour necrosis factor (TNF)-α agents or antibodies against the IL-6 receptor are used frequently in TAK. In a cohort from the Mayo Clinic, ~90% patients treated with anti-TNF-α achieved CR after a median treatment duration of 23 months.[21] In a prospective study from a TAK cohort in France, ~89% of patients gained CR after 6 months of tocilizumab treatment.[22] These data indicated that TAK treatment targeting inflammatory cytokines such as IL-6 or TNF-α could achieve better efficacy than conventional immunosuppressants. This explained (at least in part) the better treatment effect of TOF in TAK because it can also inhibit the signalling pathway of IL-6.[8]

Relapse is frequent in TAK, especially during tapering of the GCs dose. In the present study, the relapse rate during 12 months was 11.54% in the TOF group. A significant difference was not found between the TOF group and MTX group, but patients on TOF treatment achieved a longer duration of CR. According to Barra and colleagues, the relapse rate can reach >50% for patients on cDMARDs at 1-year follow-up.[5] In contrast, biological agents, including anti-TNF-α or tocilizumab, have been shown to reduce the relapse rate to ≤31%.[5] Due to the heterogeneity (naive and refractory) of patients in the present study, further studies are warranted to clarify the value of TOF to prevent relapses in TAK.

In the present study, the tapering of GCs in both groups was performed according to a predesigned protocol. After treatment, both TOF and MTX were conducive to a decrease in GCs usage. The differences of GCs dose were also observed at the 3rd and 9th month between these two groups, which probably reflected a relative higher starting GCs dose in the MTX group in contrast to that in the TOF group. The side effects of long-term use of GCs have been documented. Thus, the GCs dose should be reduced as low as possible if disease is in remission. According to 2018 EULAR recommendations, a target dose of GCs ≤10 mg/day is suggested after 1 year of treatment.[3] In the present study, we discovered that 74.07% of patients and 61.54% of patients in the TOF group could reach GCs ≤10 mg/day at 9 months and ≤7.5 mg/day at 12 months, respectively, indicating that a lower dose of GCs was achievable in TAK under effective treatment. However, the GCs-sparing effect of TOF needs further research in future studies.

Another advantage of TOF was that it could reduce the levels of inflammatory parameters, data that are consistent with previous reports.[13] In the present study, although TOF could inhibit both ESR and CRP levels, its effect on CRP was more prominent. This was probably due to the regulation of CRP by the JAK/STAT signalling pathway.[23] Consistently, decrease of another acute-phase reactant, IL-6, has been reported in another TAK study on TOF treatment.[14] In an animal model study, TOF could suppress innate and adaptive immunity in the vascular walls and inhibit the production of IFN-γ, IL-17 and IL-21.[11] Thus, TOF has a strong effect on suppressing the inflammatory reaction, which is probably the main mechanism involved in the treatment of active TAK.

Furthermore, no serious side effects were observed in patients of the TOF group during 12 months of treatment, which indicated a good safety profile of TOF in TAK treatment. According to previous short-term studies, the main side effect was infection (especially herpes zoster and upper respiratory tract infection).[24 25] A long-term safety study of TOF in treatment of rheumatoid arthritis over 9 years also reported other adverse events: malignancies, thrombosis, cardiovascular events and gastrointestinal perforation.[26] Thus, for patients with over 12 months of TOF treatment, tumour biomarkers, coagulation function, cardiovascular events and adverse effects in the gastrointestinal system should be monitored closely.

Our study also showed that baseline ESR levels, systemic symptoms and TOF treatment were associated with CR at 12 months. Patients with lower baseline ESR levels, systemic symptoms and TOF treatment were more likely to obtain disease remission. Interestingly, baseline CRP levels and constitutional symptoms have been reported to be associated with event-free survival in a TAK study of tocilizumab treatment.[22] On the one hand, this finding indicated that TOF treatment was beneficial for disease remission. On the other hand, this finding implied that the treatment response was closely related to the initial systemic or inflammatory conditions of patients.

Our study had limitations. According to reports of MTX in TAK treatment[27–30] that showed low dose of MTX to be efficacious and safe, MTX was not titrated at a higher dose in the present study. However, whether a lower or higher dose of MTX

has different treatment effects on TAK is not known. Thus, treatment effects between TOF and MTX must be evaluated further with different doses in future studies. In addition, our results need further validation due to unmatched population compositions (naive vs refractory) between TOF and MTX groups and the relatively small cohort. Moreover, the observation time was relatively short. Thus, the treatment effects of TOF in TAK should be confirmed further in a large cohort with longer follow-up.

CONCLUSIONS

TOF was superior to MTX for CR induction, a tendency to prevent relapse and tapering of the GCs dose in TAK treatment. A good safety profile for TOF was also documented in patients with TAK.

Contributors XK was responsible for data analysis and manuscript writing. YS was responsible for data collection of patients treated with MTX. XD helped data collection of patients treated with tofacitinib. LW helped with MRI analysis in this study. ZJ, HC and LM were responsible for the follow-up of patients. LJ was responsible for the whole study design.

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TRANSLATIONAL SCIENCE

Novel aspects of regulatory T cell dysfunction as a therapeutic target in giant cell arteritis

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ABSTRACT

Objectives Giant cell arteritis (GCA) is the most common primary vasculitis, preferentially affecting the aorta and its large-calibre branches. An imbalance between proinflammatory CD4$^+$ T helper cell subsets and regulatory T cells (Tregs) is thought to be involved in the pathogenesis of GCA and Treg dysfunction has been associated with active disease. Our work aims to explore the aetiology of Treg dysfunction and the way it is affected by remission-inducing immunomodulatory regimens.

Methods A total of 41 GCA patients were classified into active disease (n=14) and disease in remission (n=27). GCA patients' and healthy blood donors' (HD) Tregs were sorted and subjected to transcriptome and phenotypic analysis.

Results Transcriptome analysis revealed 27 genes, which were differentially regulated between GCA-derived and HD-derived Tregs. Among those, we identified transcription factors, glycolytic enzymes and IL-2 signalling mediators. We confirmed the downregulation of forkhead box P3 (FOXP3) and interferon regulatory factor 4 (IRF4) at protein level and identified the ineffective induction of glycoprotein A repetitions predominant (GARP) and CD25 as well as the reduced T cell receptor (TCR)-induced calcium influx as correlates of Treg dysfunction in GCA. Inhibition of glycolysis in HD-derived Tregs recapitulated most identified dysfunctions of GCA Tregs, suggesting the central pathogenic role of the downregulation of the glycolytic enzymes. Separate analysis of the subgroup of tocilizumab-treated patients identified the recovery of the TCR-induced calcium influx and the Treg suppressive function to associate with disease remission.

Conclusions Our findings suggest that low glycolysis and calcium signalling account for Treg dysfunction and inflammation in GCA.

Key messages

What is already known about this subject?

► Tregs, displaying reduced suppressive function and increased expression of IL-17, have been implicated in the pathogenesis of giant cell arteritis (GCA).

What does this study add?

► Comparative transcriptomic and protein expression analysis of GCA-derived and health blood-donor-derived Tregs identified aberrations of GCA Tregs such as downregulation of transcription factors, glycolytic enzymes as well as low activation-induced calcium signalling and induction of effector molecules.

How might this impact on clinical practice or future developments?

► We identify novel pathogenic correlates of GCA activity, which may be useful for monitoring disease activity, especially in tocilizumab-treated patients.
► Treg dysfunction may represent a new target for the treatment of GCA.

INTRODUCTION

Giant cell arteritis (GCA) is the most common form of systemic vasculitis, affecting the elderly, with a peak incidence at the age of 70–80 years.[1] GCA typically involves the aorta and/or its large-calibre branches.[2] The localisation and type of affected arteries largely determines the clinical manifestations of GCA, which include cranial symptoms such as headache and masticatory claudication, polymyalgia and non-specific systemic symptoms, that is, fever, night sweat and unintended weight loss. The histological hallmark of GCA is focal granulomatous inflammation.[3] Different studies suggested infectious agents, such as herpes simplex virus, varicella zoster virus, parvovirus B19 and *Chlamydia pneumoniae* as likely disease triggers.[4–6] Such infectious agents or an alternative trigger have been suggested to cause abnormal maturation of dendritic cell in the adventitia and the consequent activation of CD4$^+$ T cells.[7–9] The Th1-interferon γ (IFNγ) axis and the IL-6-Th17 axis are the main immune responses that dominate the GCA inflammation. While glucocorticoids or tocilizumab (TCZ), a monoclonal antibody against the IL-6 receptor, effectively suppress the IL-6-Th17 axis, the Th1 pathway appears to be less amenable to treatment.[8 9]

Various autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus and systemic sclerosis have been associated with regulatory T cell (Treg) dysfunction.[10–12] The forkhead box P3 (FOXP3) is indispensable for the development and function of Treg. Several studies have associated reduced expression of FOXP3 with the loss of immune tolerance and autoimmune inflammation.[13–15] Besides FOXP3, the suppressive potential of Tregs critically depends on an array of molecules, which stabilise their polarisation and/or directly mediate their effector

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functions. Notable examples include the interferon regulatory factor 4 (IRF4), the α chain of the interleukin 2 receptor (IL-2Rα/CD25), the cytotoxic T lymphocyte protein 4 and the glycoprotein A repetitions predominant (GARP).[16–22] Genetic variants affecting the function or the expression of these molecules have been reported to underlie monogenic inborn errors of immunity, which cause immune dysregulation[18–22] or to confer susceptibility for autoimmune diseases.[23–25]

An imbalance between proinflammatory $CD4^+$ T helper (Th) cell subsets, that is, Th1 and Th17 cells, and Tregs is thought to be involved in the pathogenesis of GCA. There is scarce evidence regarding the role of regulatory T cells (Tregs) in GCA inflammation. In particular, two studies reported reduced Treg counts in peripheral circulation of patients with GCA, which, however, did not associate with the GCA activity.[26 27] A more recent study identified increased Treg counts as a correlate of TCZ-induced remission of GCA.[28] Furthermore, Tregs in GCA were reported to display proinflammatory Th17-like properties at the expense of their suppressive function.[28] In this study, we aimed to delineate the dysfunction of Tregs in GCA. To this end, we integrated transcriptomic and proteomic data from Tregs, collected from patients with different disease activity and variable immunomodulatory regimens.

MATERIALS AND METHODS

Information on the study population and the experimental methods employed in the present work, including RNA-sequencing, the phenotypic and functional characterisation of regulatory T cells as well as the statistical analysis, is provided in the online supplementary text.

RESULTS

Study population

Studied subjects characteristics are summarised in table 1. Information on GCA patients' disease activity status and treatments at blood sampling is provided in online supplemental table 1.

Transcriptomic profiling of GCA Tregs

First, we performed differential transcriptome analysis between Tregs from patients with GCA (n=12; active disease, n=6, in remission, n=6; see online supplemental table 2 for patients' characteristics) and healthy blood donors (HD, n=6). Pairwise comparison of active GCA versus HD-derived Tregs, using adjusted p value <0.05 and cut-off fold change >1.47 ($log_2FC=0.56$), identified 27 differentially expressed genes (DEGs) (figure 1A). Among DEGs, we highlighted an enrichment for genes related to three molecular classes: Treg transcription factors (*FOXP3, IRF4* and *IKZF4*), glycolytic enzymes (*ENO1, PFKP, LDHA*) and molecules downstream to IL-2 signalling (*CISH, SOCS2*). Furthermore, relative quantification showed an overall lower expression of these transcripts in GCA Tregs, especially in the active cases, as compared with healthy Tregs (figure 1B). To evaluate the influence of glucocorticoids on the observed differences in transcript expression, we reanalysed transcriptome data after classifying patients with GCA (both active and inactive) into glucocorticoid-receiving (n=7) and those without glucocorticoid treatment (n=5). This identified no significant differences (*CISH*: p value=0.5025; *ENO1*: p value=0.3308; *FOXP3*: p value=0.9773; *IKZF4*: p value=0.7096; *IRF4*: p value=0.7096; *LDHA*: p value=0.6010; *PFKP*: p value=0.7424; *SOCS2*: p value=0.7096), suggesting that differential transcript expression by GCA Tregs was independent of the treatment with glucocorticoids.

Differential expression of *FOXP3* and *IRF4* at the level of transcript was evaluated at protein level by flow cytometry (figure 2). In line with the transcriptome data, both FOXP3 and IRF4 levels were lower in GCA Tregs than HD Tregs. Lower expression levels of FOXP3 in GCA Tregs did not associate with significant differences in FOXP3-positivity within $CD4^+$ $CD25^{hi}CD127^{lo}$ Tregs (online supplemental figure 1). Treg from patients in remission and those with active GCA displayed similar expression levels of FOXP3 and IRF4. On the other hand, TIGIT, whose transcript levels were reduced in most GCA samples, displayed similar expression among different groups of patients and HD.

Treg dysfunction in GCA

Despite the fact that patients with GCA and HD displayed similar $CD4^+$ $CD25^{hi}CD127^{lo}$ Treg counts (figure 3A), we identified several qualitative abnormalities with respect to the expression of effector molecules by GCA Tregs. GARP is involved in TGF-β maturation and the suppressive potential of Tregs both in vitro and in vivo depends on its expression.[19 29] After 18 hours of

Table 1 Characteristics of studied subjects at blood sampling

	HD (n=28)	Active GCA (n=14)	Inactive GCA (n=27)	P†
Age (years)—median (IQR)	61.8 (58.9–76.8)	68.3 (63.3–77.7)	69.3 (61.5–77.1)	0.3548 (ns)
Sex, female—no (%)	15 (53.6)	8 (57.1)	16 (59.2)	0.9122 (ns)
N.European ethnicity—no (%)	27 (96.4)	13 (92.3)	23 (85.2)	0.3260 (ns)
Disease duration (years)—median (IQR)	–	1.1 (0.1–4.1)	3.5 (0.8–6.2)	0.1446 (ns)
CRP (mg/L)—median (IQR)	–	30.9 (11.1–60.6)	1 (0.5–2.6)	<0.001‡
ESR 1 hour (mm)—median (IQR)	–	45 (33–80.5)	8 (5–22)	<0.001‡
Relapsed cases—no (%)	–	8 (57.1)	–	–
TCZ—no (%)	–	–	12 (44.4)	–
Duration of TCZ treatment, median (years)—median (IQR)	–	–	1 (0.4–2.8)	–
Corticosteroids as monotherapy—no (%)	–	3 (21.4)	8 (29.6)	0.7186 (ns)
Prednisolone or prednisolone equivalent dose (mg)—median (IQR)	–	0 (0–5)	2.5 (0–5)	0.2040 (ns)
MTX—no (%)	–	–	7 (25.9)	–
LFN—no (%)	–	1 (7.1)	1 (3.7)	–

*P<0.05.
†P<0.001.
‡ns, non-significant.
CRP, C reactive protein; GCA, giant cell arteritis; HD, healthy blood donor; LFN, leflunomide; MTX, methotrexate; ns, non-significant; TCZ, tocilizumab.

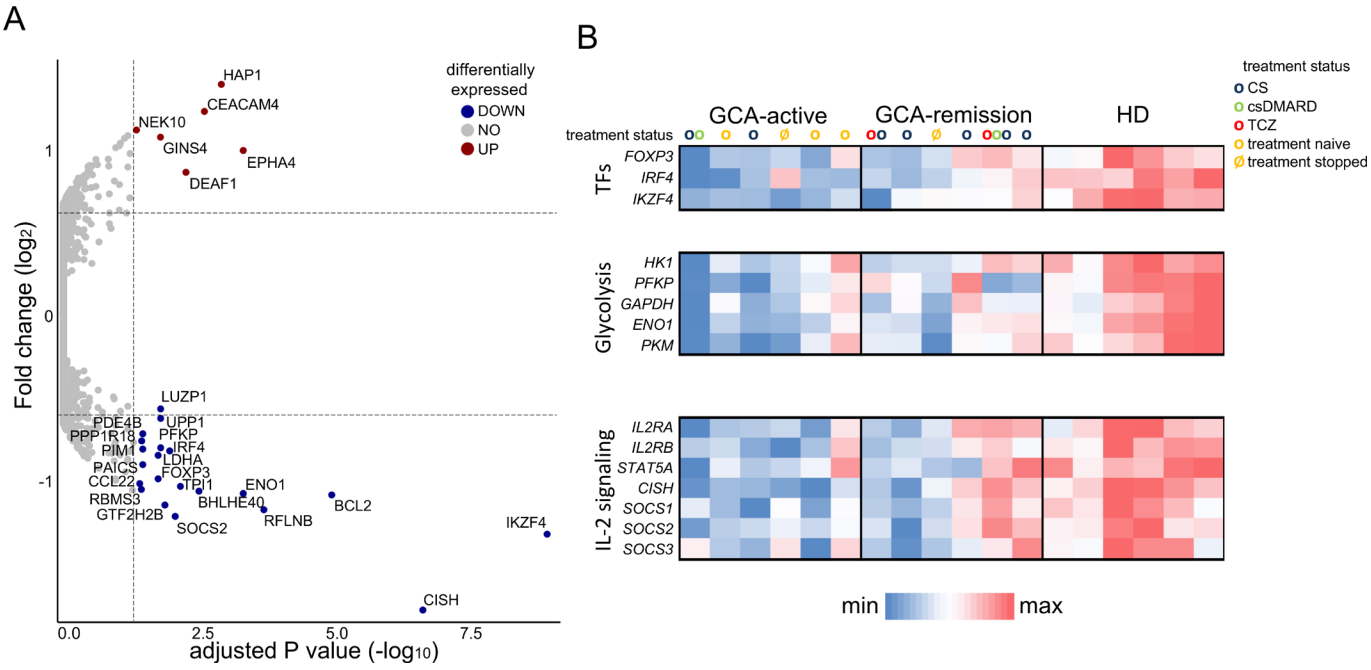

Figure 1 Transcriptional, metabolic, and signalling disturbances in GCA Tregs. (A) Volcano plot showing differentially expressed genes between active GCA Tregs vs healthy Tregs (adjusted p value<0.05, $\log_2$(fold change)>0.56). (B) Heatmap analysis showing differential expression of selected genes encoding transcription factors, glycolytic molecules, and IL-2/STAT-5 signalling pathway, in Tregs from different groups (active GCA cases, GCA in remission, healthy donors). Treatment of each studied patient is indicated: CS, corticosteroids; csDMARDs, conventional synthetic disease-modifying antirheumatic drug; GCA, giant cell arteritis; TCZ, tocilizumab.

CD3/CD28 stimulation with Dynabeads, GCA Tregs expressed significantly lower levels of GARP than HD Tregs (figure 3B). The suppressive function of Tregs critically depends on CD25, whose reduced expression by Tregs has been linked to diverse autoimmune diseases.[30 31] Similar to GARP, GCA Tregs displayed impaired induction of CD25 after CD3/CD28 stimulation (figure 3C). However, we observed no difference in GARP or CD25 expression between Tregs from active and inactive GCA. TCR-induced calcium signalling has been linked to both Treg development and suppressive function.[32] Here, we identified a marked reduction in immediate TCR-induced calcium influx in GCA Tregs, especially in those from patients with active disease (figure 3D,E). In contrast, the difference in calcium flux between GCA Tregs in remission and healthy Tregs was not statistically significant (p value=0.1936). It has been demonstrated that the exon 2 of FOXP3 physically binds RORγT to prevent Th17 polarisation, and that patients with GCA display a higher frequency of IL-17 producing and FOXP3-exon 2 deficient (FOXP3Δ2) Tregs, which could play a pathogenic role in GCA.[28] We were able to recapitulate these observations in our cohort (figure 3G–I). In addition, we observed that at single cell level, FOXP3Δ2 Tregs expressed less CD25, as compared with their FOXP3 exon 2-expressing counterparts. To evaluate the relevance of identified phenotypic abnormalities of GCA Tregs, we performed a suppression assay evaluating the proliferation of conventional T cells ($CD4^+$ $CD25^{lo}CD127^{hi}$) in the presence or absence of $CD4^+$ $CD25^{hi}CD127^{lo}$ Tregs (figure 3H). The results show significantly reduced suppressive potential of GCA Tregs. Furthermore, the fact that the suppressive potential of Tregs from active patients was significantly lower to the one of patients in remission, suggests the association of Treg dysfunction with disease activity. Differences in differentiation state of Tregs could account for Treg dysfunction in GCA. To evaluate this, we measured the frequencies of activated Tregs and resting Tregs, as those were defined by Miyara *et al*.[33] In line with previous reports,[27 28] the latter revealed comparable proportions of activated to resting Tregs between GCA patients and HD (online supplemental figure 2), suggesting the occurrence

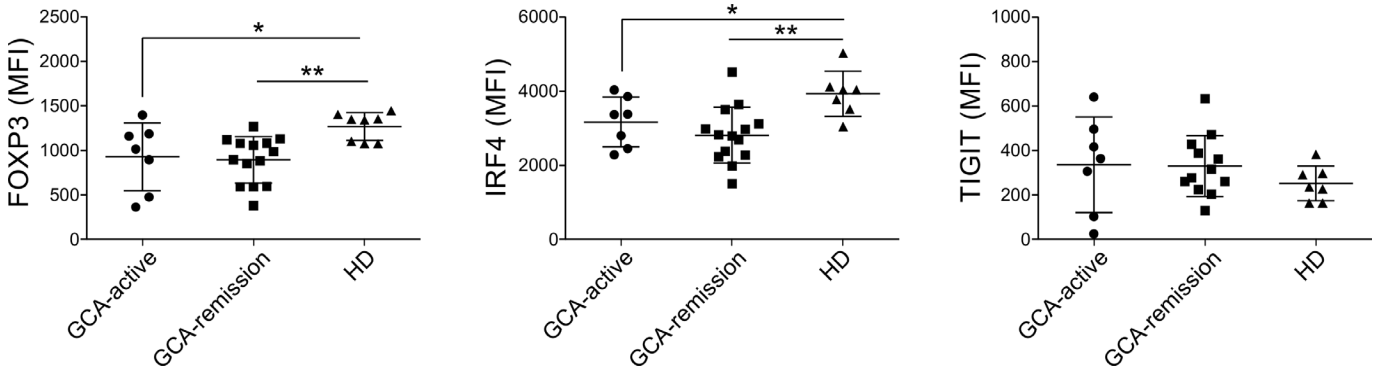

Figure 2 Reduced expression of FOXP3 and IRF4 by GCA Tregs, measured by flow cytometry as MFI. Bars represent the means±SDs. GCA, giant cell arteritis; HD, healthy blood donor; MFI, median fluorescence intensity.

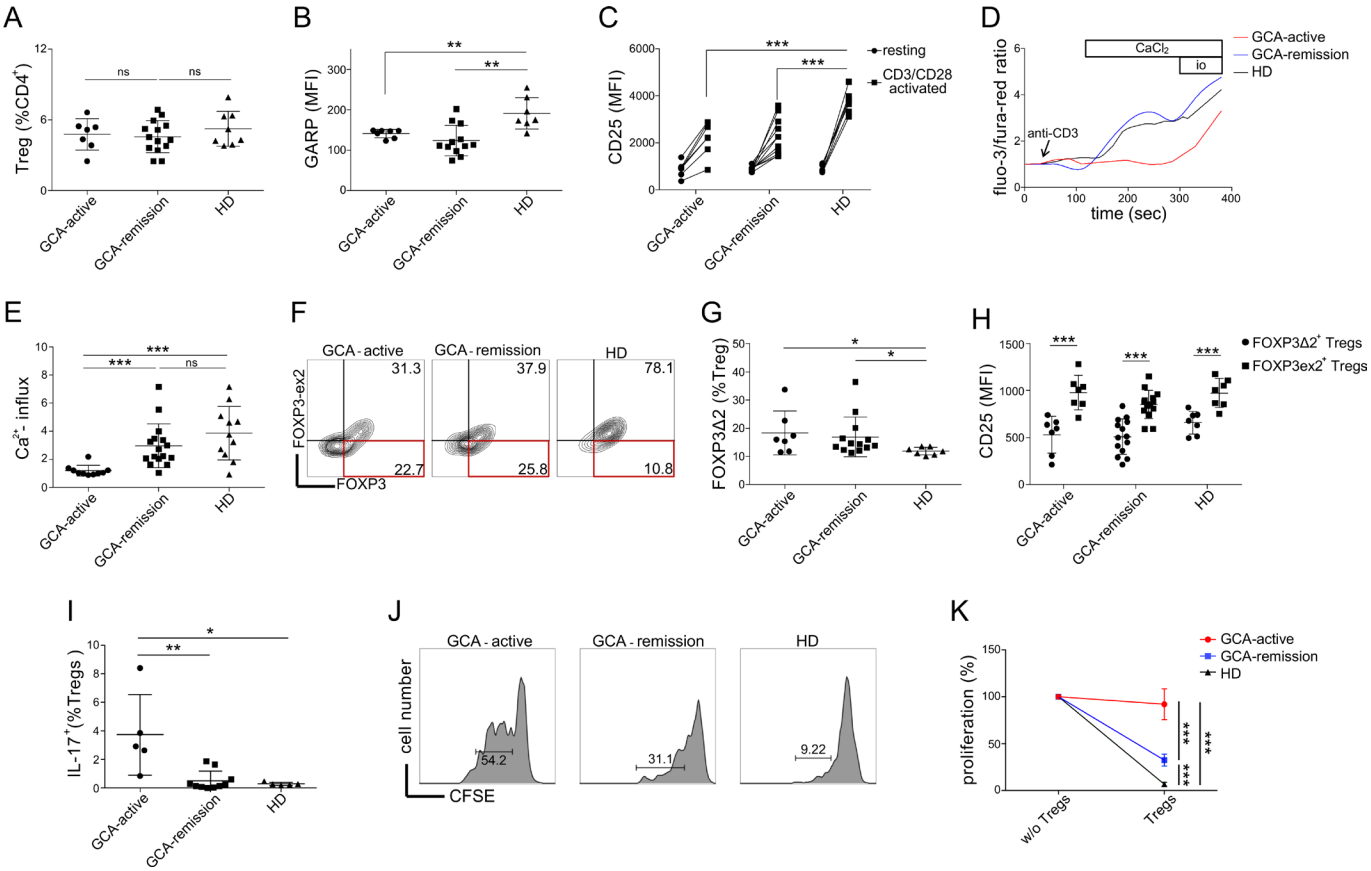

Figure 3 Treg dysfunction in GCA. (A) Frequencies of Tregs ($CD4^+CD25^{hi}CD127^{lo}$) in different groups (active GCA, GCA in remission, healthy donors). (B) Protein expression of GARP after 18 hours of CD3/CD28 stimulation. (C) Protein expression of CD25 at rest and after 18 hours of CD3/CD28 stimulation. (D) Representative calcium flux tracing (GCA patients vs healthy donors). At 60 s, anti-CD3 (clone: OKT3) was added (5 µg/mL). At 180 s, $CaCl_2$ was added (7 mM). At 330 s, ionomycin was added (14 µg/mL). (E) Calcium flux in Tregs (normalised to baseline) after $CaCl_2$ addition. (F) Representative gating strategy to quantify FOXP3Δ2 cells, using anti-FOXP3 clone 236A/E (total FOXP3) and clone 150D (measuring only exon 2). (G) Frequencies of FOXP3Δ2 cells (% Tregs). (H) Single-cell expression level of CD25 in FOXP3Δ2 Tregs versus FOXP3 exon 2-expressing Tregs at the basal state. (I) Frequencies of IL-17 producing Tregs (IL-17A+) (% Tregs, CD4 +CD25 hi CD127lo). (J) Treg suppression assay, conventional T cell proliferation in the presence of autologous Tregs: representative plots from patients with GCA (active and in remission) and a HD are shown. (K) Summary of cell proliferation normalised to the positive control of each sample (without Tregs) in patients with GCA (GCA-active: n=5, GCA-remission: n=10) and HD (n=5). GCA, giant cell arteritis; HD, healthy blood donor; MFI, median fluorescence intensity.

of Treg dysfunction in the presence of normal Treg population dynamics.

Glycolysis inhibition recapitulates dysfunction of GCA Tregs

As presented above, transcriptome analysis revealed lower expression of glycolytic enzymes, such as phosphofructokinase (*PFKP*) and enolase 1 (*ENO1*), in GCA Tregs (figure 1B). Despite several controversies regarding Treg metabolism, recent studies have shown that glycolysis promotes FOXP3 expression, and under certain circumstances, the suppressive function of human Tregs.[34 35] Therefore, to evaluate the likely role of reduced glycolysis in human Treg dysfunction, we evaluated the effect of glycolysis inhibition on Treg phenotypes, using 2-deoxyglucose (2-DG). Glycolysis inhibition in healthy Tregs led to failure of GARP and CD25 upregulation after 18 hours of TCR stimulation (figure 4A,B). Furthermore, TCR-induced calcium influx was effectively abolished by glycolysis inhibition (figure 4C,D). These findings suggests a direct link between glycolysis and calcium signalling in human *ex vivo* Tregs. On the other hand, glycolysis inhibition in GCA Tregs had no significant additive effect on reduced upregulation of GARP or CD25 (online supplemental figure 3A,B). Similar was the case with TCR-induced calcium influx in Tregs from patients with active GCA (online supplemental figure 3C). Finally, as glycolysis has also been linked to alternative splicing of FOXP3 in human iTregs,[36] we tested whether glycolysis inhibition in healthy Tregs could lead to higher frequencies of FOXP3Δ2 Tregs, which was the case (figure 4E). Similar to *ex vivo* GCA Tregs, FOXP3Δ2 cells expressed less CD25 than FOXP3 exon 2-expressing Tregs (figure 4F).

TCZ partially normalises GCA Treg dysfunction

As TCZ has been shown to enhance the suppressive function of Treg in GCA,[28] we evaluated the previously identified phenotypical changes in GCA Tregs in the subgroup of TCZ-treated patients, which all were in remission. As shown in figure 5A,B, TCZ treatment appears to enhance IRF4 but not FOXP3 expression in Tregs. Furthermore, the induction of GARP and CD25 remained impaired, also in Tregs from TCZ-treated patients (online supplemental figure 4). Similar to the rest of patients in remission, treatment with TCZ appears to normalise TCR-induced calcium influx in Tregs (figure 5C). Finally, we were able to recapitulate the previously described reduction in the frequency of FOXP3Δ2 Tregs in TCZ-treated patient with CGA

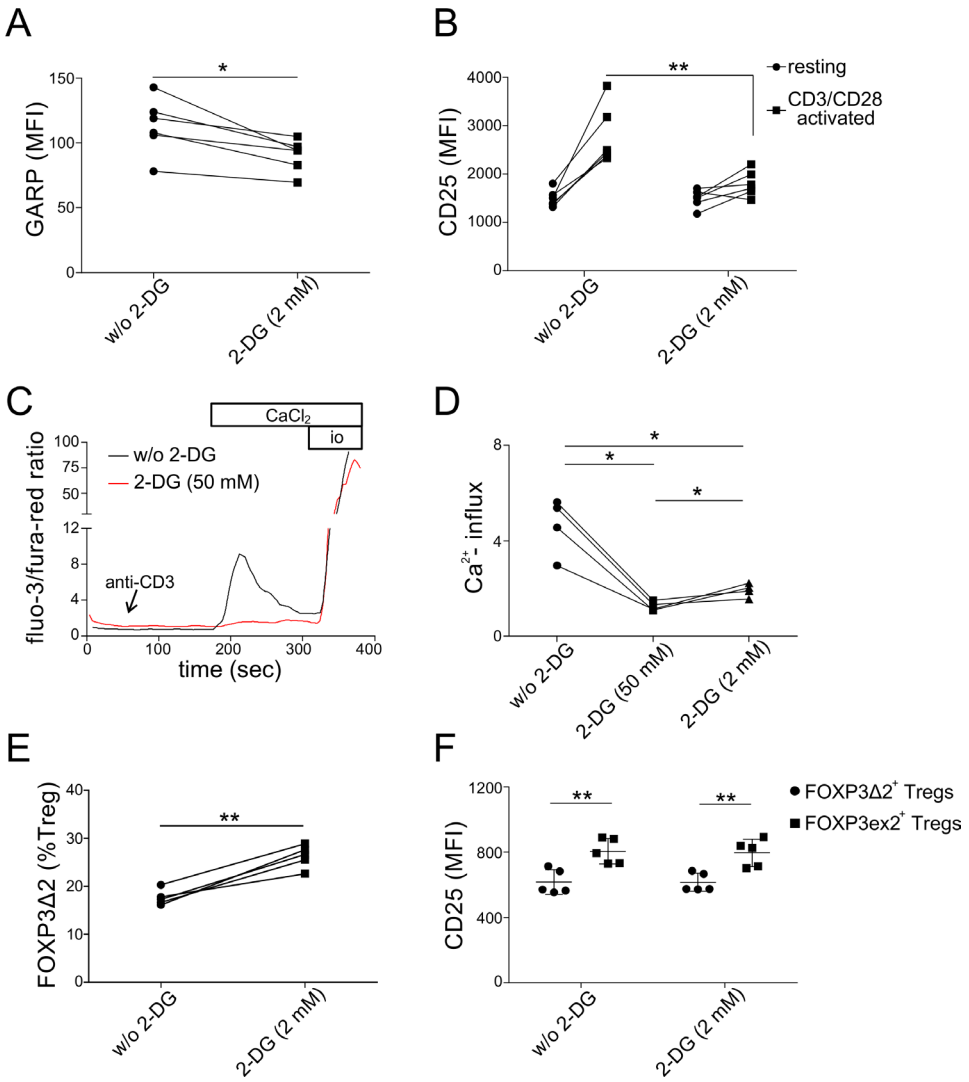

Figure 4 Glycolysis inhibition of healthy Tregs. (A) Protein expression of GARP after 18 hours of CD3/CD28 stimulation, following 48 hours of pre-incubation with 2-deoxyglucose (2-DG) (2 mM). (B) Protein expression of CD25 at rest and after 18 hours of CD3/CD28 stimulation, following 48 hours of 2-DG (2 mM). (C) Representative calcium flux tracing. Sorted Tregs were analysed in the presence or absence of 2-DG (50 mM). Anti-CD3, $CaCl_2$, and ionomycin were added at timepoints as described for figure 3D. (D) Calcium flux in Tregs (normalised to baseline) after $CaCl_2$ addition. Two concentrations of 2-DG were used for glycolysis inhibition (50 mM and 2 mM). (E) Frequencies of FOXP3Δ2 cells (% Tregs). (F) Single-cell expression level of CD25 in FOXP3Δ2 Tregs versus FOXP3 exon 2-expressing Tregs at the basal state, with and without 2-DG (2 mM). GARP, glycoprotein A repetitions predominan; MFI, median fluorescence intensity.

(figure 5D).[28] Partial reversion of Treg dysfunction by TCZ, is suggested by the improved suppressive potential of Tregs in the context of the suppression assay (figure 5E). Altogether, these findings demonstrate that IL-6 receptor blockade seems to improve calcium signalling and the suppressive function of Tregs in GCA. Most evaluated Treg parameters, especially TCR-induced calcium influx and the suppressive function, whose recovery correlates with remission, were comparable in GCA remission with or without TCZ (figure 5B–E). This together with the similar results after performing a subanalysis of patients in remission with or without methotrexate (online supplemental figure 5), suggest that improved function of Tregs is rather the consequence of effective immunomodulation in GCA and not a medication-specific effect.

DISCUSSION

Here we explored the aetiology of Treg dysfunction in GCA and identified for the first time the downregulation of IRF4 and FOXP3, which are critical transcription factors for both the polarisation and suppressive functions of Tregs, the reduction of TCR-induced calcium signalling as well as the insufficient upregulation of effector molecules as causes of Treg pathogenicity in GCA. Further, using transcriptome analysis we identify a marked downregulation of glycolytic enzymes in GCA Tregs, which may play a central role in the aetiology of most identified Treg dysfunctions.

Imbalances in the expression of FOXP3 isoforms have been reported in various autoimmune diseases, including rheumatoid arthritis and autoimmune thyroiditis.[37] Among FOXP3 isoforms, expression of the exon 2 containing FOXP3 appears critical for the suppressive function of human iTregs.[36] The fact that patients with *FOXP3* variants selectively affecting the expression of exon 2 develop IPEX, provides additional evidence on its *in vivo* regulatory function.[38] Mechanistically, based on murine Treg findings, it has been suggested that the regulatory role of the exon 2 of FOXP3 can be explained by the

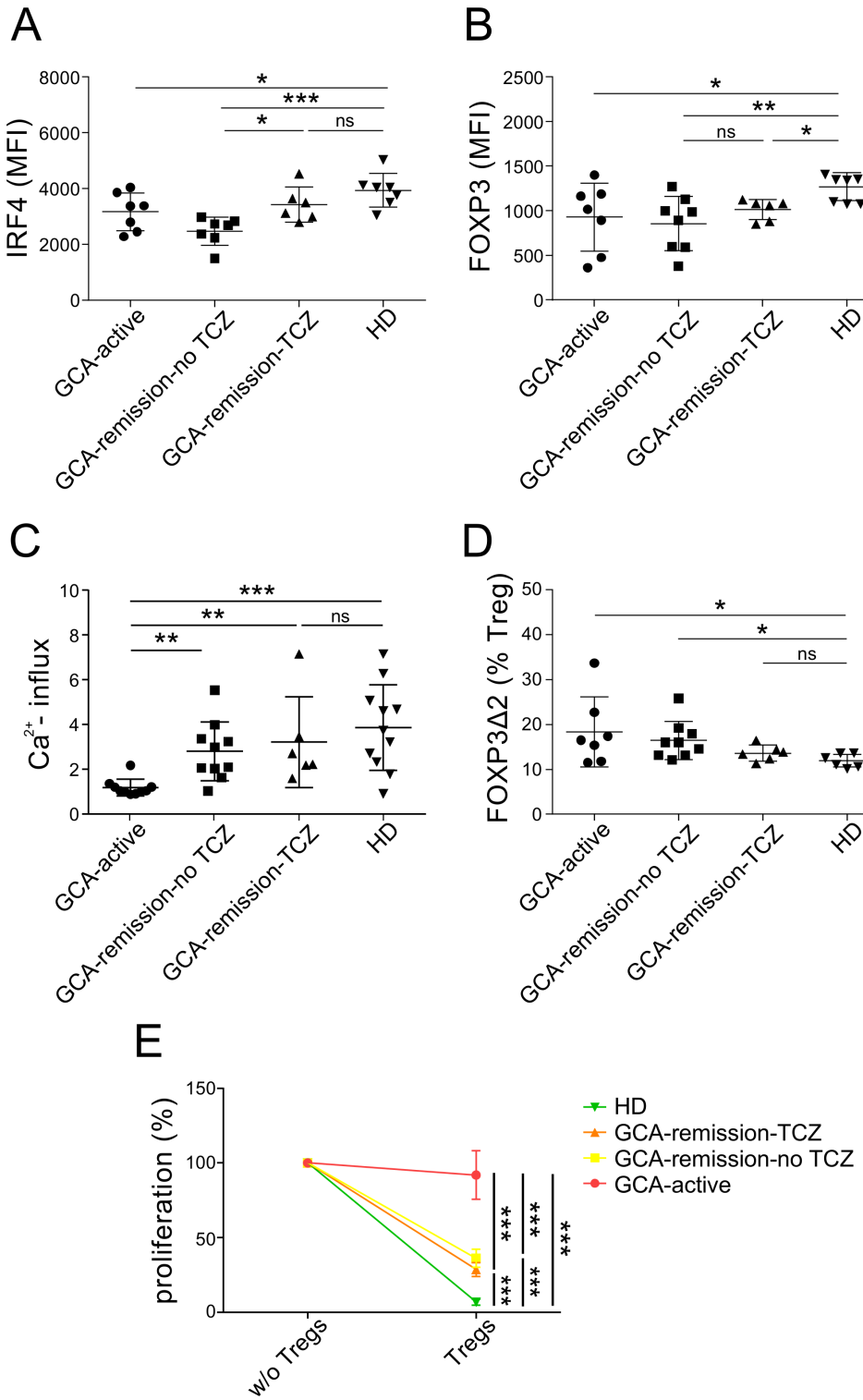

Figure 5 Tocilizumab normalised IRF4, FOXP3Δ2, and calcium flux. (A) Protein level of IRF4 in Tregs. (B) Protein level of FOXP3 in Tregs. (C) Frequencies of FOXP3Δ2 (% Tregs). (D) Calcium flux in Tregs (normalised to baseline) after $CaCl_2$ addition. (E) Treg suppression assay, conventional T cell proliferation in the presence of autologous Tregs: summary of cell proliferation normalised to the positive control of each sample (without Tregs) in patients with GCA (GCA-active: n=5, GCA-remission-TCZ: n=5, GCA-remission-TCZ: n=5) and HD (n=5). GCA, giant cell arteritis; HD, healthy blood donor; TCZ, tocilizumab.

fact that it physically antagonises RORγT, and exon 2-skipping increases the propensity of Tregs to produce IL-17, which potentially exacerbates inflammation.[39 40] The finding by Miyabe *et al* that GCA patients display higher frequencies of FOXP3Δ2 Tregs, which we confirmed, proved evidence on the pathogenicity of alternative FOXP3 splicing and especially reduced expression of the exon 2 of FOXP3 in GCA.[28]

TCR-induced calcium signalling largely depends on the pathway of store-operated calcium entry (SOCE).[41] Conditional deletion of SOCE mediators in murine Tregs and the consequent

loss of TCR-induced calcium influx, affected both their polarisation and effector differentiation, resulting in systemic autoimmunity.[32] Human STIM1 deficiency abrogates TCR-induced calcium influx and besides immunodeficiency causes autoimmunity,[42] which given the role of SOCE in murine Tregs could be explained by Treg dysfunction. Here we identify reduced TCR-induced calcium influx in GCA Tregs, which appeared to normalise in Tregs from TCZ-treated and the rest of patients in remission, suggesting the direct correlation of this finding with GCA inflammation.

Despite the longstanding belief that Tregs are not glycolytic but rather rely on the oxidative pathway of glucose metabolism,[43 35] several recent studies have identified various aspects of human Treg biology, including *FOXP3* alternative splicing, cell migration, proliferation and IL-2 signalling, which all depend on glycolysis.[35 36 43–46]Likewise, reduced glycolysis and the consequently compromised suppressive potential of Tregs has been implicated in pathogenesis of autoimmune diseases, such as multiple sclerosis and type 1 diabetes mellitus.[36 47] Mechanistically, de Rosa *et al* have shown that glycolysis controls *FOXP3* splicing and enhances the expression of exon 2-containing FOXP3, which is involved in the suppressive activity of Tregs.[36] Furthermore, a murine T cell study reported that the glycolytic metabolite phosphoenolpyruvate enhances TCR-induced calcium influx and that calcium mobilisation in T cells was reduced after 2-DG-mediated inhibition of glycolysis.[48] In this study, side-by-side characterisation of GCA Tregs and in vitro glycolysis-inhibited Tregs demonstrate that GCA-associated Treg abnormalities, such as the increased frequency of FOXP3Δ2 Tregs and the reduced TCR-induced calcium influx, can be recapitulated by glycolysis inhibition in healthy Tregs. Considering the central role of exon 2-expressing FOXP3 and the direct link between glycolysis and calcium signalling in Tregs, which we identify in the present study, our findings suggest that the downregulation of glycolytic enzymes in GCA Tregs is a central event in the aetiology of Treg dysfunction in GCA.

Longer term follow-up of GiACTA trial revealed that a minority of treated patients, that is, 42%, maintained clinical remission after stopping treatment with TCZ.[49] The requirement of long-term treatment together with the limited reliability of acute phase reactants under TCZ treatment can render the monitoring of disease activity expensive, necessitating vascular imaging such as PET.[50] Therefore, laboratory biomarkers reflecting disease activity independently of the acute phase response may be useful for evaluating disease activity in TCZ-treated patients. Our findings suggest the TCR-calcium response and the expression of IRF4 by Tregs as markers of GCA remission in TCZ-treated patients.

Apart from the above-mentioned correlates of remission, GCA Tregs – even from TCZ-treated patients – still display a largely dysfunctional phenotype, including lower activation-induced expression of CD25 and GARP. This suggests the need for novel therapeutic approaches with a broader effect on Treg dysfunction. On the other hand, the fact that TCZ or csDMARD-induced remission associated with normalised calcium influx only, highlights the central pathogenic role of compromised calcium signalling in GCA Tregs. The lack of steroid-sparing effect of the calcineurin inhibitor cyclosporine in GCA[51] may stem from the critical role of calcium signalling for Treg function and comes in line with the aforementioned finding.

The strengths of our study include the analysis of $CD4^+CD25^{hi}CD127^{lo}$ cells as this gating strategy reliably distinguishes *ex vivo* Tregs,[52 53] and the phenotypic analysis of *ex vivo* unprimed Tregs, which better reflects the in vivo setting. The use of RNA-Seq for transcriptomic profiling has been suitable to enumerate pathologies in GCA Tregs, which we validated at protein level and experimentally with in vitro analysis of healthy Tregs. Our patient cohort was representative of GCA, including patients in remission and active cases as well as treatment naïve patients. Weaknesses of the study include the small number of tested patients, especially for the transcriptome analysis, where a bigger number of patients could potentially have led to the detection of more DEGs. Study of a larger number of patients with GCA, including pretreatment samples as well as samples from patients with same disease activity status, receiving similar treatment, may have aided evaluation of the possible differential effects of the immunomodulatory agents on the Treg phenotype. On the other hand, small sample size can have underpowered the detection of differences between TCZ-treated patients and HD and may also account for the identification of normal Treg frequency in GCA, in the present study, which deviates from the reported reduced Treg counts in some of the previous studies on GCA Tregs.[26 27] In addition, we have not demonstrated the metabolic disturbance directly with a metabolic assay, most well-established of which is the Seahorse metabolic flux assay, due to limitations both regarding the assay sensitivity and the cell availability.[54] Another point that requires further research is the characterisation of Tregs from inflamed arteries, whose study would necessitate fresh samples and/or the development staining protocols reliably identifying Tregs and the expression levels of key tolerogenic molecules.

In summary, we present novel abnormalities of Treg function in GCA, suggesting the pathogenic role of low glycolysis and calcium signalling in GCA Tregs. Our findings may aid the development of therapeutic approaches targeting Treg dysfunction in GCA and provide new correlates of disease remission, which may be useful for monitoring disease activity, especially in case of TCZ-treated patients.

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

Ethics approval This study was conducted in accordance with the Declaration of Helsinki and was also approved from the Ethical committee of the Hannover Medical School (approval number: 8875). All patients signed an informed consent form.

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EPIDEMIOLOGICAL SCIENCE

Native joint infections in Iceland 2003–2017: an increase in postarthroscopic infections

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ABSTRACT

Objectives Nationwide study on the epidemiology, clinical characteristics and outcomes among patients with native joint infection (NJI) in Iceland, 2003–2017.

Methods All positive synovial fluid culture results in Iceland were identified and medical records reviewed.

Results A total of 299 NJI (40 children and 259 adults) were diagnosed in Iceland in 2003–2017, with a stable incidence of 6.3 cases/100 000/year, but marked gender difference among adults (33% women vs 67% men, p<0.001). The knee joint was most commonly affected, and *Staphylococcus aureus* was the most common isolate in both adults and children, followed by various streptococcal species in adults and *Kingella kingae* in children. NJI was iatrogenic in 34% of adults (88/259) but comprised 45% among 18–65 years and a stable incidence. Incidence of infections following arthroscopic procedures in adults increased significantly compared with the previous decade (9/100 000/year in 1990–2002 vs 25/100 000/year in 2003–2017, p<0.01) with no significant increase seen in risk per procedure. The proportion of postarthroscopic NJI was 0.17% overall but 0.24% for knee arthroscopy. Patients with postarthroscopic infection were more likely to undergo subsequent arthroplasty when compared with other patients with NJI (p=0.008).

Conclusions The incidence of NJI in Iceland has remained stable. The proportion of iatrogenic infections is high, especially among young adults, with an increase seen in postarthroscopic infections when compared with the previous decade. Although rare, NJI following arthroscopy can be a devastating complication, with significant morbidity and these results, therefore, emphasise the need for firm indications when arthroscopic treatment is considered.

Key messages

What is already known about this subject?

- There are few studies on native joint septic arthritis (native joint infection, NJI), which address the impact of iatrogenic infections.

What does this study add?

- This study confirms a high proportion of iatrogenic NJI in Iceland, with an increase seen in postarthroscopic infections when compared with the previous decade.
- Our nationwide analysis estimated the frequency of postarthroscopic NJI to be 0.17% rising to 0.24% for the knee joint.

How might this impact on clinical practice or future developments?

- Although rare, there are serious complications associated with arthroscopy, including infection which can cause considerable morbidity and potential sequelae.
- Appreciation of these risks and establishment of firm indications for arthroscopic procedures is essential.

INTRODUCTION

Bacterial septic arthritis (SA) is an uncommon yet serious infection which can lead to rapid destruction of the joint. These infections often require a lengthy hospital stay and prolonged treatment with intravenous antibiotics, resulting in high patient and healthcare burden. Delayed or inadequate treatment can result in permanent loss of joint function as well as life-threatening septicaemia. Even with treatment, overall mortality rates related to SA can be significant in adults, ranging from 3% to 23% with 30-day mortality rates of 2%–10%[1–8] and a poor functional outcome in 24% of cases.[9]

The incidence of SA is increasing according to some studies.[5 10 11] This can potentially be explained by an ageing population, increased use of immunosuppressive drugs and an increase in invasive diagnostic and therapeutic joint procedures such as joint injections, arthroscopies and open joint surgery. Arthroscopic procedures have increased steadily in the last two decades, largely supplanting open joint surgery (other than joint replacement surgery). The most common indications for arthroscopic procedures in adults are degenerative or traumatic meniscal tears and arthroscopic partial meniscectomy (APM) is one of the most commonly performed orthopaedic operations.[12 13] However, several studies have failed to show an advantage of arthroscopic surgery over conservative management or placebo surgery for these patients.[13–15]

There are few recent epidemiological and clinical studies on native joint infections (NJIs), which address the impact of iatrogenic infections. Two recent studies from New Zealand demonstrated that 16.9% and 17% of NJI were iatrogenic, respectively.[4 7] A previous study in Iceland, 1990–2002, showed a rising incidence of SA, primarily due to

cases related to open joint surgery and arthrocentesis.[10] The proportion of iatrogenic infections was 41.8% among adults, making further studies on this problem imperative.

The primary objective of this retrospective, nationwide study was to describe the epidemiology, clinical characteristics and outcomes of NJI in Iceland during the subsequent 15 years, 2003–2017, and in particular, to assess the impact of iatrogenic infections in native joints.

MATERIALS AND METHODS

Setting, data sources and identification of positive cultures

According to Statistics Iceland the country had 288 471 inhabitants at the beginning of the study period and 348 450 at the end. All inhabitants with residency of 6 months or more are covered by the Icelandic national health insurance, which is funded by taxes. Secondary care is provided by eight hospitals in the country, but vast majority of patients with joint infections are treated at two hospitals providing both secondary and tertiary care; most cultures of synovial fluid are performed in those two locations. Inpatient care is multidisciplinary, involving consultants in infectious disease, rheumatology and orthopaedics. A nationwide computerised and manual search for positive synovial fluid cultures was performed, covering a 15-year period, 1 January 2003–31 December 2017, in all microbiology departments in Iceland. In cases where no antibiotic therapy was administered and/or symptoms were not compatible with infection, the isolate was considered a contaminant.

Data collection, case definitions and exclusions

Medical records of all patients with positive synovial fluid cultures taken from a native joint were reviewed for the following data: patient age, sex, comorbidities including presence of underlying joint disease, immunosuppressive treatment, joints involved, history of recent arthrocentesis, arthroscopy or joint surgery, clinical presentation, concomitant infection, laboratory test results, microbiology results, antimicrobial treatment and surgical management. Patients with contaminated cultures; those without compatible symptoms and/or those who did not receive any antimicrobial therapy were excluded. In a few cases of NJI, there was doubt whether the isolated pathogen was the real cause of infection or contamination. These cases were included if the patient had clinical signs of infection and received full antibiotic therapy for NJI.

*Streptococcus pyogene*s isolates were *emm*-typed and serotypes of *Streptococcus pneumoniae* were identified using standard methods.

Iatrogenic NJI was defined as infections diagnosed within 8 weeks following arthrocentesis or arthroscopy; or within 6 months from open joint surgery. Relapse of infection was defined as readmission with NJI of the previously infected joint within 6 months after completing treatment with reidentification of the offending organism.

Definitive treatment, with a microbiologically appropriate drug, was defined as therapy administered for more than 50% of the total duration of parenteral antimicrobial therapy.

Epidemiology

Overall, gender-specific and age-specific incidence rates were calculated by dividing the number of cases by the overall, gender-specific and age-specific Icelandic population as listed by Statistics Iceland and expressed as cases/100 000 individuals/year.

The total number of arthroscopic procedures performed during the study period was acquired from the Directorate of Health in Iceland and used as the numerator in incidence calculations for arthroscopy-related infections as described above.

The prevalence of psoriatic arthritis (PsA) in Iceland is 0.14%[16] and the prevalence of rheumatoid arthritis (RA) is estimated between 0.8% and 1% based on previous Nordic studies.[17] These numbers were used when calculating the incidence rates of NJI in patients with RA and PsA, presented as cases/1000 patients/year.

Patient involvement

There was no active patient or public involvement in this retrospective study.

Statistical analysis

Statistical analysis was performed using R V.3.1.3 (R Core Team, Vienna, Austria) and GraphPad Prism V.9.0 (GraphPad Software, San Diego, California, USA). Poisson regression analysis was performed for the age specific incidence of NJI. The χ^2 test and Fisher's exact test were used for categorical variables and Mann-Whitney U test for continuous, non-normally distributed data. Multivariable logistic regression, adjusted for age and gender, was used with joint replacement surgery as the dependent variable and postarthroscopic NJI as the independent variable. Two-tailed testing was performed and $p<0.05$ used as the level of significance.

RESULTS

Identification of confirmed cases

The process for identification of confirmed infections is shown in figure 1. Over the 15-year period, 893 microbiological samples from joint fluid were registered as positive. In 257 cases, the sample was incorrectly marked as joint fluid, most often coming from an infected bursa and 199 were contaminants. Therefore, culture-confirmed cases were identified in 437 patients, of which 299 were NJI.

Demographics and clinical characteristics

Overall, 40 children under the age of 18 and 259 adults fulfilled criteria for NJI, with a significant gender difference seen among adults (33% women vs 67% men, $p<0.001$). The average age of adults was 60.2 years (SD 19) and children 6.4 years (SD 6.1) the youngest patient was 2 months old and the oldest 99 years old. The clinical and laboratory characteristics of NJI are shown by age in tables 1 and 2, respectively.

NJI occurred in nine patients with a history of injection drug use. In this patient group infection of the axial joints was significantly more common compared with non-injection drug users (4/9 vs 9/250, $p<0.001$).

A total of seven adult patients (2.7%) were diagnosed with concurrent endocarditis and there was an increase during the study period with one case diagnosed in 2003–2010 and six cases in 2011–2017 (1/156 vs 6/143, $p=0.05$).

Incidence

The overall average incidence of culture confirmed NJI was 6.3/100 000 residents/year for the period 2003–2017, ranging from 3.7 to 10 cases/100 000 residents (figure 2A). There was no significant change over the 15-year time period. Age-specific and gender-specific incidence is shown in figure 2B, demonstrating the highest incidence rates at the extremes of the age spectrum, and consistent gender differences across almost all age groups.

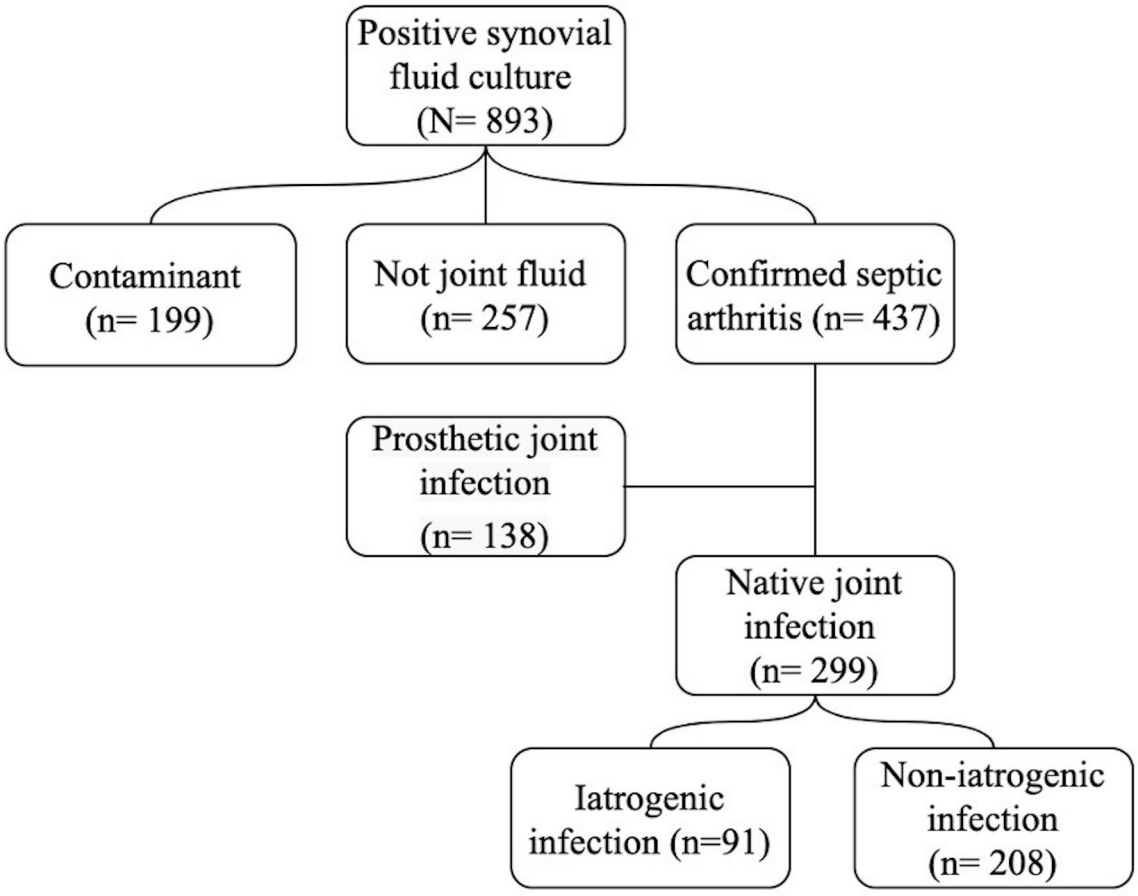

Figure 1 Flow chart showing identification of culture confirmed NJI in Iceland, 2003–2017. NJI, native joint infection.

Iatrogenic infections

NJI was considered iatrogenic in 7.5% of paediatric cases (3/40) and 34% of adult cases (88/259). Infection was diagnosed following arthroscopy in 22.4% (58/259) of cases, arthrocentesis in 7.3% (19/259) of cases and open joint surgery (other than joint replacement surgery) in 4.3% (11/259) of cases in adults (table 3). The median time from arthrocentesis to diagnosis of infection was 10 days (IQR 5.5–13 days) vs 13 days from arthroscopy (IQR 8–20.5 days) (p=0.03). The median time from open joint surgery to diagnosis was 52 days (IQR 26.5–65 days). In adults 18–65 years of age the overall percentage of iatrogenic infections was 45%, significantly higher than the percentage of iatrogenic infections among older adults (≥65 years) (67/149 vs 21/110, p<0.01). The difference was mostly due to postarthroscopic infections which were primarily observed in younger adults (53/149 vs 5/110, p<0.01) whereas postarthrocentesis NJI was more common in older adults (7/149 vs 12/110, p=0.09) (table 1).

When compared with a previous nationwide study covering 1990–2002, there was no significant change in the overall incidence of iatrogenic infections among adults (40/100 000/year in 1990–2002 vs 37.5/100 000/year in 2003–2017). There was a significant increase in infections among adults following arthroscopic procedures (9/100 000/year in 1990–2002 vs 25/100 000/year in 2003–2017, p<0.01). At the same time, the incidence of infections following arthrocentesis and open joint surgery (other than joint replacement surgery) decreased (17/100 000/year vs 8/100 000/year for arthrocentesis, p=0.1; 13.5/100 000/year vs 5/100 000/year, p=0.06 for open joint surgery, respectively).

During the whole study period at least 22 033 arthroscopic procedures were performed. However, due to incomplete data before 2010 the number of iatrogenic infections following arthroscopic procedures during 2010–2017 was used to calculate the risk per procedure. The estimated ratio of infections per arthroscopic procedure is shown in table 4. The knee joint was analysed separately since the majority of iatrogenic infections followed procedures on the knee.

Contributing risk factors

Overall, 82% of adults had a potential risk factor for NJI (online supplemental table 1). Underlying joint disease was present in 49% (127/259), with osteoarthritis being most common. Inflammatory rheumatic disease was present in 24% with crystal arthropathy being most common. There was a decrease in the incidence of infections in patients with RA and PsA during the study period (6.7 cases/1000 patients/year in 2003–2010 vs 2 cases/1000 patients/year in 2011–2017, p=0.04 and 15.9 cases/1000 patients/year vs 5.8 cases/1000 patients/year, p=0.3, respectively).

Microbial aetiology

Bacterial species isolated from joint samples are shown in table 5. Methicillin-susceptible-*Staphylococcus aureus* was most common in children and adults, followed by different streptococcal species in adults. Coagulase-negative staphylococci were significantly more common in NJI following any kind of joint procedure (33/88 vs 10/171, p<0.01). *Kingella kingae* was the second most common isolate in children and was only found in

Table 1 Clinical characteristics on admission among children and adults with NJI in Iceland, 2003–2017

	Children <2 years	2–18 years	Total	Adults 18–65 years	>65 years	Total	P value
No of cases	19	21	40	149	110	259	
Time to presentation (days)	2	3	3	4	2	3	
Joint pain	16/19 (84)	21/21 (100)	37/40 (93)	146/149 (98)	108/110 (98)	254/259 (98)	0.08
Swollen joint*	14/18 (78)	20/21 (95)	31/39 (79)	144/148 (97)	101/107 (94)	245/255 (96)	**<0.001**
DROM†	19/19 (100)	20/20 (100)	39/39 (100)	141/142 (99)	104/107 (97)	245/249 (98)	1
Joint redness¶	8/15 (53)	7/13 (54)	15/28 (54)	43/112 (38)	47/90 (52)	90/202 (45)	0.4
Warm joint‡	8/12 (67)	11/13 (85)	19/25 (76)	89/115 (77)	54/72 (75)	143/187 (76.5)	1
Infected joint:							
Knee	4/19 (21)	9/21 (43)	13/40 (33)	95/149 (64)	53/110 (48)	148/259 (57)	**0.006**
Shoulder	3/19 (16)	0	3/40 (7.5)	7/149 (5)	18/110 (16)	25/259 (10)	1
Elbow	2/19 (11)	7/21 (33)	9/40 (23)	1/149 (1)	0	1/259 (0.5)	**<0.001**
Hip	1/19 (5)	3/21 (14)	4/40 (10)	12/149 (8)	8/110 (7)	20/259 (8)	0.5
Ankle	6/19 (32)	2/21 (9.5)	8/40 (20)	11/149 (7)	7/110 (6)	18/259 (7)	**0.01**
Wrist	1/19 (5)	0	1/40 (2.5)	3/149 (2)	14/110 (13)	17/259 (6.5)	0.5
Hands and feet	0	0	0	5/149 (3)	6/110 (5)	11/259 (4)	–
Other joints§	2/19 (11)	0	2/40 (5)	15/149 (10)	4/110 (4)	19/259 (7)	1
Polyarticular infection	0	0	0	7/149 (5)	12/110 (11)	19/259 (7)	–
Iatrogenic infection	0	3/21 (14)	3/40 (7.5)	67/149 (45)	21/110 (19)	88/259 (34)	**<0.001**

Data are no/no (%).
P value, children vs adults.
P values <0.05 are shown in bold.
*Information on joint swelling found in: 18/19 cases <2 years, 21/21 cases 2–18 years, 148/149 cases 18–65 years and 107/110 >65 years.
†DROM: Information found in: 19/19 cases <2 years, 20/21 cases 2–18 years, 142/149 cases 18–65 years and 107/110 >65 years.
‡Information on temperature change over joint found in: 12/19 cases <2 years, 13/21 cases 2–18 years, 115/149 cases 18–65 years and 72/110 >65 years.
§Adults: 8 sternoclavicular, 7 acromioclavicular, 2 sacroiliac, 2 vertebral facet joint infections. Children: 2 sacroiliac infections.
¶Information on joint redness found in: 15/19 cases <2 years, 13/21 cases 2–18 years, 112/149 cases 18–65 years and 90/110 >65 years.
DROM, decreased range of motion; NJI, native joint infection.

children <3 years of age. *S. aureus* was the causative pathogen in 78% of infections among injection drug users and 56% of patients with RA. There was an increase in the number of NJI caused by *S. pyogenes* during the study period, with two infections diagnosed in 2003–2010 and 8 in 2011–2017 (2/156 vs 8/143, p=0.05).

Treatment and outcomes

Empiric and definitive antimicrobial therapy are summarised in online supplemental table 2, treatment and outcomes are summarised in online supplemental table 3. Median duration of parenteral antimicrobial treatment was significantly longer in adults compared with children, 29 days in adults (IQR=21–42 days) vs 13.5 days in children (IQR=10–28.5 days) (p<0.01). In adults, 24 patients (9%) received a prosthetic joint following infection. The average time from diagnosis to arthroplastic surgery was 3.5 years. Of these patients, 62.5% (15/24) had a history of iatrogenic infection with 46% (11/24) having postarthroscopic infection (11/58 vs 13/201, p=0.008) and 16.7% (4/24) having infection following arthrocentesis (4/19 vs 20/240, p=0.08). Postarthroscopic NJI was independently associated with the need for subsequent arthroplastic surgery after adjusting for age and gender with OR 3.6 (95% CI 1.3 to 10.3). The average time from postarthroscopic NJI to insertion of a prosthetic joint was 3.2 years.

Relapse of infection after treatment occurred in 4.6% of adult cases (12/259) with no relapse noted in paediatric cases of NJI. The average age of these patients was 61 years and 50% (6/12) had an iatrogenic infection (postarthroscopy four patients, postarthrocentesis one patient, open joint surgery one patient; p=0.2). *S. aureus* was the pathogen in 75% (9/12) and the median time of parenteral therapy before relapse was 29 days (IQR=21–41).

The overall 30-day mortality rate was 4.7% (14/299) and in adults the mortality was 5.4% (14/259). The average age of these patients was 81 years. Of those who died 50% had *S. aureus* infection. Deaths could be directly attributed to the infection in 57% of cases (8/14) while it was contributory in 43%.

DISCUSSION

This comprehensive nationwide study provides epidemiological, clinical and prognostic analysis of patients with culture-proven NJI over a 15-year period. The average incidence of NJI in Iceland 2003–2017 was 6.3 cases/100 000 residents per year, with the highest incidence rates seen at the extremes of the age spectrum, as found in previous studies.[10 11 18] The male predominance observed among NJI is also in line with earlier reports.[10 11 19 20]

The median time from the onset of symptoms to healthcare presentation and subsequent diagnosis in our study was 3 days, a markedly shorter time than previously reported.[1 2 21] One explanation for this shorter time may be universal ease of access to healthcare services in Iceland.

The proportion of patients with iatrogenic infection was 34% in adults, considerably higher than has been reported by others.[4 7] In young adults (18–65 years), nearly half of the infections were iatrogenic, which is alarmingly high. Previously, the mean number of iatrogenic infections rose from 2.8 annual infections in 1990–1994 to 9 infections/year in 1998–2002,[10] but according to our results they have since remained stable. The infections most often followed arthroscopic procedures while in the previous decade infection following arthrocentesis was most

Table 2 Investigations and laboratory results on diagnosis among children and adults with NJI in Iceland, 2003–2017

	Children <2 years	2–18 years	Total	Adults 18–65 years	>65 years	Total	P value
No of cases	19	21	40	149	110	259	
Admission data:							
Temperature (median, °C)	38	38	38	37.7	37.6	37.7	**0.02**
WCC (median, x10^9/L)	13.2	10.9	11.4	9.9	11.6	10.5	0.4
ESR (median, mm/hour)	34	35	35	54	71	57	**0.006**
CRP (median, mg/L)	49	48	49	123	180	148	**<0.001**
Synovial fluid WCC (median, x10^6)*	86 675	31 075	31 075	50 400	69 000	58 794	0.3
Positive blood culture†	3/16 (19)	7/15 (47)	10/31 (32)	32/100 (32)	42/79 (53)	74/179 (41)	0.4
Crystal arthropathy¶	–	–	–	5/60 (8)	22/63 (35)	27/123 (22)	–
Normal admission values:‡							
Temperature <37.8°C	6/19 (32)	8/21 (38)	14/40 (35)	78/141 (55)	59/102 (58)	137/246 (56)	**0.02**
WCC <10.5×10^9/L	6/19 (32)	10/21 (48)	16/40 (40)	84/143 (59)	41/109 (38)	125/252 (50)	0.3
CRP <10 mg/L	2/19 (10.5)	4/21 (19)	6/40 (15)	4/143 (3)	6/107 (6)	10/250 (4)	**0.01**
ESR <20 mm/hour	1/10 (10)	1/7 (14)	2/17 (12)	11/70 (16)	7/49 (14)	18/119 (15)	1
Other infective syndromes:							
Adjacent osteomyelitis	3/19 (16)	9/21 (43)	12/40 (30)	16/149 (11)	13/110 (12)	29/259 (11)	**0.005**
Endocarditis	0	0	0	3/149 (2)	4/110 (4)	7/259 (3)	–
Cellulitis	0	0	0	3/149 (2)	3/110 (3)	6/259 (2)	–
Other§	0	0	0	3/149 (2)	5/110 (5)	8/259 (3)	–

Data are no/no (%).
P value, children vs adults.
P values <0.05 are shown in bold.
*Information on number of WCC in the synovial fluid obtained was found in 171 adult cases and 12 paediatric cases.
†Information on blood culture (whether taken, positive or negative) was found in: all paediatric cases, 141 cases in age group 18–65 years and 103 cases >65 years. Blood culture was performed in 16/19 <2 years, 15/21 cases 2–18 years, 100/149 cases 18–65 years and 79/110 >65 years.
‡Information on (A) temperature was found in: all paediatric cases, 144/149 cases 18–65 years and 102/110 >65 years. (B) WCC: all paediatric cases, 143/149 cases 18–65 years and 109/110 >65 years. (C) CRP: all paediatric cases, 143/149 cases 18–65 years, 107/110 >65 years. (D) ESR: 10/19 cases <2 years, 7/21 cases 2–18 years, 70/149 cases 18–65 years and 49/110 >65 years.
§Pneumonia 5 (all patients were >65 years), urinary tract infection 2, streptococcal toxic shock syndrome one patient.
¶Information on concurrent crystal arthropathy in adults was found in: 60/149 cases 18–65 and 63/110 >65 years.
CRP, C reactive protein; ESR, erythrocyte sedimentation rate; NJI, native joint infection; WCC, white cell count.

common (online supplemental figure 2). The frequency of postarthroscopic NJI in older reports ranges from 0.1% to 0.5%[10 22] with more recent publications reporting 0.15%–0.84%.[23–25] Our study estimated the frequency of postarthroscopic NJI to be 0.17%, with 0.24% for the knee joint, which is consistent with these reports.

Although the frequency of postarthroscopic arthritis per procedure has remained stable over the last 15 years, the number of infections has increased due to an increase in the number of arthroscopic procedures. The Directorate of Health in Iceland published a report in 2017 on the yearly numbers of arthroscopic knee procedures performed in Iceland. From 2012 to 2016, the frequency was estimated to be 590 arthroscopies/100 000 residents/year and 890 procedures/100 000 residents over the age of 50.[26] These numbers are considerably higher than those published from other Nordic countries. In 2011, the frequency of arthroscopies among patients older than 55 years in Denmark was 322 procedures/100 000 residents and in 2012 the frequency of knee arthroscopies in patients older than 18 years in Finland was 347/100 000.[27 28] For further comparison the frequency in Iceland in 2012 in patients older than 18 years was 770 knee arthroscopies/100 000 residents.[26] This indicates that arthroscopic knee procedures are overused in Iceland and since the evidence of their benefit is weak with previous studies consistently showing that APM, one of the most commonly performed arthroscopic procedure, offers no benefit over conservative treatment or placebo surgery, the indications for these procedures should be reviewed.[12–15] Additionally, other potential complications including pulmonary embolism, cast this wide practice in doubt.[29]

A high number of adult NJI patients had an underlying joint disease (49%) of which 24% had inflammatory rheumatic disease and just over 6% of patients had RA, which is considered to be a major risk factor for NJI. This proportion of patients with RA was lower than reported in some studies,[1 21] yet similar to some.[3 4 18] These data, therefore, suggest that it may be inflammatory joint disease itself rather than the specific disease process which raises risk of NJI. A decrease was seen in the incidence of NJI in patients with RA and PsA over the study period. Although immunosuppressive drugs such as disease-modifying antirheumatic drug and TNF-alpha inhibitors are considered to increase the risk of infections, it is possible that their role in preserving function and subsequent decreased need for arthrocentesis and intra-articular steroids may offer overall protection against infection.

The median duration of parenteral antimicrobial treatment for NJI was significantly longer for adults (4 weeks) compared with children (2 weeks) (online supplemental table 3). The optimal duration of antimicrobial therapy in children has been studied with prospective, randomised trials showing that treatment with intravenous antibiotics for short periods (4–7 days), followed by oral therapy was as successful as longer courses of parenteral therapy[30 31] and guidelines for treatment were changed accordingly in 2011. There is less consensus on the duration of treatment for NJI in adults. The general recommendation is 2–4 weeks of intravenous treatment followed by oral therapy for at

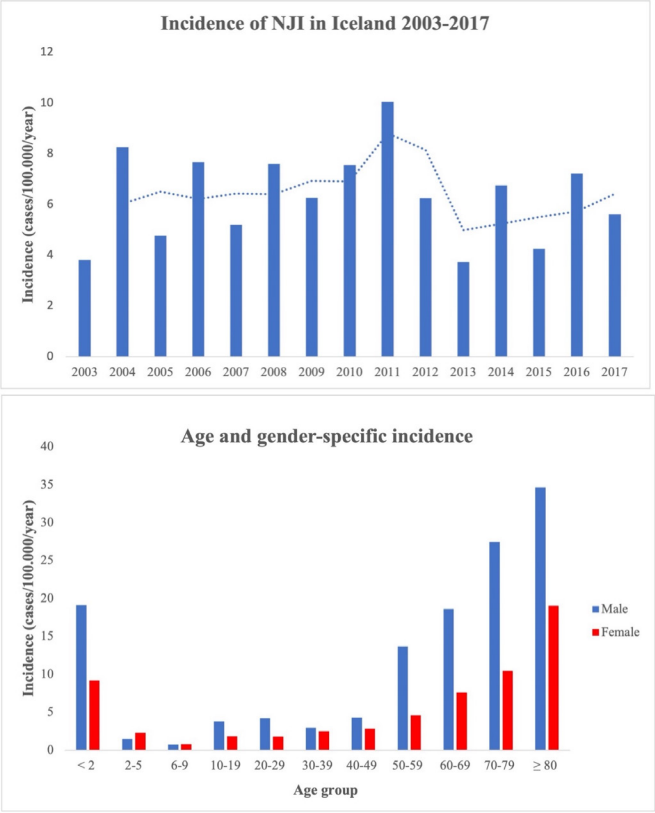

Figure 2 (A) Overall annual incidence of culture confirmed NJI in Iceland, 2003–2017. The dashed line shows running 2-year average. (B) Age-specific and gender-specific incidence of culture confirmed NJI in Iceland, 2003–2017. NJI, native joint infection.

least 7–14 days. This is consistent with the median duration of parenteral antibiotic therapy in adults in our study, but it is probable that the time could have been shortened in selected cases, as suggested by more recent studies.[7 32]

The mortality rate in our study was 5.4% in adults, which is in the lower range compared with previous reports.[1 2 4–6 8] We found that 9% of adults in our cohort required arthroplastic surgery, all within 9 years of diagnoses of NJI. A large retrospective cohort study on patients who received arthroscopic knee washout for NJI between 1997 and 2017 in England, found that within 15 years, 9% of patients had knee arthroplasty, corresponding to an annual rate of arthroplasty about six times higher than in the general population.[8] These findings highlight the potential risk of long-term consequences following NJI. Our study found that patients with a history of postarthroscopic infection are significantly more likely to require arthroplastic surgery compared with other patients with NJI, after adjusting for age and gender. Previous cohort studies suggest that progression of osteoarthritis may be more rapid in those who have undergone arthroscopic procedures, possibly accelerating the need for arthroplastic surgery.[33 34] When bacterial infection complicates these procedures, more rapid destruction of the joint is likely to further accelerate the need for joint replacement.

The comprehensive nationwide approach of this study as well as the microbiological confirmation of cases is the main strengths of this study. Moreover, detailed clinical and follow-up information was available for most cases and historical information was available for epidemiological comparisons. Nevertheless, this study has important limitations. The number of NJI in our study is underestimated due to limitation of case ascertainment to culture positivity. Case ascertainment using diagnostic codes or Newmans's modified criteria would likely have increased our case numbers. In particular, cases of small joint NJI are underrepresented in our cohort since synovial fluid sampling is often not performed when small joint NJI is suspected as these joints often do not have enough volume to support needle aspiration.[7] Similarly, it is likely that the incidence of NJI in young children is underestimated in our study as cultures of synovial fluid are the mainstay of diagnosis while PCR for *K. kingae* has been shown to

Table 4 Ratio of infections per arthroscopic procedure, 2010–2017

Joint	No of SA following arthroscopy	No of arthroscopic procedures	Ratio, %
All joints	37	21 342	0.17
Knee joint	32	13 290	0.24
Other joints*	5	8052	0.06

*Shoulder joint: 4, ankle joint: 1.
SA, septic arthritis.

Table 3 Iatrogenic NJI in adults in Iceland 2003–2017

	Arthrocentesis	Arthroscopy	Open joint surgery	Total
No of cases	19	58	11	88
Age (years, median)	69	54	63	57
Time from procedure to diagnosis (days, median)	10	13	52	13
Male/female ratio	1.4	2.6	2.7	2.3
Infected joint				
Knee	11/19 (58)	52/58 (90)	8/11 (73)	71/88 (81)
Shoulder	3/19 (16)	4/58 (7)	1/11 (9)	8/88 (9)
Other*	5/19 (26)	2/58 (3)	2/11 (18)	9/88 (10)
Synovial culture				
Staphylococcus aureus	11/19 (58)	23/58 (40)	4/11 (36)	38/88 (43)
CNS	4/19 (21)	23/58 (40)	6/11 (55)	33/88 (38)
Streptococci†	3/19 (16)	5/58 (9)	1/11 (9)	9/88 (10)
Other‡‡	1/19 (5)	7/58 (12)	0	8/88 (9)

Data are no/no (%). Median values for age and time from procedure to diagnosis are shown.
*Acromioclavicular, ankle, hip, proximal interphalangeal and wrist joint.
†Streptococcus, viridans group: 3, Streptococci group C or G: 4, *Streptococcus mitis:* 1, *Streptococcus pneumoniae:* 1.
‡*Enterobacter cloacae*, gram positive rods, *Klebsiella oxytoca*, *Lactococcus cremoris*, Micrococcus species.
CNS, coagulase-negative staphylococci; NJI, native joint infection.

Table 5 Results of synovial fluid culture in NJI in Iceland 2003–2017

Bacterial isolates	Children (<18 years) n=40	Adults (≥18 years) n=259	Total (%) n=299
Staphylococcus aureus††	19 (48)	137 (53)	156 (52)
Staphylococcus lugdunensis	0	7 (3)	7 (2)
Coagulase-negative Staphylococci	1 (2.5)	43 (17)	44 (15)
Streptococcus agalactiae	0	8 (3)	8 (3)
*Streptococcus pneumoniae**	1 (2.5)	8 (3)	9 (3)
Streptococcus pyogenes†	1 (2.5)	9 (3.5)	10 (3)
Streptococci group C or G	1 (2.5)	20 (8)	21 (7)
Other Streptococci	2 (5)	8 (3)	10 (3)
All Streptococci	5 (12.5)	53 (20)	58 (19)
Escherichia coli	0	3 (1)	3 (1)
Enterobacter cloacae	0	4 (1.5)	4 (1)
Kingella kingae	11 (28)	0	11 (4)
Other gram-negative rods‡	1 (2.5)	6 (2)	7 (2)
All gram-negative rods	12 (30)	13 (5)	25 (8)
Neisseria gonorrhoea	0	1 (0.4)	1 (0.5)
Gram positive rods§	3 (7.5)	4 (1.5)	7 (2)
Other¶	1 (2.5)	6 (2)	7 (2)
Total**	41	264	305

Data are no/no (%).
*Serotypes in year 2003: 22, 2004: 4, 12, 35, 2007: 19F, 6B, 2009: 6B, 2014: 21, 2016: 15B/C.
†Information on *emm* types was available in 9/10 isolates. Overall, seven different *emm* types were identified, the most common type was *emm*3 (33%), all three cases isolated after 2013. Other types were emm1, 12, 28, 81, 87, 89.
‡*Burkholderia pseudomallei, Escherichia hermannii, Klebsiella oxytoca, Pasteurella multocida, Pseudomonas aeruginosa, Rosemonas* species, *Stenotrophomonas maltophilia*.
§*Bacillus* species, *Clostridium perfringens, Corynebacterium* species, gram positive rods unspecified.
¶*Aerococcus urinae, Enterococcus faecium, Lactococcus cremoris, Micrococcus* species, anaerobic gram-positive cocci unspecified.
**Four patients had two bacterial species isolated, one had three.
††Only Methicillin-susceptible *S. aureus* was isolated.
NJI, native joint infection.

have significantly better sensitivity. It is, however, unlikely that this will influence the rate of complications as the clinical course of *K. kingae* NJI is usually benign.[35 36]

CONCLUSION

This study suggests that the incidence of NJI in Iceland has remained stable over the past 15 years. However, the proportion of iatrogenic infections is high, seen in 45% of young adults, most often following arthroscopic procedures which are increasingly being performed on relatively young patients with joint complaints. Although the overall frequency of postarthroscopic infections remains unchanged, the incidence of these infections has significantly increased when compared with the previous decade due to more widespread use. Our study suggests that patients with postarthroscopic infection are more likely to relapse after treatment when compared with other patients with NJI and are more likely to receive arthroplastic surgery. Despite being rare, there are serious complications associated with arthroscopy, including infection which entails considerable morbidity. Appreciation of these risks and establishment of firm indications for arthroscopic procedures is essential.

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Contributors MG had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design: SLG, HE, SG and MG. Acquisition of data: SLG, HE and MG. Analysis and interpretation of data: SLG and MG. All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published.

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Genetic predisposition (HLA-SE) is associated with ACPA-IgG variable domain glycosylation in the predisease phase of RA

In addition to Fc glycans, IgG can carry *N*-linked glycans in the variable domain. The abundant presence of disialylated variable domain glycans (VDGs) is a special feature of anti-citrullinated protein antibody (ACPA)-IgG and possibly other autoantibodies. The introduction of glycosylation sites is mediated by somatic hypermutation (SHM), a T-cell dependent process.[1] The high frequency of glycosylation sites does not correlate with the number of SHM, pointing towards a selective advantage of B cells expressing variable domain glycosylated ACPA.[2] Previously, we observed that ACPA-IgG VDGs are already present in the phase preceding rheumatoid arthritis (RA) onset and predictive for disease development.[3] In addition, we provided first evidence that the human leucocyte antigen (HLA) 'shared epitope' (SE) alleles, the most prominent genetic risk factor for ACPA-positive RA, are associated with the presence of VDG on ACPA-IgG predisease.[4] Hence, VDG could possibly explain the contribution of HLA-SE restricted T cells in the maturation of the ACPA response. Building on these results, we now hypothesised that HLA-SE alleles may not be associated with ACPA positivity as such, but with the specific presence of variable domain glycosylated ACPA-IgG, a favourable factor for the development of this multifactorial disease.

To substantiate our hypothesis, we expanded the set of presymptomatic individuals (n=228) and RA-patients (n=126) from Sweden and analysed two additional cohorts comprising ACPA-positive Dutch subjects with arthralgia (n=239) and ACPA-positive healthy Japanese individuals (n=58) (online supplemental table S1). We determined the presence/percentage of ACPA-IgG VDG using liquid chromatography[5] and assessed associations with HLA-SE (online supplemental materials and methods). In particular, we focused on the most prominent glycan peak (GP24) found on top of the variable domain,[1] which carries a bisecting *N*-acetylglucosamine and two terminal sialic acids (G2FBS2) (figure 1A). ACPA-IgG VDG were, with a median of 58%, already abundantly expressed in healthy individuals (online supplemental table S1), in contrast to conventional IgG molecules that yield 12% of VDG.[6] VDG (p=0.047) and GP24 (p=0.003) were significantly higher in HLA-SE + Dutch individuals with arthralgia compared with the HLA-SE-negative group (figure 1B and online supplemental tables S2 and S3). HLA-SE DR4+ (HLA-DRB1*04:01, *04:04, *04:05, *04:08 and *04:10 alleles) individuals showed the strongest increase in VDG (p=0.009) and GP24 (p=0.005) compared with HLA-SE-negative subjects (figure 1B). Even though we observed a strong correlation between VDG and ACPA levels (online supplemental figure S1), we could not identify an association between ACPA levels and HLA-SE (p=0.66) (online supplemental table S4). Moreover, in line with our hypothesis, the association between HLA-SE and GP24 remained significant after correcting for ACPA levels in a multivariable analysis (HLA-SE: p=0.03, HLA-SE DR4+: p=0.07) (online supplemental table S3), indicating that HLA-SE primarily associates with abundantly variable domain glycosylated ACPA.

Interestingly, subjects with an 'incomplete' VDG (lower than the median of 75%) (online supplemental table S1) were more prone to transition to RA, if they were HLA-SE DR4+ (HR: 2.74, p=0.029) (figure 1C). Conceivably, HLA-SE restricted T cells increase SHM and hence the formation of glycosylation-sites, impacting on a subsequent increased risk to develop disease.[3] Likewise, although underpowered and statistically not significant, VDG and GP24 were numerically increased in the healthy ACPA-positive subjects from Japan, mainly in the HLA-SE DRB1*04:05+ group, the predominant HLA-SE alleles in this population (figure 1D).

The association between HLA-SE and increased VDG percentages was not present in the Swedish dataset, possibly because all subjects transition to RA (online supplemental online supplemental table S5). However, the findings replicated our previous data, as HLA-SE alleles associated with the presence of ACPA-IgG VDG in the pre-RA phase, after correcting for ACPA positivity (OR: 1.998, p=0.040) (online supplemental table S7). No association was found between HLA-SE and ACPA in a reciprocal analysis (ie, after correcting for the presence of VDG) (OR: 0.620, p=0.254) (online supplemental table S8). Similar to our preceding analyses, this correlation was only found predisease as we could not identify a link between HLA-SE and VDG in established disease (OR: 0.305, p=0.269) (online supplemental table S9), likely because most ACPA-IgGs carry an abundant amount of VDG by then (online supplemental table S1). Thus, in the phase preceding RA, HLA-SE alleles are associated with ACPA harbouring elevated amounts of VDG. Additionally, HLA-SE + individuals showed a significant increase in VDG towards disease onset (matched paired analysis; figure 1E,F).

Hence, the data presented support a concept in which HLA-SE restricted T cells stimulate the introduction of glycosylation sites in ACPA-expressing B cells, an event taking place before the development of ACPA-positive disease. HLA-SE can thus be considered as an 'accelerating factor' causing the abundant expression of VDG on ACPA-IgG (figure 1G). Our data also provide an explanation for why HLA-SEs do not associate with ACPA in healthy individuals[7 8] as these are not yet abundantly glycosylated in their variable domains. The fact that all ACPA-IgGs are heavily glycosylated in established RA explains why HLA-SE associate with ACPA in this disease stage and emphasises the possibility that VDGs serve as an important 'hit' involved in the unrestrained expansion of the RA-specific autoreactive B-cell response.

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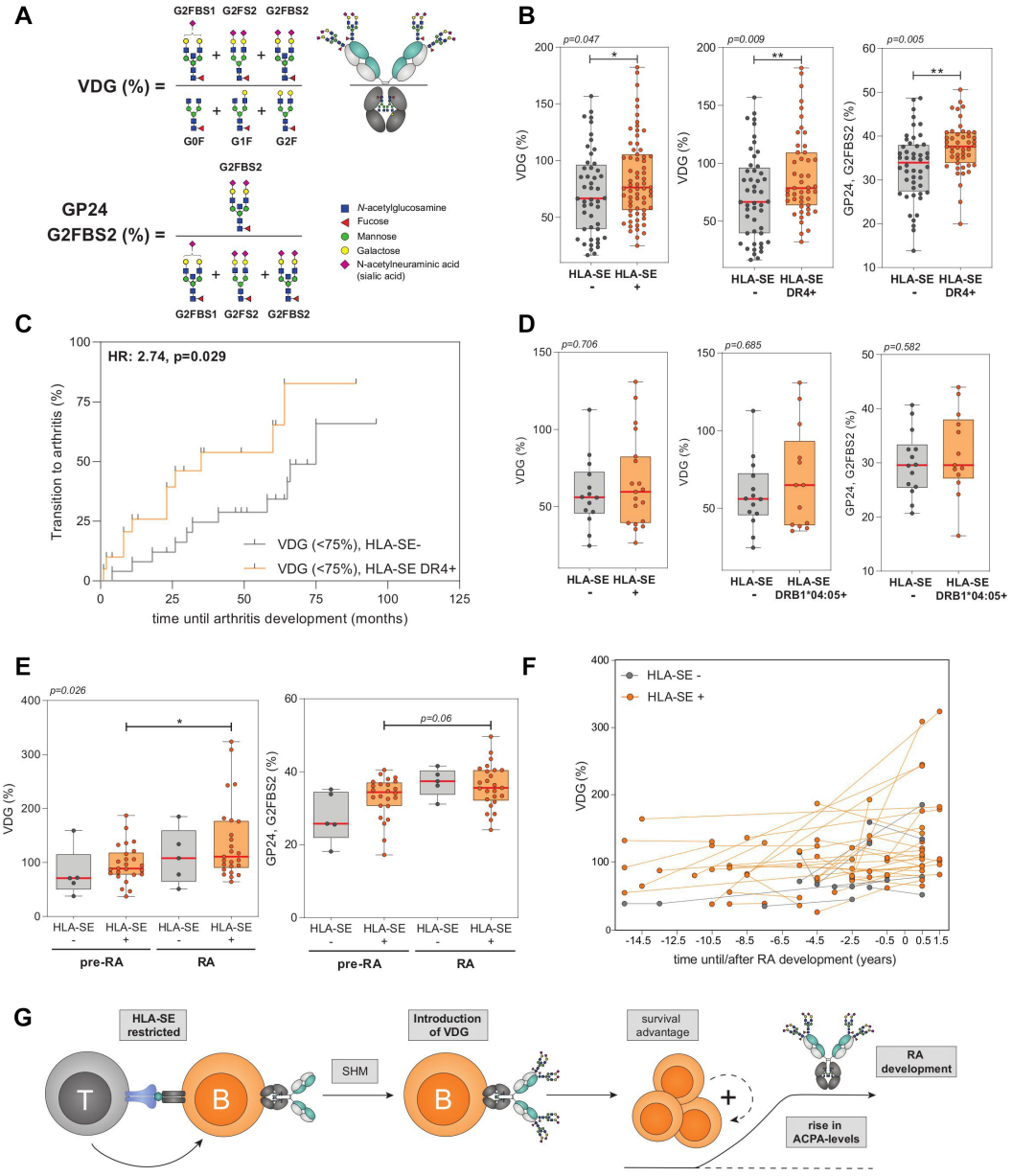

Figure 1 Percentage of anti-citrullinated protein antibody (ACPA)-IgG variable domain glycosylation (VDG) and glycan peak 24 (GP24, G2FBS2) in HLA-SE- and HLA-SE + individuals. (A) Formulas to calculate the percentage of ACPA-IgG VDG and the most common complex-type disialylated glycan peak found on top of the variable domain, GP24. ACPA-IgGs were captured; glycans were released using PNGaseF; 2-AA was labelled, and hydrophilic interaction liquid chromatography-solid phase extraction (HILIC SPE) was purified and analysed using ultra-high performance liquid chromatography (UHPLC). The formulas presented are based on the abundance of the liquid chromatography determined Fc glycan traits G0F, G1F and G2F, and VD glycan traits G2FBS1, G2FS2 and G2FBS2. The respective glycans and their locations on the antibody molecule are schematically illustrated. Agalactosylated (G0), monogalactosylated (G1), digalactosylated (G2), fucose attached to the core GlcNAc (F), bisecting GlcNAc (B), monosialylated (S1), disialylated (S2). Blue square: GlcNAc, green circle: mannose, yellow circle: galactose, red triangle: fucose, pink diamond: *N*-acetylneuraminic acid. (B) ACPA-IgG + individuals with arthralgia from the Netherlands (Amsterdam). Increased ACPA-IgG VDG of HLA-SE+ (n=67) compared with HLA-SE- (n=48) individuals. Significantly higher ACPA-IgG VDG and GP24 in HLA-SE DR4+ (n=47) individuals. (C) ACPA-IgG + individuals with arthralgia from the Netherlands (Amsterdam) with a VDG of <75% (n=49). ACPA-IgG + individuals with arthralgia with a VDG lower than 75% are more prone to transition to disease and transition earlier, if they are HLA-SE DR4+ (HR 2.74, 95% CI 1.07 to 7.00; p value: 0.029). (D) ACPA-IgG + symptom-free healthy individuals from Japan (Nagasaki). Statistically not significant trend for an increased percentage of ACPA-IgG VDG and GP24 in HLA-SE+ (n=19) particularly HLA-SE DRB1*04:05 (n=13) healthy individuals compared with the HLA-SE- (n=14) group. (E,F) Matched pairs of samples from pre-symptomatic individuals and patients with RA from Sweden (Umea) (n=59). HLA-SE + individuals show a significant increase in their percentage ACPA-IgG VDG and GP24 towards disease onset. HLA-SE + presymptomatic individuals (n=24) show already high VDG levels up to 15 years before RA onset. (G) Graphical illustration of concluding hypothesis. HLA-SE restricted T cells give help to ACPA-IgG expressing B cells, which results in SHM and the introduction of *N*-linked glycan sites, and consequently VDG (associations between HLA-SE and VDG). These ACPA-IgG VDG + B cells expand, leading to a rise in ACPA levels and ultimately towards disease development. Mann-Whitney U tests or linear regression analysis was performed between non-paired and Wilcoxon signed-rank test between matched paired samples. The comparison of the survival curves was performed using a Mantel-Cox test. Significant differences are denoted by * or **, and the respective p values are represented. GlcNac, *N*-acetylglucosamine; HLA-SE, human leucocyte antigen-shared epitope; RA, rheumatoid arthritis; SHM, somatic hypermutation; VDG, variable domain glycan.

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Competing interests HUS, TWJH and REMT are mentioned inventors on a patent on ACPA-IgG V-domain glycosylation.

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B-cell targeted therapy is associated with severe COVID-19 among patients with inflammatory arthritides: a 1-year multicentre study in 1116 successive patients receiving intravenous biologics

Dear Editor,

A potential association between rituximab and more severe COVID-19 outcomes has been previously raised, based on case reports, retrospective studies and mostly declarative registries.[1–4] To further investigate this association, we focused on patients with inflammatory arthritides (IA) receiving intravenous biological agents at day hospitals to limit selection and recall bias, as well as missing data.

All patients with IA treated in day hospitals with intravenous biological agents (rituximab, abatacept, infliximab or tocilizumab) in seven clinical centres in France (Strasbourg, Colmar, Mulhouse, Nancy, Reims, Clermont-Ferrand and Saint-Antoine hospitals in Paris) were enrolled in the study. Data were collected from 1 September 2019 (5 months before the outbreak of the epidemic in France, so that all enrolled patients had been exposed to a biologic prior to the start of the epidemic) to 1 January 2021.[3] In each centre, we obtained the list of all patients receiving intravenous biological agents from the hospital pharmacist. Therefore, all patients receiving one of the four drugs within the time frame of the study were enrolled in each centre. The occurrence of hospitalised COVID-19 was the primary outcome criterion, that is, SARS-CoV-2 presence confirmed by PCR and resulting in hospitalisation or death. Data were analysed with Bayesian methods in univariate and multivariate analyses using weakly informative prior (specifying that $0.05 < OR_x < 20$ a priori) or priors derived from recently published data.[3] A prior distribution is a probability distribution that expresses what is already known on the parameter of interest, such as the OR, through either theoretical consideration and/or past observations, and is a fundamental part of Bayesian methods and inference. Using a prior distribution allows decreasing, at least partially, concerns about the potential lack of statistical power. In order to ensure that any difference in risk with rituximab was not primarily due to baseline differences between rituximab and other biological groups, we performed multivariate analyses accounting for risk factors of severe COVID-19 based on literature.

A total of 1116 patients receiving intravenous biological agents were enrolled: 449 with infliximab, 392 with rituximab,

Table 1 Univariate and multivariate models assessing the association between the occurrence of hospitalised COVID-19 and each variable

Variables	Rituximab (n=392)	Other bDMARDs (n=724)	Univariate OR of hospitalised COVID-19 (95% CrI)	Multivariate OR of hospitalised COVID-19 (95% CrI) Model #1	Multivariate OR of hospitalised COVID-19 (95% CrI) Model #2
bDMARDs (RTX vs other bDMARDs)			**8.5 (2.4 to 38.6) Pr (OR >1)≈1.0**	**7.7 (1.7 to 44.7)**	**4.4 (1.8 to 11.1)**
Median age (years)	64 (56–71)	57.3 (47.0–67.0)	**1.0 (1.0 to 1.1) Pr (OR >1)≈1.0**	1.0 (1.0 to 1.0)	1.0 (1.0 to 1.0)
Female	285 (72.7)	426 (58.8)	0.6 (0.2 to 2.0) Pr (OR >1)=0.2	0.5 (0.1 to 2.1)	0.5 (0.2 to 1.0)
IA diagnosis					
RA	366 (95.6)	305 (42.4)	RA versus SPA 0.3 (0.0 to 1.4) Pr (OR >1)=0.06	RA versus SPA 1.0 (0.1 to 7.4)	RA versus SPA 0.6 (0.1 to 3.7)
Spondyloarthritis (including psoriatic arthritis)	0	364 (50.6)	RA versus other 2.2 (0.4 to 8.7)	RA versus other 2.3 (0.3 to 13.2)	RA versus other 2.1 (0.3 to 10.7)
Other*	17 (4.4)	51 (7.1)	Pr (OR >1)=0.8		
Comorbidities†					
Cardiovascular disease	60 (15.4)	167 (23.1)	0.5 (0.1 to 2.1) Pr (OR >1)=0.2	3.6 (0.9 to 16.6)	2.7 (1.3 to 5.8)
Cerebrovascular disease	10 (2.6)	29 (4.0)	0.5 (0.0 to 4.2) Pr (OR >1)=0.3	0.5 (0.0 to 4.0)	0.5 (0.0 to 4.3)
Chronic lung disease	92 (23.5)	84 (11.6)	1.9 (0.5 to 6.4)	1.0 (0.2 to 3.9)	1.8 (0.9 to 3.8)
Diabetes	48 (12.3)	68 (9,4)	2.8 (0.6 to 9.6) Pr (OR >1)=0.8	1.7 (0.4 to 7.4)	2.1 (0.5 to 5.4)
Median BMI (kg/m²) (IQR)	25.8 (23.2–29.4)	27.3 (23.4–31.2)	Normal BMI vs BMI >25 0.2 (0.0 to 1.4) Pr (OR >1)=0.1	Normal BMI vs BMI >25 0.1 (0.0 to 1.0)	Normal BMI vs BMI >25 0.2 (0.0 to 1.1)
BMI >30 kg/m²	67 (24.4)	120 (32.3)	Normal BMI vs BMI >30 0.5 (0.1 to 2.6) Pr (OR >1)=0.2	Normal BMI vs BMI >30 0.4 (0.1 to 2.9)	Normal BMI vs BMI >30 0.4 (0.1 to 2.2)
Treatments					
Conventional synthetic DMARDs	242 (61.7)	374 (51.7)	0.6 (0.2 to 1.9) Pr (OR >1)=0.2	0.6 (0.2 to 2.1)	0.5 (0.2 to 1.0)
Other immunosuppressive agents	7 (1.8)	5 (0.7%)	3.0 (1.4 to 6.5) Pr (OR >1) ≈ 1.0	4.0 (0.5 to 30.4)	2.1 (1.0 to 4.4)
Oral glucocorticoids Median dose (mg/day) — (IQR)	1 (0–5)	0 (0–0)	No steroids vs 0–10 mg/day 3.0 (0.7 to 11.4)	No steroids vs 0–10 mg/day 1.7 (0.4 to 7.2)	No steroids vs 0–10 mg/day 1.7 (0.4 to 7.5)
No steroids	190 (48.5)	486 (37.1)	Pr (OR >1)=0.9	No steroids vs >10 mg/day 1.3 (0.1 to 10.9)	No steroids vs >10 mg/day 1.5 (0.1 to 11.1)
0–10 mg/day	114 (29.1)	90 (12.4)	No steroids vs >10 mg/day 2.9 (0.3 to 20.5)		
>10 mg/day	13 (3.3)	10 (1.4)	Pr (OR >1)=0.8		

Model #1: weakly informative prior (specifying that $0.05<OR_i<20$ a priori).
Model #2: taking into account prior according to a recent publication by Strangfeld *et al*.[3]
Bold indicates statistically significant results.
*Other IA includes vasculitides n=16, juvenile idiopathic arthritis n=12, connective tissue diseases n=11, polymyalgia rheumatica n=10 and others n=19: uveitis, inflammatory bowel disease, stiff-person syndrome, sarcoidosis, inflammatory myositis, calcium pyrophosphate deposition disease, familial Mediterranean fever, Blau syndrome and McCune-Albright syndrome.
†Comorbidities: 'cardiovascular disease' includes abnormal heart rhythms or arrhythmias, aorta disease, coronary artery disease (narrowing of the arteries), heart attack, heart failure, cardiomyopathy, heart valve disease, hypertension, pericardial disease, peripheral vascular disease; 'cerebrovascular disease' includes history of stroke and transient ischaemic attack; 'chronic lung disease' includes asthma, chronic obstructive pulmonary disease, interstitial pneumopathy and pulmonary fibrosis, asbestosis, pneumonitis, obstructive sleep apnea–hypopnea syndrome and history of pulmonary embolism; diabetes includes type I and II diabetes.
bDMARD, biologic disease-modifying anti-rheumatic drug; BMI, Body Mass Index; IA, inflammatory arthritides; RA, rheumatoid arthritis; RTX, rituximab; SPA, spondyloarthr.

Table 2 Informative prior used in multivariate analysis Model #2

Variables	Gaussian prior distribution on log(OR), N(mu, sigma²)	Prior OR (95% CrI)
RTX versus other bDMARDs	N(1.04, 0.281)	2.8 (1.0 to 8.0)
Median age (years)	N(–0.079, 0.0016)	0.9 (0.85 to 1.0)
Gender	N(–0.757, 0.189)	0.5 (0.2 to 1.1)
Cardiovascular disease	N(0.752, 0.191)	2.1 (0.9 to 5.0)
Chronic lung disease	N(0.752, 0.191)	2.1 (0.9 to 5.0)
Conventional synthetic DMARDs	N(–0.757, 0.191)	0.5 (0.2 to 1.1)
Other immunosuppressive agents	N(0.64, 0.145)	1.9 (0.9 to 4.0)
Other variables	N(0, 0.428)	1.0 (0.05 to 20)

bDMARD, biological disease-modifying antirheumatic drug; CrI, credibility interval; DMARD, disease-modifying antirheumatic drug; RTX, rituximab.

170 with tocilizumab and 105 with abatacept. Ten cases of severe COVID-19 occurred: 9 in patients treated with rituximab (2.3% of total patients treated with rituximab) and 1 in a patient treated with infliximab (0.1% of patients treated with biological agents other than rituximab, 0.2% of patients treated with infliximab) (table 1 and online supplemental table 2). Four deaths occurred during follow-up, but none were related to COVID-19 (a dialysed 50-year-old man treated with tocilizumab for systemic sclerosis who developed a serious non-COVID infection, an 86-year-old woman treated with rituximab for rheumatoid arthritis, who developed a serious pulmonary bacterial infection; and a 62-year-old woman and a 70-year-old man treated with infliximab for psoriatic arthritis, who died of unexplained sudden death). In univariate analysis, the proportion of hospitalised COVID-19 was higher for patients receiving rituximab than other biological agents (9/392 vs 1/724, OR=8.5, 95% credibility interval (CrI) 2.6 to 38.6, Pr (OR >1)≈1; tables 1 and 2). Rituximab remained the only factor associated with risk of hospitalised COVID-19 (OR 7.7, 95% CrI 1.7 to

44.7) in multivariate analyses (table 1). In patients with hospitalised COVID-19 (online supplemental table 1), the median delay from last infusion to infection was 3.5 months (IQR 1.8–5.0). One patient was admitted to intensive care. The sensitivity analysis, in patients with moderate-to-severe and critical COVID-19 (ie, individuals who had SpO_2 <94% on room air at sea level and who required oxygen), yielded the same results as the main analysis in patients with hospitalised COVID-19 (online supplemental table 2).

The present work joins previous studies to confirm the risk of B-cell depletion with regard to the development of hospitalised and severe COVID-19.[1–3] Of note, the low number of events and the number of covariates limit the robustness of the statistical analysis, which might explain that classical risk factors such as age, sex, comorbidities, body mass index and corticosteroids were not associated with severe COVID-19 in the present study. In addition, the present study is the first to provide a prevalence of severe SARS-CoV-2 infection in a cohort which includes the totality of patients receiving intravenous biological treatment. In this study, approximately 2% of rituximab-treated patients developed hospitalised COVID-19, compared with only one patient (0.1%) among those treated with infliximab, tocilizumab or abatacept.

These results strongly indicate the increased risk of severe COVID-19 in patients receiving B-cell targeted therapy. Among patients with IA, those receiving rituximab should be prioritised for vaccination against SARS-CoV-2, sufficiently in advance of treatment infusion/reinfusion.

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SARS-CoV-2 infection after vaccination in patients with inflammatory rheumatic and musculoskeletal diseases

Patients with inflammatory rheumatic and musculoskeletal diseases (iRMDs) are often treated with immunomodulatory or immunosuppressive medications; consequently, they have been excluded alongside other immunocompromised patients from late stages of SARS-CoV-2 vaccine trials. SARS-CoV-2 vaccine efficacy in this population is unclear, though initial data are reassuring overall.

Table 1 Summary of 38 cases of SARS-CoV-2 infection ≥14 days after the first/single SARS-CoV-2 vaccine dose in the European Alliance of Associations for Rheumatology COVID-19 and COVAX registries, and breakdown by vaccination status

	All patients (N=38, N (%))	Fully vaccinated (n=10, N (%))	Partially vaccinated (n=28, N (%))
Sex			
Female	29 (76)	7 (70)	22 (79)
Male	9 (24)	3 (30)	6 (21)
Age, median (IQR)	58 (49–65)	62.5 (49–72)	57 (49–64)
Country			
Belgium	1 (3)	1 (10)	
Croatia	2 (5)	1 (10)	1 (4)
France	17 (45)	5 (50)	12 (43)
Greece	2 (5)		2 (7)
Hungary	1 (3)		1 (4)
Italy	1 (3)		1 (4)
Netherlands	1 (3)		1 (4)
Portugal	2 (5)		2 (8)
Slovakia	3 (8)	1 (10)	2 (8)
Spain	1 (3)		1 (4)
Turkey	3 (8)	2 (20)	1 (4)
UK	4 (11)		4 (14)
Comorbidities Only collected in COVID-19 registry (n=8 cases), shown as N (%) of 8			
Obstructive lung disease	1 (13)	1 (10)	
Hypertension	3 (38)	1 (10)	2 (7)
Cardiovascular disease	2 (25)		2 (7)
Cerebrovascular disease	1 (13)		1 (4)
Other	1 (13)		1 (4)
Rheumatic disease diagnoses			
ANCA-associated vasculitis (eg, GPA, EGPA)	2 (5)	1 (10)	1 (4)
Axial spondyloarthritis	9 (24)	1 (10)	8 (29)
Giant cell arteritis	1 (3)		1 (4)
Inflammatory myopathy	1 (3)		1 (4)
Polymyalgia rheumatica	1 (3)		1 (4)
Rheumatoid arthritis	17 (45)	5 (50)	12 (43)
Sjogren's syndrome	2 (5)	1 (10)	1 (4)
Systemic lupus erythematosus	3 (8)		3 (11)
Systemic sclerosis	3 (8)	1 (10)	2 (7)
Undifferentiated connective tissue disease	1 (3)	1 (10)	
Other	1 (3)		1 (4)
Inflammatory rheumatic disease activity			
Remission	18 (47)	8 (80)	10 (36)
Low	13 (34)	2 (20)	11 (39)
Moderate	5 (13)		5 (18)
Missing	2 (5)		2 (7)
Rheumatic disease medication and medication changes as a result of COVID-19 vaccination			
None	5 (13)	1 (10)	4 (14)
Abatacept	1 (3)		1 (4)
Antimalarials (including hydroxychloroquine, chloroquine and mepacrine/quinacrine)	5 (13)	2 (20)	3 (11)
Cyclosporine	1 (3)		1 (4)
Denosumab	1 (3)		1 (4)
Glucocorticoids	12 (32)	3 (30)	9 (32)
IL-6 inhibitors (including tocilizumab and sarilumab)	3 (8)		3 (11)
Stopped/held before COVID-19 vaccination	1		1
Stopped/held after COVID-19 vaccination	1		1
IVIG	1 (3)	1 (10)	
JAK inhibitors (including tofacitinib, baricitinib and upadacitinib)	2 (5)	1 (10)	1 (4)

Continued

Table 1 Continued

	All patients (N=38, N (%))	Fully vaccinated (n=10, N (%))	Partially vaccinated (n=28, N (%))
Methotrexate	10 (26)	3 (30)	7 (25)
Stopped/held after COVID-19 vaccination	2		2
Mycophenolate mofetil/mycophenolic acid	3 (8)	1 (10)	2 (7)
Rituximab	1 (3)	1 (10)	
Stopped/held before COVID-19 vaccination	1	1	
Stopped/held after COVID-19 vaccination	1	1	
Sulfasalazine	2 (5)		2 (7)
TNF inhibitors (including infliximab, etanercept, adalimumab, golimumab, certolizumab and biosimilars)	10 (26)	2 (20)	8 (29)
Other	4 (11)		4 (14)
COVID-19 vaccine type			
Pfizer-BioNTech	30 (79)	8 (80)	22 (79)
Moderna	1 (3)		1 (4)
AstraZeneca/Oxford	4 (11)		4 (14)
CoronaVac/Sinovac	3 (8)	2 (20)	1 (4)
COVID-19 vaccine type: N of reinfections/total N of vaccine in registries (% of reinfection per vaccine)			
Pfizer-BioNTech	30/3038 (1)	8/1919 (<1)	22/1119 (2)
Moderna	1/375 (<1)	0/204 (0)	1/171 (1)
AstraZeneca/Oxford	4/730 (1)	0/181 (0)	3/549 (1)
Janssen/Johnson & Johnson	0/40 (0)	0/1 (0)	0/39 (0)
Sputnik V	0/4 (0)	0/4 (0)	
CoronaVac/Sinovac	3/49 (6)	2/41 (5)	1/8 (13)
Other	0/2 (0)	0/2 (0)	
Unknown	0/120 (0)	0/60 (0)	0/60 (0)
COVID-19 outcome			
Deceased due to COVID-19	3 (8)	2 (20)	1 (4)
Vital status not known at this time	1 (3)		1 (4)
Full recovery	28 (74)	8 (80)	20 (71)
Resolved, with sequelae	3 (8)		3 (11)
Missing	3 (8)		3 (11)
Number of days from COVID-19 vaccine to infection, median (IQR)			
COVID-19 registry, most recent dose	23 (17–30)	22 (22–22)	24 (17–30)
COVAX registry, first dose	26.5 (20–52)	76 (52–97)	23 (18–27)
COVAX registry, second dose	24 (13–55)	45 (24–58)	7.5 (3.5–11.5)
COVAX registry, third dose	26.5 (23–30)	26.5 (23–30)	
Number of vaccine doses administered before COVID-19 diagnosis			
One dose	23 (61)		23 (82)
Two doses	13 (34)	8 (80)	5 (18)
Three doses	2 (5)	2 (20)	

All data are N (%) of the column unless stated otherwise.
ANCA-associated vasculitis, anti-neutrophil cytoplasmic antibody-associated vasculitis; COVID-19, Coronavirus disease 2019; EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; IL-6 inhibitors, interleukin-6 inhibitors; IVIG, Intravenous immunoglobulin; JAK inhibitors, Janus kinase inhibitors; TNF inhibitors, tumour necrosis factor inhibitors.

However, a slightly lower SARS-CoV-2 immunogenicity of vaccines has been documented in some patients with iRMD.[1 2] Some common rheumatic and musculoskeletal disease (RMD) medications have been highlighted as possible influential factors on immunogenicity, particularly rituximab (RTX), mycophenolate mofetil (MMF), methotrexate (MTX), abatacept and glucocorticoids.[3–7]

The European Alliance of Associations for Rheumatology (EULAR) launched a COVID-19 registry in March 2020, capturing COVID-19 outcomes in the European RMD population. Questions on reinfection and vaccination were added in January 2021. A further EULAR registry (COVAX) was launched in February 2021 to collect data on COVID-19 vaccination and related adverse events among patients with RMD. Here we describe a series of patients who contracted SARS-CoV-2 infection after COVID-19 vaccination between 19 January 2021 and 27 July 2021.

The series consists of 38 adults with iRMDs, 8 from the COVID-19 registry (<1%, out of 9118 patients with iRMD diagnosed with COVID-19) and 30 from the COVAX registry (<1%, out of 4393). Cases were deemed eligible if they were 'partially vaccinated' (≥14 days after dose 1 to <14 days after dose 2) or 'fully vaccinated' (≥14 days after dose 2/single dose), as per Centers for Disease Control and Prevention definitions[8] (17 cases were excluded for this reason). A quarter (26%) were fully vaccinated and 28 cases (74%) were partially vaccinated.

As shown in table 1, 76% of the series is female, with a median age of 58 (IQR 49–65) from 12 countries. The most frequent

Table 2 Summary of 34 cases of SARS-CoV-2 infection ≥14 days after the first/single SARS-CoV-2 vaccine dose in the European Alliance of Associations for Rheumatology COVID-19 and COVAX registries, stratified by COVID-19 outcome (excluding cases with missing/unknown COVID-19 outcome, N=4)

	Deceased, n=3 (N)	Full recovery, n=28 (N)	Resolved, with sequelae, n=3 (N)
Sex			
Female	1 (RA+SjS)	21	3
Male	2 (RA, SSc)	7	
Age, median (IQR)	>80 (SSc) >70 (RA, RA+SjS)	58 (49.5–65.0)	50 (49–61)
Rheumatic disease diagnoses			
ANCA-associated vasculitis		2	
Axial spondyloarthritis		7	1
Giant cell arteritis		1	
Inflammatory myopathy		1	
Polymyalgia rheumatica		1	
RA	1	11	2
Sjogren's syndrome		1	
RA+Sjogren's syndrome	1		
Systemic lupus erythematosus		2	
SSc	1	2	
Undifferentiated connective tissue disease		1	
Other		1	
Rheumatic disease activity			
Remission		16	
Low	2 (RA, RA+SjS)	9	1
Moderate	1 (SSc)	3	
Unknown			2
COVID-19 vaccine type			
Pfizer/BioNTech	3	22	2
Moderna		1	
AstraZeneca/Oxford		3	
CoronaVac/Sinovac		2	1
Other			1
COVID-19 vaccination status			
Partially vaccinated	1 (SSc)	20	3
Fully vaccinated	2 (RA, RA+SjS)	8	
Rheumatic disease medication			
None		4	
Abatacept			1
Antimalarials		4	
Cyclosporine		1	
Denosumab		1	
Glucocorticoids	1 (RA)	8	
IL-6 inhibitors		1	1
IVIG		1	
JAK inhibitors		2	
Methotrexate		9	
MMF		2	
MMF+glucocorticoids	1 (SSc)		
Rituximab	1 (RA+SjS)		
Sulfasalazine		1	1
TNF inhibitors		8	1
Other		4	
Number of days from COVID-19 vaccine to infection, median (IQR)			
COVID-19 registry (most recent vaccine dose)		23 (17–30)	
COVAX, first dose	18 (SSc)	29 (21.5–72.0)	26 (18–31)

Continued

Table 2 Continued

	Deceased, n=3 (N)	Full recovery, n=28 (N)	Resolved, with sequelae, n=3 (N)
COVAX, second dose	22 (RA) 32 (RA+SjS)	45 (19–58)	10 (10–10)
COVAX, third dose		26.5 (23–30)	

All data are N (%) of the column unless stated otherwise.
ANCA-associated vasculitis, anti-neutrophil cytoplasmic antibody-associated vasculitis; COVID-19, Coronavirus disease 2019; IL-6, Interleukin-6; IVIG, intravenous immunoglobulin; JAK, janus-kinase; MMF, mycophenolate mofetil; RA, rheumatoid arthritis ; SjS, Sjogren's syndrome; SSc, systemic sclerosis; TNF, tumour necrosis factor.

iRMD diagnoses were rheumatoid arthritis (RA, 45%), axial spondyloarthritis (axSpA, 24%), systemic sclerosis (SSc, 8%) and systemic lupus erythematosus (8%). Most were in remission (47%) or had low disease activity (34%). The top iRMD medications were glucocorticoids (32%), MTX (26%) and tumour necrosis factor inhibitors (TNFi, 26%). The median glucocorticoid dose in users was 5 mg/day (IQR 5–10).

The most common comorbidities among COVID-19 registry cases were hypertension (38%) and cardiovascular disease (25%). Comorbidities are not reported in the COVAX registry. Out of the 30 COVAX cases, 29 had no SARS-CoV-2 infection prior to vaccination, and this was unknown in one case. These data are not collected in the COVID-19 registry.

Seventy-nine per cent received the Pfizer/BioNTech vaccine; 11% received AstraZeneca; 8% received CoronaVac/Sinovac; and 3% received Moderna. Sixty-one per cent had one vaccine dose before COVID-19; 34% had two; and 5% had three. Median times from vaccination to infection are shown in table 1.

Most patients (74%) fully recovered from the SARS-CoV-2 infection; however, several patients recovered with ongoing sequelae (8%) and three patients died (8%).

Two of the deceased patients were male: one >80-year-old man with SSc, treated with glucocorticoids (10 mg/day) and MMF, who received one Pfizer vaccine 18 days prior to SARS-CoV-2 infection (therefore this patient was not fully vaccinated); one >70-year-old man with RA, treated with glucocorticoids (5 mg/day) who received two Pfizer doses (44 and 22 days before SARS-CoV-2 infection). The other patient was female: a >70-year-old woman with RA and Sjogren's syndrome, treated with RTX (the most recent RTX infusion was 195 days before the first vaccine), who received two Pfizer vaccines (60 and 32 days prior to infection) (table 2).

The three patients who recovered with ongoing sequelae had axSpA and RA, and were treated with abatacept, interleukin-6 inhibitors, sulfasalazine and TNFi (table 2).

Overall, the low numbers of SARS-CoV-2 infection post-vaccination in both registries are encouraging. Some observations described here have already been highlighted in existing research; for example, all three deceased patients were treated with medications that are potential negative influences on post-vaccination SARS-CoV-2 immunogenicity in the RMD population.[3 7] However, no vaccine has perfect efficacy; thus, a small number of postvaccination diagnoses of SARS-CoV-2 infections were expected, similarly to existing clinical trial observations; the influence of RMD medications on immunity after vaccination is still unclear.

There are significant limitations to this case series. The sample size is not sufficiently powered to evaluate associations between iRMD population-specific factors and SARS-CoV-2 infection after COVID-19 vaccination or to calculate a vaccine failure rate. Both the EULAR COVID-19 and COVAX registries rely on voluntary case submission, leading to selection bias in the data. No information is provided concerning the presence or the titre of postvaccine antibodies at the time of the infection. No causal conclusions can be drawn from this dataset, and the observations highlighted here cannot be extrapolated onto the wider iRMD population. Further research is needed to more deeply examine possible links between iRMD and medication-specific factors and SARS-CoV-2 infection after vaccination.

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data capture, (2) audit trails for tracking data manipulation and export procedures, (3) automated export procedures for seamless data downloads to common statistical packages, and (4) procedures for data integration and interoperability with external sources.

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Correspondence on 'Shared epitope defines distinct associations of cigarette smoking with levels of anticitrullinated protein antibody and rheumatoid factor' by Ishikawa *et al*

We read with great interest the paper of Ishikawa *et al*,[1] which addressed the link between smoking and the levels of anti-citrullinated protein antibodies(ACPA) and rheumatoid factor (RF) in a total of 6239 Japanese rheumatoid arthritis (RA) patients. We particularly appreciate the detailed smoking history collected that allowed a very detailed analysis. The authors collected information about the number of cigarette packs smoked per day, the years smoking and the time of smoke cessation when it was present. They also distinguished the ever smoker patients in three categories: smokers at disease onset, ex-smokers before onset and smokers after onset, which looks like a very pertinent stratification for exploring the pathogenic role of cigarette smoking. The study found a significant and dose-dependent association between smoking and antibody positivity for ACPA or RF antibodies, as well as, an association with high titres of ACPA or RF antibodies, which was only dose-dependent regarding RF. In both cases, the association was stronger with RF than ACPA. Moreover, the association of smokers with ACPA levels only was significant in the subgroup of patients with shared epitope (SE) alleles in the HLA-DRB1. In contrast, the association of smoking with RF was significant independently of the SE alleles. The authors conclude that these results suggest that the development of RF and ACPA is driven by different mechanisms without further detailing the implications. Perhaps, it is possible to obtain more information about these differences from the Ishikawa *et al* data. This would be very useful because there is currently a lot of interest in the relationship between smoking and the autoantibody-defined subgroups of RA patients.[2–6] Specifically, we think it will be informative to know if smoking introduced a differential association between the two subsets of patients with SE and high ACPA described in figure 3B in the Ishikawa *et al* article. Also, we think that a conditional analysis of one antibody on the other autoantibody, or stratified analyses on the serologically defined subgroups, could clarify the interpretation of the results regarding specific autoantibodies given the common concurrent presence of RF and ACPA. An analysis of this type has been recently reported in a large Swedish study that also counted with high-quality information on cigarette smoking.[2] In this study, both smoking and the presence of the SE conferred independent disease risk for RA with the two antibodies, ACPA and RF, whereas the $ACPA^{-}/RF^{+}$ patients showed an increased risk of disease among smokers, which was only marginally affected by the presence of the SE, and the $ACPA^{+}/RF^{-}$ patients were predominantly associated with the SE. These results have been interpreted as meaning the smoking may be a critical driver of RF production whereas the SE could be the main driver of ACPA development.[3] In this inquiry on the specific factors contributing to the RA subsets, we think the additional information regarding the Japanese population studied by Ishikawa *et al* will be very welcomed.

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Response to: 'Corresponence on 'Shared epitope defines distinct associations of cigarette smoking with levels of anticitrullinated protein antibody and rheumatoid factor' by Ishikawa *et al*' by Regueiro and Gonzalez

We are pleased to welcome the correspondence by Regueiro and Gonzalez[1] on our work.[2]

As they wondered if cigarette smoking (CS) introduced a differential association between the two subsets of patients with shared epitope (SE) and high anticitrullinated cyclic peptide/protein antibody (ACPA) described in figure 3B of the original manuscript, we add further explanation as follows; as shown in figure 3B of the original manuscript, smokers at the time of disease onset (SaO) with SE alleles have higher (OR 3.10, 95% CI 1.82 to 5.11, $p=2.3\times10^{-5}$) than never smokers with SE alleles (OR 2.24, 95%CI 1.56 to 3.24, $p=1.5\times10^{-5}$), while SaO without SE alleles does not have significant risk of high ACPA levels (OR 0.49, 95%CI 0.16 to 1.53, p=0.22). This indicates that CS does not independently affect ACPA production, but rather interacts with SE alleles and further increases the risk. Furthermore, we presented the linear association between SaO with SE alleles and ACPA levels (not presence of high ACPA level) in online supplementary figure S5 of the original manuscript, where the β coefficient of SaO with SE alleles (0.85, 95%CI 0.50 to 1.20, $p=2.8\times10^{-6}$) is higher than that of never smokers with SE alleles (0.55, 95%CI 0.34 to 0.77, $p=6.8\times10^{-7}$) implicating the former group of patients have higher ACPA titres. Furthermore, SaO without SE did not show even a positive trend of an association. These data clearly show the interactive effect of CS and SE alleles on ACPA production in patients with rheumatoid arthritis (RA).

They also suggested that a conditional analysis of one autoantibody on the other autoantibody, or stratified analyses on the serologically defined subgroups could clarify the interpretation of the results regarding specific autoantibodies given the common concurrent presence of rheumatoid factor (RF) and ACPA. While high levels of one autoantibody independently associated with high levels of the other autoantibody, conditional analysis by each autoantibody in addition to SE alleles still shows the same effects of SaO (and SE alleles) on high ACPA and RF levels as seen in figure 3A of the original manuscript (figure 1). Stratifying the patients according to the level of each autoantibody, ACPA(+) without high RF and RF(+) without high ACPA, did not change the association patterns observed in Figure 3B of the original manuscript (figure 1B). Moreover, stratifying the patients by different serotypes, ACPA(+) RF(-) (excluding RF(+) subjects from ACPA(+) subjects) and RF(+) ACPA(-) (excluding ACPA(+) subjects from RF(+) subjects), still showed the same trends, but lost significance due to the limited numbers of patients in some subgroups (figure 1C). Taken together, these two additional analyses indicate that the associations among SE, CS and each autoantibody production were not confounded by the presence of the other autoantibody frequently found in patients with RA.

Accordingly, our data further show the distinctive effect of CS on the presence and levels of ACPA and RF in Japanese RA patients. Importantly, our findings well fit with the work by Hedström *et al*,[3] indicating that the distinctive effects of CS on ACPA and RF are not limited on a certain ethnic groups or specific SE epitope alleles. Indeed, we also showed that amino acid (AA) position 74 has the most significant effect on the presence and high levels of ACPA by the omnibus analyses as in figure 5 of the original manuscript, meaning that this AA position, instead of specific SE alleles, might be a critical driver for ACPA development and the effect of CS on ACPA levels might also be dependent on this AA position.

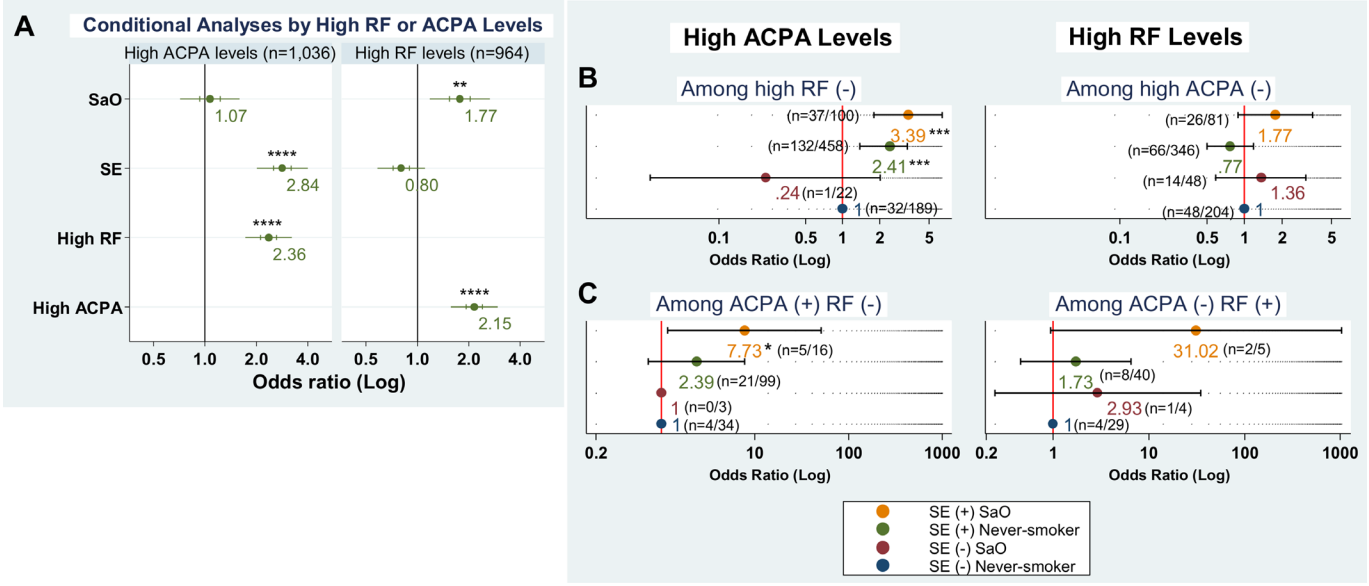

Figure 1 Cigarette smoking affects ACPA levels only in patients with SE alleles while cigarette smoking per se affects high RF levels regardless of SE allele status. (A) The association of smoking at the time of disease onset (SaO) with high ACPA or RF levels were evaluated conditioning on presence of SE alleles and high levels of RF or ACPA. ORs are indicated by dots and numbers, and 95% CIs are indicated by two-sided lines. (B, C) The associations of SaO with high ACPA or RF levels with or without SE alleles were evaluated referring never-smokers without SE alleles. Patients were stratified according to levels of autoantibodies (B; high ACPA without high RF or high RF without high ACPA) or serotypes (C; ACPA+RF or ACPA-RF+). ORs are indicated by dots and numbers, and 95% CIs are indicated by two-sided lines. The numbers of patients (case/total) are also indicated. High ACPA or RF: top quartile of ACPA or RF positive patients. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. ACPA, anticitrullinated cyclic peptide/protein antibody; RF, rheumatoid factor; SaO, smokers at the time of onset; SE, shared epitope.

The importance of this AA position for ACPA development was also reported by the studies on Caucasian populations[4 5] as well as Japanese populations,[6] further implicating a transethnic effect of this AA position on ACPA development. Further studies focusing on precise molecular mechanisms will be of particular interest.

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Causal association of gut microbiome on the risk of rheumatoid arthritis: a Mendelian randomisation study

I read with interest the articles by Inamo[1] and Alpizar-Rodriguez *et al*[2] regarding the effects of the gut microbiome on the risk of rheumatoid arthritis (RA). The Mendelian randomisation (MR) study suggested that dysbiosis may be a secondary phenomenon, rather than a trigger, in the pathogenesis of RA,[1] while the cohort study by Alpizar-Rodriguez *et al* suggested a role for intestinal dysbiosis in the development of RA.[2] However, some methodological issues in the MR study must be discussed. First, I applied a two-sample MR analysis in the MR base platform to the same data analysed with the MR by Inamo.[1] From this analysis, I could obtain 32 single nucleotide polymorphisms as instrumental variables. The MR estimates determined using inverse variance weighted (IVW) and MR-Egger regression analyses support a causal association between gut microbiome and the occurrence of RA (IVW: beta=−0.024, SE=0.007, p=0.0006; MR-Egger: beta=−0.027, SE=0.009, p=0.005), while the weighted median approach yielded no evidence of a causal association between gut microbiome and RA (beta=−0.005, SE=0.003, p=0.144). Unlike the MR results by Inamo,[1] a 'leave-one-out' analysis demonstrated that the IVW method without rs1230666 remained significant (p=0.034) and no single single nucleotide polymorphism (SNP) was driving the IVW point estimate. Second, MR studies are susceptible to bias from pleiotropy. Therefore, sensitivity analysis is required to verify the validity of conclusions drawn from the MR study.[3] Two methods are commonly used for sensitivity testing: a weighted median estimator, which provides valid estimates even if 50% of the SNPs are not valid instruments[4] and MR-Egger regression, which tests for unbalanced pleiotropy and estimates the causal effect of an exposure on an outcome.[5] Here, I found that the results of the MR analysis are supported by significant findings of the MR-Egger analysis (also similar to the IVW estimates), thus providing additional confidence in these findings. Considering that the weighted median estimator allowing 50% of the instruments to be invalid may be a conservative method and no method can provide an infallible test of causation,[3] the MR data may provide support for previous observational studies that have shown an association between microbiome and RA.[2]

Thus, I believe that the findings of this MR study should be interpreted by taking the aforementioned methodological concerns into consideration. In conclusion, the MR analysis results may support epidemiological evidence for a relationship between gut microbiome and RA,[2] suggesting further investigation on how much gut microbiome affects the development of RA.

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Response to: 'Causal association of gut microbiome on the risk of rheumatoid arthritis: a Mendelian randomisation study' by Lee

I am grateful to Dr Young Ho Lee[1] for response to my article.[2] Although my study demonstrated non-causal association between gut microbiome and the risk of rheumatoid arthritis (RA), the author demonstrated significant association between them.

First, the reason why different conclusions were drawn from two studies is that I extracted only 'Gut microbiota (bacterial taxa) (unit decrease)' from variables of exposures in harmonised dataset before conducting Mendelian randomisation (MR) (R script is in online supplementary file), because other exposures did not have significant association with the risk of RA in the preliminary analysis. This additional operation brought less single nucleotide polymorphisms as instrumental variables in my study and the different result of 'leave-one-out analysis', in particular the influence of rs1230666 (*MAGI3*) on MR estimate. In addition, although the author demonstrated significant association by both inverse-variance weighted (IVW) and MR-Egger regression analyses, it does not mean that gut dysbiosis might cause the development of RA because beta were less than zero (if dysbiosis has causal effect against the development of RA, beta might be positive value).

More importantly in this instance, as commented in response from Alpizar-Rodriguez *et al* to my correspondence,[3] to conclude causal association of dysbiosis against RA by MR, we need more appropriate genome-wide association study dataset which represent a relevant measure of dysbiosis in RA. To my knowledge, no study has found strong influence of genetics on specific bacterial taxa which is considered to be involved in the pathogenesis of RA, such as *Prevotella* spp abundance.[4 5] Thus, to interpret the result of MR appropriately, we don't have enough evidence to conclude causal effect of dysbiosis against the risk of RA yet. However, considering gut is one of the main sites of immune response, it is reasonable to accept the concept that dysbiosis could trigger RA. Further investigation with refined approach is required to clarify this question.

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Polyfunctional TEM cells in psoriatic arthritis synovium skewed towards Th17 cells

We read the article by Wade *et al*[1] with great interest. With very elegant experiments, they have reported enrichment of polyfunctional T-lymphocytes in the synovial tissue of psoriatic arthritis (PsA); they have also noticed that polyfunctional synovial T-cells were positively associated with Disease Activity Index for Psoriatic Arthritis. We agree there are only limited studies in human autoimmune diseases which have addressed regulatory role of T-cell polyfunctionality of the activated effector memory T cells (TEM) in single cell suspensions. We have been working on TEM cell subpopulations in PsA and over years have demonstrated Th17, Th9 and mucosal-associated invariant T cells in PsA,[2–4] which are also polyfunctional. In respect to studying polyfunctional T cells in PsA instead of focusing on Th1, Th17 and exTh17 cells,[1] we took a different approach to look into the cytokine profile and polyclonality of these TEM cells in respect their profound role in the disease process of PsA such as (IL-17, IL-23R, IL-22 and TNFα) and also compared with that polyfunctional T cells of rheumatoid arthritis (RA) to see whether a role of polyfunctional T cells can be reproduced in an another autoimmune arthritis and if so to identify the differences in degree/quality of the TEM polyfunctional cells at single cell level in these two different autoimmune arthritis.

We have studied synovial fluid T cells compared with the synovial tissue extracted T cells by Wade *et al*.[1] Here, we are sharing our data which substantiates regulatory role of synovial polyfunctional T-cells in PsA as reported by Wade *et al*; further, it indicates that despite being polyfunctional (IL-17A+, IL-22+, TNFα+, IFNγ+), the key pathological TEM cells in the inflamed joints of PsA are skewed towards Th17 cells joints compared with that of RA.

From untreated age/sex matched, patients with PsA and RA (n=15/each) with active disease peripheral blood mononuclear cells (PBMC) and synovial fluid mononuclear cells (SFMC) were collected. $CD3^+$ T cells were magnetically sorted and isolated from SFMCs and PBMCs. Isolated $CD3^+$ T cells (10^6 cells/mL) were activated with antihuman CD3/CD28 cocktail; cells were cultured in the RPMI medium for 5 days (figure 1). Hi-D FACS studies were done (i) to identify activated memory ($CD11a^+CD45RO^+$) T cells ($CD3^+CD4^+$/$CD8^+$) and (ii) to evaluate relevant Th1/Th17 cytokine profile: TNFα, IFNγ,IL-17A, IL-22. Mean fluorescence intensity and the percentages of each cell population were analysed using a Flow Jo software. Experiments were carried-out in triplicates; results are described as Mean±SEM. One-way analysis of variance with Tukey multiple comparison test was used to compare results among more than two groups; $p \leq 0.05$ was considered statistically significant.

Both in PsA and RA, marked polyfunctionality was noticed in T cells of PBMC/SFMC. However, compared with the matched blood T cell cytokine, polyfunctionality was more evident in the synovial cells. In PsA SFMCs, the numbers for IL-23R (8.5%±1%), IL-17A (15.5%±1.4%) and IL-22 (8.4%±1.3%) secreting cells were significantly higher ($p<0.001$) than the PsA PBMCs (1.2%±0.7%, 3.8%±0.3% and 1.1%±0.5%, respectively), whereas in PsA, the % of TNFα T cells in PBMCs (20.7±0.66) was more than PsA SFMCs (8.16±0.11, $p<0.001$); IFNγ secreting cells in SFMCs and PBMCs were 3% and 6%, respectively. SFMCs of RA (figure 1) had significantly higher numbers ($p<0.001$) of IL-23R (2.1%±0.4%), IL-17A (5.9%±1.2%), IL-22 (2.5%±0.5%), TNFα (24.3%±0.2%) secreting cells compared with RA PBMCs (1.0%±0.5%, 1.8%±0.1%, 0.8%±0.2% and 14.3%±0.2%, respectively). PBMCs of RA had more numbers of IFNγ producing cells ($p<0.001$) than SFMCs of RA (15.8%±0.5% vs 8.6±0.2%).

Consistent to report by Wade *et al*[1] and our earlier reports, we noticed that activated CD4 memory T cells[3 4] were the major source for the lesional cytokines. More intriguing result is that both in RA and PsA the localised pathological TEM in the inflamed synovial fluid were vastly polyfunctional. However, though both in RA and PsA the key TEM cells in the synovium were polyfunctional, they were different and unique in their cytokine profile: (i) compared with RA (figure 1), the TEM cells in PsA SFMCs were skewed towards Th17 cells and this is likely because of higher expression of IL-23R in TEM cells of PsA; (ii) in RA, TEM cells were skewed towards Th1 cytokine profile; (iii) probably, this explains superior efficacy for anti-IL-17A targeted therapies in PsA compared with that of RA.

Our study demonstrates that despite the TEM cells are being polyfunctional at the single cell level still in PsA, the TEM cells

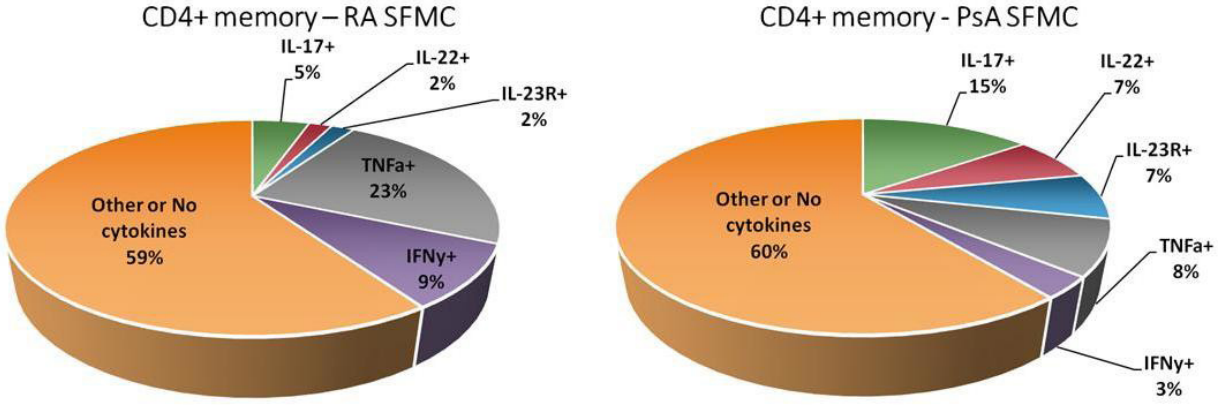

Figure 1 Polyfunctional synovial fluid effector memory T cells and its variations in RA and PsA. 10^6/mL SFMCs were incubated for 6 days in 24-well plates precoated with 5 µg/mL anti-CD3 (UCHT1, eBioscience) and 2 µg/mL soluble anti-CD28 (CD28.2, eBioscience) antibodies. On day 6, cells were activated with PMA (50 ng/mL) and ionomycin (1 ug/mL); and for intracellular staining, Monensin was added to the cell culture (2 µM, Sigma). FlowJo software was used to gate and analyse the $CD3^+CD4^+CD11a^+CD45RO^+$ cells. This figure is adapted from original publication by Raychaudhuri *et al*.[5] PsA, psoriatic arthritis; RA, rheumatoid arthritis; SFMC, synovial fluid mononuclear cells.

are skewed more towards the Th17 cells when compared with RA. At different time points of a disease process, it is expected that the kinetics of the polyfunctionality of these cells along with their cytokine profile would vary. Thus, this could be a possible explanation for treatment failure or variations in responsiveness to a specific anticytokine therapeutic agent in a patient with RA or in a patient with PsA.

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Response to: 'Polyfunctional TEM cells in psoriatic arthritis synovium skewed towards Th17 cells' by Raychaudhuri *et al*

We read with interest the research letter by Raychaudhuri *et al*, which examines the frequencies of cytokine producing CD4+ memory T cells in psoriatic arthritis (PsA) and rheumatoid arthritis (RA) synovial fluid mononuclear cells (SFMC) compared with peripheral blood mononuclear cells (PBMC).[1] The authors examined the frequencies of single cytokine-producing T cells, specifically interleukin (IL)-17A+, IL-22+, tumour necrosis factor (TNF)+, interferon gamma (IFNγ)+ or IL-23R+ and report that Th17 cells are enriched in PsA SFMC, while RA is skewed to a Th1-like profile. In our previous publication, Wade *et al*,[2] we reported the frequencies of both single cytokine-producing and multiple cytokine (polyfunctional)-producing T cells, in addition to the frequencies of Th1, Th17 and exTh17 cells by using the Th17 lineage marker CD161. In our study, however, we reported these findings in synovial tissue biopsies from PsA patients, as opposed to PsA SFMC (reported by Raychaudhuri *et al*), demonstrating enrichment of polyfunctional T cells, specifically triple-positive cytokine-producing T cells isolated from PsA synovial tissue.

While discrepancies in immune cell frequencies and phenotypes have been reported between SFMC and synovial tissue,[3] the study by Raychaudhuri *et al* report an increase in CD4+ IL-17a+ T cells in PsA SFMC compared with PBMC, an observation which we also described in PsA synovial tissue.[2] Moreover, Raychaudhuri *et al* also report a decrease in TNFα production in PsA SFMC compared with PBMC, similar to that observed in our study in PsA synovial tissue .[2]

However, we noted that while Radchaudhuri *et al* report the identification of polyfunctional T cells in PsA and RA, we believe these data should be interpreted cautiously. Radchaudhuri *et al* demonstrate an increase in a number of single cytokine-producing T cells, specifically IL23R, 1L-17A and IL-22 in PsA compared with RA, however, they do not show that these cytokines are being co-produced by the same T cells (ie, polyfunctionality). Polyfunctionality or co-production of multiple cytokines within the same T-cell population is best evaluated using advanced flow cytometric algorithm analysis.[2 4] In this manner, polyfunctionality within a specific T cell can be accurately analysed. Moreover, we reported that it is these polyfunctional T cells that correlate with disease activity for psoriatic arthritis (DAPSA) and not the single cytokine-producing T cell subsets. Additionally, when we used a PDE4 inhibitor in our ex vivo synovial tissue single cell cultures, again it was the polyfunctional T-cell population and not the single-producing cytokine populations which responded, suggesting that the polyfunctional T cells are significantly contributing to disease pathogenesis and response in PsA. Further studies by Radchaudhrui *et al* to examine polyfunctional T cells within their dataset in addition to the single positive cytokine-producing T cells which they have reported in their study would extend their current findings. This would allow the comparative evaluation of these polyfunctional effector memory T cells in the periphery versus site of inflammation providing additional insight into their potential role in autoimmune disease.

Interestingly, in addition to examining the synovial environment in PsA, the authors also examined T effector memory (TEM) cells within RA SFMC and report a more Th1-like profile. Previous studies by Basdeo *et al* report an accumulation of ex-Th17 cells or non-classical Th1 cells in RA SFMC.[4] It is now known that Th17 cells can lose their ability to produce IL-17 and instead switch to predominantly producing IFNγ.[5] These ex-Th17 cells can no longer be distinguished from Th1 cells purely on the production of IFNγ, given that both subsets produce this cytokine in high amounts. Therefore, the Th17 plasticity marker CD161 is used to delineate Th1 cells (CD161− IFNγ+) from ex-Th17 cells (CD161+ IFNγ+). Thus, future analysis of the IFNγ+ TEM population in the Raychaudhuri *et al*'s study could be performed to ascertain through the use of CD161 expression if the RA SFMC in their study display increased levels of Th1 cells or non classical Th1/exTh17 cells.

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Frequency of MRI changes suggestive of axial spondyloarthritis in the axial skeleton in a large population-based cohort of individuals aged <45 years

I read with great interest the recent report by Baraliakos *et al* published in the Annals of Rheumatic Disease, about a general population cohort study of healthy volunteers that examined the presence of bone marrow oedema and fatty lesions on MRI of the spine and sacroiliac joints.[1] Over the past decade, imaging techniques such as the MRI have revolutionised the application of radiographic findings in the early diagnosis of axial spondyloarthritis (axSpA).[2]

In this study, the authors elegantly identified an increased frequency of fatty lesions as well as bone marrow oedema in the vertebral corners of the spine, particularly in the lower part of the thoracic spine. I agree with the authors' comment that the vertebral MRI lesions found in the healthy volunteers could be induced by mechanical load or early osteoarthritis; however, given the distribution and the characteristics of the lesions in some patients, these findings could be reflective of diffuse idiopathic skeletal hyperostosis (DISH).

DISH is a common skeletal condition of unknown aetiology characterised by calcification of spinal ligaments and enthesis, and radiographic evidence of flowing ossification along with the vertebral bodies, most commonly in the thoracic spine.[3] From an epidemiologic perspective, DISH has an incidence rate of 4%–7% and could be associated with symptoms of inflammatory back pain and stiffness among patients, mimicking axSpA.[4 5]

In the current study, the authors pointed out that older patients had increased frequency of the thoracic lesions on MRI, a characteristic radiographic feature of DISH, although the prevalence of the disease increases with age, DISH may affect patients under the age 45.[6 7] Moreover, a recent study that evaluated the spine of 53 DISH patients with the use of MRI, demonstrated bone marrow oedema and fat deposition in at least one vertebral corner, in 76% and 67% of patients, respectively, similar results seen in axSpA.[8]

In conclusion, we believe caution should be taken regarding the interpretation of the MRI of the spine findings in order to avoid an erroneous diagnosis of axSpa, and consequently, the use of unnecessary, expensive and potentially harmful treatments. Future studies are warranted to elucidate the long-term progression of the inflammatory spinal lesions in healthy adults and to provide us with a better understanding of the natural history of conditions such as DISH.

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Response to: 'Frequency of MRI changes suggestive of axial spondyloarthritis in the axial in a large population-based cohort of individuals aged <45 years' by Parperis

We agree with the authors of the comment that caution in the interpretation of MRI is needed, though we think this is generally the case for any kind of imaging techniques. Regarding the axial skeleton including the spine and the sacroiliac joints (SIJs) this is particularly critical if identification of patients with axial spondyloarthritis (axSpA) is pursued. In this context our study[1] confirms earlier data.[2] We conclude that false positive MRI findings account for much of the confusion that has been created in relation to the Assessments in Spondyloarthritis International Society (ASAS) classification criteria.[3]

However, Dr Parperis has a different issue[4] since he proposes that some subjects in our study with described fatty changes may already have or potentially develop diffuse idiopathic skeletal hyperostosis (DISH). This condition may be difficult to differentiate from ankylosing spondylitis especially in older ages. In fact, we cannot preclude the presence of DISH since we do not have X-ray or CT images for comparison which are considered the gold standard for diagnosis of DISH. Further, no follow-up data of the individuals are available yet. However, the likelihood of a high prevalence of DISH in a population with a mean age of 38 years is below 1%.[5] Considering the dramatic increase of DISH in older age groups, only a small subset of participants in our study might have represented early cases of DISH. The balanced distribution of sex in participants affected by fatty lesions in our cohort mitigates this potential bias since DISH is usually more prevalent in males being associated with the metabolic syndrome.[6] Slightly more females had bone marrow edema (BME) and slightly more males had fatty lesions (FL) in the spine. These results were similar in subanalyses based on body mass index (BMI), where BME was not found in volunteers with higher BMI, whereas FL were only slightly increased in those in higher BMI categories. Furthermore, there were no differences in the distribution of study participants with different spinal back pain levels in the last 3 months prior to the MRI examination. All of them showed a similar distribution of both BME and FL in the spine. In addition, the distribution of lesions (particularly in the lower part of the thoracic spine) in our study does not support a diagnosis of DISH, since those patients rather present with pathological lesions in the middle part of the thoracic spine, which is in contrast to other pathological finding that rather occur in the thoracolumbar area.[7]

Finally, we would like to stress that it was not the intention of our study to make diagnoses of axSpA but to provide background population based data which questions the sensitivity and specificity of previously proposed MRI criteria for classification purposes.[3] Nevertheless, our results may also be relevant for clinical purposes and the design of future prospective studies to assess the natural course of degenerative changes including DISH.

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Correspondence on: 'Irritable bowel syndrome symptoms in axial spondyloarthritis more common than among healthy controls: is it an overlooked comorbidity?' by Wallman *et al*

We read with interest the letter by Wallman *et al*, that reported the prevalence of irritable bowel syndrome (IBS) in patients with axial spondyloarthritis (axial SpA).[1] The authors present data from the population based SPondylARtrit TvÄrsnittsKohort Universitetssjukhuset i Skåne (SPARTAKUS) study,[2] showing that symptoms meeting IBS criteria were significantly more frequent among patients with axial SpA without known inflammatory bowel diseases (IBD) (30%), than in controls (16%; OR: 2.5 (95% CI 1.1 to 5.7)). Authors conclude that IBS may be an overlooked frequent comorbidity of axial SpA warranting further research and increased awareness. In our view, this suggested high prevalence of IBS in patients with axial SpA might be overestimated for multiple reasons. Even though Wallman *et al* discussed the limitations of their approach, we would weight them differently resulting in a different appraisal.

First, the clinically evident IBD is present in 6%–14% of axial SpA patients, undiagnosed in a significant part of them.[3] Microscopic gut inflammation—which does not have to be associated with a pronounced clinical picture of IBD—is even more common, reaching around 60% in axial SpA patients.[3] As IBD was solely retrospectively excluded based on the clinical history in the study population of patients with axial SpA, with no endoscopy performed, symptoms meeting IBS criteria could also be explained by the presence of an undiagnosed IBD in this group of patients. Authors speculate that it is not likely based on no differences in IBS symptoms between patients with axial SpA with elevated versus normal C-reactive protein (CRP) and faecal calprotectin levels. However, both CRP and faecal calprotectin levels may be elevated in patients with axial SpA even without IBD.[4] At the same time, more than 40% of the axial SpA patients included in the SPARTAKUS study were treated with tumour necrosis factor inhibitors,[2] that could influence bothCRP and faecal calprotectin levels.

Second, the authors report that abdominal symptoms were observed almost twice more frequently in patients receiving non-steroid anti-inflammatory drugs (NSAID). Furthermore, NSAID related microscopic colitis or known side effects similar to IBD and IBS symptoms were not excluded as well.

Third and most importantly, authors defined IBS as gut symptoms meeting ROME III criteria for IBS.[5] According to these criteria, IBS should be a diagnosis of exclusion and may be considered when other potential causes for bowel symptoms were actively excluded. Given that, using these criteria in such a retrospective analysis—where exclusion of other causes of bowel symptoms that may be attributable to IBD is not fully possible—can lead to false conclusions.

Therefore, we think that the prevalence of IBS presented by the authors may be overestimated—given the fact, that in the described setting other explanations of bowel symptoms are more likely—and should therefore be handled with caution. Making the diagnosis of IBS in a clinical context of axial SpA without performing the full diagnostic work-up and having full information (eg, endoscopy) one could miss IBD as a manifestation of axial SpA or side effects of NSAIDs treatment that in both cases would have relevance for the proper management strategy.

Fabian Proft ,[1] **Mikhail Protopopov** ,[1] **Valeria Rios Rodriguez,**[1] **Murat Torgutalp,**[1] **Britta Siegmund,**[1] **Denis Poddubnyy** [1,2]

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Response to: 'Correspondence on: irritable bowel syndrome symptoms in axial spondyloarthritis more common than among healthy controls: is it an overlooked comorbidity?' by Proft *et al*.

We appreciate the correspondence by Proft *et al* regarding our study entitled 'Irritable bowel syndrome symptoms in axial spondyloarthritis more common than among healthy controls: is it an overlooked comorbidity?',[1 2] and thank *Annals of the Rheumatic Diseases* for the opportunity to respond. We also acknowledge the major contributions of Proft *et al* to the field of spondyloarthritis (SpA) research.

Regarding our main result, that irritable bowel syndrome (IBS) symptoms are significantly more common among patients with axial SpA without known inflammatory bowel disease (IBD) (n=182) than in healthy controls (n=50), Proft *et al* point out that the increased prevalence is likely due to other causes than actual clinical IBS (in particular gut inflammation and side effects of non-steroidal anti-inflammatory drug (NSAID) use).

In response to this, we would first like to draw attention to the rule-out character of the ROME III criteria used to diagnose IBS, as also brought up by Proft *et al*. According to these, a clinical IBS diagnosis requires both a typical constellation of gastrointestinal symptoms, as defined by the criteria, and the exclusion of organic causes such as IBD or malignancies. The main finding of our study, that 30% of the axial SpA patients in the well-characterised SPARTAKUS cohort reported IBS symptoms, as opposed to 16% of healthy controls (sex/age-adjusted OR 2.5; p=0.036), refers to self-reported symptoms, as defined by the ROME III criteria, but irrespective of their underlying cause (and hence not per se meeting the exclusion condition). This important distinction—between IBS symptoms and a clinical IBS diagnosis—is made throughout our report.

In the second part of our study, we then performed a hypothesis-generating analysis of potential drivers behind the observed IBS symptoms. Similar to Proft *et al*, a priori we also considered gut inflammation (in the form of microscopic gut inflammation or even undiagnosed IBD) and NSAID side effects among the more plausible explanations behind these symptoms.

Regarding gut inflammation, faecal calprotectin (F-calprotectin) is a standard clinical and highly sensitive biomarker of IBD disease activity. In SpA, F-calprotectin (as well as C-reactive protein) has also been shown to be significantly elevated among patients displaying microscopic (histological) gut inflammation at ileocolonoscopy, with a reported optimal F-calprotectin cut-off of 85 mg/kg for the detection of such cases.[3] Moreover, two other studies have demonstrated the presence of macroscopic inflammatory lesions at capsule endoscopy and/or ileocolonoscopy in SpA patients with previously undiagnosed IBD to be significantly associated with elevated F-calprotectin (>100 mg/kg).[4 5] In our study, adjustment for F-calprotectin levels did not at all change the results of our main analysis, comparing the prevalence of gut symptoms meeting ROME III criteria for IBS between axial SpA patients and controls. Nor did we find any overall association between the presence of IBS symptoms and F-calprotectin elevation in the axial SpA group, when applying different previously suggested F-calprotectin cut-offs, and the geometric means of F-calprotectin in patients with and without IBS symptoms were very similar (31 vs 35 mg/kg). Finally, the association between IBS symptoms and female sex in the present work (adjusted OR 2.4 vs males; p=0.017) contrasts with the strong male predominance of microscopic (histological) gut inflammation (OR 8.9 vs females; p=0.035) observed by van Praet *et al* among axial SpA patients without diagnosed IBD,[6] lending further support to the view that undiscovered gut inflammation is unlikely to be the main driver behind the reported IBS symptoms in our study.

In respect to antitumour necrosis factor (TNF) therapy, initiation of adalimumab in axial SpA patients with newly discovered subclinical IBD has been shown to simultaneously reduce macroscopic gut lesions and F-calprotectin levels.[4] Moreover, ongoing treatment with monoclonal antibody-type anti-TNF agents among axial SpA patients without known IBD has been associated with significantly lower F-calprotectin levels than in patients receiving etanercept or no anti-TNF treatment.[7] Despite such previously demonstrated effects on potential low-grade gut inflammation, in our study IBS symptoms were reported by 40% (n=19) of the 48 patients with ongoing monoclonal antibody-type anti-TNF therapy, while the corresponding figure among the remaining 134 patients was 27% (n=36), thus rather pointing towards a disconnect between gut inflammation and IBS symptoms in our cohort.

In regards to potential NSAID side effects, adjustment for NSAID-use during the last 3 months only marginally decreased the point-estimate OR for the axial SpA patients versus controls difference in reported IBS symptoms from 2.5 to 2.2, although statistical significance was lost (p=0.067). Within the axial SpA group, there was also a positive univariate association between NSAID-use and the presence of IBS symptoms, but this did not remain statistically significant in the multivariate model. Furthermore, NSAID enteropathy is known to result in elevated F-calprotectin levels,[7 8] but we found no clear association between IBS symptoms and F-calprotectin.

In our multivariate analysis, the factors most strongly related to the presence of IBS symptoms in the axial SpA group were female sex (which is also over-represented among clinical IBS patients in the general population[9]) and comorbid fibromyalgia (known to be closely associated with clinical IBS in the general population[10]). As previously shown for SpA patients with comorbid fibromyalgia,[11] all patient-reported outcomes, but not the evaluator's global assessment of disease activity, were also significantly worse in our axial SpA group reporting IBS symptoms.

In light of the various aspects brought up above, we thus hypothesised that clinical IBS, similar to fibromyalgia, may be over-represented in axial SpA, explaining a relevant part of the observed increase in self-reported IBS symptoms relative to controls in our main analysis. We agree with Proft *et al* that the lack of endoscopic examinations is a limitation of this approach and call for future studies including such assessments to try to further elucidate what the increased frequency of IBS symptoms in the axial SpA population really represents. We note, however, that the authors of a previous study, examining SpA patients with capsule endoscopy and ileocolonoscopy, also hypothesise clinical IBS to be relatively frequent in this disease, based on common gastrointestinal complaints among patients with normal endoscopic examinations.[5] Finally, we fully share the viewpoint of Proft *et al*, that in the clinical setting, when seeing SpA patients presenting with gastrointestinal symptoms, a thorough examination of potential causes should be performed, including (but not limited to) considerations regarding gut inflammation and NSAID entheropathy.

Johan Karlsson Wallman ,[1,2] **Elisabeth Mogard** ,[1,2] **Jan Marsal**,[3,4] **Kristofer Andréasson**,[1,2] **Anna Jöud**,[5] **Mats Geijer**,[6,7] **Lars Erik Kristensen**,[8] **Elisabet Lindqvist**,[1,2] **Tor Olofsson** [1,2]

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Efficacy and improved tolerability of combination therapy with interleukin-1 blockade and MAPK pathway inhibitors for the treatment of Erdheim-Chester disease

We have read with interest the article by Cohen-Aubart *et al*[1] reporting the efficacy of infliximab in the treatment of Erdheim-Chester disease (ECD), a rare non-Langerhans histiocytosis. The anti-Tumor Necrosis Factor-α agent demonstrated a variable degree of efficacy, suggesting that infliximab might represent a therapeutic option for moderately severe ECD cases; similar results had been observed with anakinra.[2 3] Nonetheless, following the identification of causative mutations along the mitogen-activated protein kinase(MAPK) pathway,[4] severe forms of ECD are currently treated with targeted small molecule agents. Specifically, the BRAF inhibitor vemurafenib and the MAPK or extracellular-signal regulated kinase (MEK) inhibitor cobimetinib have been successfully used to treat life-threatening forms of ECD. Although life-saving, these drugs are associated with severe toxicity, which often mandates treatment discontinuation.[5] Here, we describe the efficacy and improved tolerability of combination therapy with interleukin-1 blockade and targeted MAPK inhibitors in the treatment of ECD.

Among 45 patients with a diagnosis of ECD and followed up at our institution, 25 have been treated with at least one targeted therapy (vemurafenib or cobimetinib). All patients underwent clinical evaluations every 3 months in order to assess treatment efficacy and safety; imaging restaging of disease activity and extension was performed every 6 months. Patient clinical and treatment characteristics are summarised in table 1. Sixteen patients were treated with vemurafenib, seven with cobimetinib and two were sequentially treated with vemurafenib and cobimetinib, with the former also receiving a second vemurafenib course after the discontinuation of the MEK inhibitor. Cobimetinib and vemurafenib were successful in controlling disease progression and improving clinical manifestations in all patients. However, treatment had to be discontinued in eight cases (29%); adverse reactions to vemurafenib and cobimetinib were the only cause of treatment discontinuation.

We hypothesised that combination therapy with anakinra might effectively dampen the toxicity related to MAPK pathway inhibition, thus preventing discontinuation of these life-saving drugs. Seven patients from our cohort were treated with a combination therapy including anakinra. In two of these cases, anakinra was initiated following the development of a severe inflammatory adverse event induced by MAPK inhibition. Specifically, one patient with severe ECD sequentially received vemurafenib and cobimetinib; however, treatment with both MAPK inhibitors yielded a severe systemic inflammatory reaction (fever, elevation of acute phase reactants), which mandated discontinuation. Subsequent initiation of anakinra in addition to vemurafenib effectively controlled systemic inflammation. Another patient developed myocarditis while on vemurafenib; he received anakinra as an add-on therapy, which prompted resolution of cardiac inflammation. In both cases, combination therapy with anakinra proved effective and well tolerated. Most importantly, it enabled retention of life-saving treatment regimens with MAPK inhibitors.

A previous study indicated that combination therapy with anakinra and MAPK pathway inhibitors in ECD might result in incremental efficacy.[6] The present study indicates that anakinra can effectively control and may prevent MAPK inhibitor-related adverse events. Thereby, combination therapy with anakinra might prevent discontinuation and increase retention of life-saving therapies with MAPK inhibitors, especially in the case of inflammation-mediated reactions. Combinatorial approaches might potentially encompass other cytokine-blocking agents, including infliximab.[1] However, anakinra might represent a particularly suitable add-on therapy, given an excellent record of safety and a short half-life of 6 hours, which allows for prompt treatment discontinuation on resolution of the adverse events.

Table 1 Clinical and treatment characteristics of targeted therapy courses

	Vemurafenib (n=19)	Cobimetinib (n=9)
Age at last follow-up visit, years	59.3±12.8	56.2±12.4
Male sex (%)	17 (90)	6 (66)
BRAF V600E mutation (%)	19 (100)	3 (33)
Clinical manifestations (%)		
Cardiovascular	13 (68)	56 (67)
Pleuropulmonary	13 (68)	6 (67)
Neurological and/or orbital	18 (95)	9 (100)
Retroperitoneal	16 (84)	6 (67)
Skeletal	19 (100)	9 (100)
Previous therapies (%)	16 (84)	8 (89)
Targeted therapy	1 (5)	2 (22)
Interferon-α-2a	12 (63)	3 (33)
Glucocorticoid	8 (42)	1 (11)
Methotrexate	3 (16)	1 (11)
Tocilizumab	1 (5)	0 (0)
Anakinra	0 (0)	3 (33)
Infliximab	1 (5)	0 (0)
Imatinib	1 (5)	0 (0)
Tofacitinib	0 (0)	1 (11)
Concomitant therapies (%)	8 (42)	5 (56)
Glucocorticoids	8 (42)	1 (11)
Anakinra	2 (11)	5 (56)
Adverse reactions (%)	11 (58)	4 (44)
Renal	6 (32)	1 (11)
Cutaneous	5 (26)	3 (33)
Systemic inflammation	2 (11)	1 (11)
Cardiovascular	3 (16)	1 (11)
Haematological	0 (0)	1 (11)
Mucosal/gastrointestinal	0 (0)	4 (44)
Hepatic	1 (5)	0 (0)
Treatment discontinued (%)	5 (26)	3 (33)

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Response to: 'Efficacy and improved tolerability of combination therapy with interleukin-1 blockade and MAPK pathway inhibitors for the treatment of Erdheim-Chester disease' by Campochiaro *et al*

We read with interest the letter by Campochiaro *et al*[1] that reported the efficacy of a combination regimen including anakinra and targeted therapy for the treatment of Erdheim-Chester disease (ECD), a rare multisystem inflammatory histiocytosis. Based on the involvement of inflammatory cytokines, including tumour necrosis factor-alpha and interleukin-1, in the pathophysiology and the clinical manifestations of ECD, previous reports demonstrated a variable efficacy with a good tolerance of daily subcutaneous anakinra and infliximab for the treatment of ECD.[2 3] Among 262 patients with ECD who have been seen at our institution until 2019, 31 (12%) (including 12 described in a previous analysis[2]) were treated with 100 mg (n=27) or 200 mg (n=4) daily of anakinra, with a mean duration of 29 months (range, 1–132). Patients were followed on a regular basis and evaluated as previously described.[4] The clinical and molecular details are provided in table 1. Patients treated with anakinra had more osseous and less central nervous system (CNS) involvements. The patients were treated with anakinra alone or in combination with steroids in two patients, and interferon-alpha in one patient. In the latter, failure of treatment led to the prescription of vemurafenib, a BRAF inhibitor, while anakinra was progressively stopped. She thus received a combination therapy (vemurafenib and anakinra) during 1 month without adverse event. Among the 31 patients, anakinra was well tolerated (apart from mild pain at the injection site) in 27 patients. Four (13%) presented severe side effects (sepsis in two, oedema in one and heart failure in one). The global efficacy could be assessed in 24 patients (77%). Ten (42%) underwent an improvement of clinical and/or metabolic disease, in particular at osseous sites, with an objective partial or complete response on bones hypermetabolisms in 8 (33%). Six patients (25%) were stable, whereas 8 (33%) experienced a progression of their disease while under anakinra, including a progression or occurrence of CNS (n=5), cardiac (n=3, with tamponade in 2), lung (n=3) and/or skin (n=2) disease. After receiving anakinra, 11 patients (35%) were treated with targeted therapy, including a BRAF inhibitor in eight cases and/or a MEK inhibitor in seven cases. The targeted therapy was efficacious in all cases. Among the whole cohort of 262 patients, 117 patients (45%) were treated with targeted therapies (vemurafenib or dabrafenib for BRAF inhibition, and trametinib or cobimetinib for MEK inhibition). Although these patients had more cardiac and CNS involvements, they had a better survival than the patients who were not treated with targeted therapies (HR 0.6350, 95% CI 0.4170 to 0.9945, p=0.04). Apart from a single patient who had a short combination with anakinra for 1 month described above, we did not combine targeted therapy and anakinra in our cohort.

Table 1 Clinical and molecular characteristics of patients with Erdheim-Chester disease (ECD)

	All (n=262)	Anakinra (n=31)	Targeted therapies (n=117)
Age at diagnosis, years (mean, SD)	57.7 (14.5)	55.2 (16.4)	57.2 (13.8)
Sex (male/female)	179/83 (2.2)	18/13 (1.4)	74/43 (1.7)
BRAF^{V600E}	148/228 (65%)	16/24 (67%)	95/116 (82%)
ECD involvements			
Long bones	206 (79%)	27 (87%)	101 (86%)
Cardiac	133 (51%)	15 (48%)	83 (71%)
Aorta	159 (61%)	17 (55%)	85 (73%)
CNS	96 (37%)	8 (26%)	56 (48%)
Xanthelasma	58 (22%)	7 (23%)	30 (26%)
Retroperitoneal fibrosis	164 (63%)	20 (65%)	84 (72%)
Lung	93 (35%)	9 (29%)	46 (39%)
Treatments			
IFN-alpha	164 (63%)	25 (81%)	63 (54%)
Anakinra	31 (12%)	31 (100%)	11 (9%)
Targeted therapies	117 (45%)	11 (35%)	117 (100%)
Deaths	85 (32%)	10 (12%)	25 (21%)
Median survival from diagnosis (months)	139	199	Undefined

CNS, central nervous system; IFN, interferon.

Campochiaro *et al* hypothesised that anakinra could dampen the toxicity related to targeted therapies. Most adverse events occurring during BRAF inhibitor treatment, with or without MEK inhibitors, are mild or moderate and can be managed with careful monitoring, dose reduction and supportive care.[5] The most severe adverse events (≥grade 3) usually occur during the first cycle of treatment. Even if the addition of a MEK inhibitor to BRAF inhibition improves outcomes and decreases the incidence of squamous skin cancers and other skin-related toxicities, it is well known that cardiovascular adverse events are more frequent with combined MEK and BRAF inhibitors than with BRAF inhibitors alone (particularly the decrease in left ventricular ejection fraction).[6] The mechanisms of such cardiac toxicities are not fully understood but MAPK has been shown protective for the heart. Although Campochiaro *et al* reported the improvement of vemurafenib-induced myocarditis with anakinra, as it was previously reported in non-toxic myocarditis,[7] we believe that further evaluation is needed to use this treatment for toxic myocarditis. Recent BRAF and MEK inhibitors, encorafenib and binimetinib, have less frequent adverse events, which are usually manageable, reversible and infrequently associated with discontinuation. Notably, fever was less frequently seen than with classical BRAF and MEK inhibitors in a trial of 570 patients.[8] Such drugs could represent interesting drugs for ECD treatment and should be further evaluated.

Previous studies reported the efficacy of therapies targeting the MAPK pathway for treating ECD, with patients treated after anakinra failure.[9] Even if anakinra proved effective in patients with mild disease, a subset of patients experienced a progression of their disease with life-threatening manifestations including tamponade in two and CNS in five. We therefore believe that anakinra should be restricted to 'mild forms' of ECD, in particular patients with bone disease, because of its acceptable profile of tolerance and efficacy for this specific involvement. Patients should be regularly assessed to detect the occurrence of organ dysfunction (cardiac or CNS) that can occur despite anakinra, and then switched to targeted therapies if appropriate. For patients who receive targeted therapies, the security profile requires regular monitoring of heart, skin and retina. The benefit of adding anakinra for improving these toxicities remains to be established.

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Correspondence on 'Immune checkpoint inhibitor-induced inflammatory arthritis persists after immunotherapy cessation' by Braaten *et al*

We read with interest the study published by Braaten and colleagues, analysing the long-term outcomes of 60 patients developing persistent inflammatory arthritis (IA) after immune checkpoint inhibitors (ICIs) cessation. The most relevant result of the study was the presence of active arthritis in more than half of the patients at the last follow-up visit.[1]

We report here our experience in the context of a joint oncology/rheumatology outpatient clinic, in order to evaluate the risk of developing IA in patients treated by anti-PD1 drugs. During 1-year period, we consecutively assessed all the adult patients candidate to anti-PD1 treatment, referring to the Oncology Unit at the Sapienza University of Rome. After treatment starts, in the case of musculoskeletal manifestations, patients were referred to the Sapienza Arthritis Center, Rheumatology Unit, Sapienza University of Rome. Arthritis was defined as the occurrence of at least one episode of clinical synovitis, with morning stiffness lasting at least 30 min. IA activity was assessed by disease activity score on 28 joints by ESR (DAS28-ESR).[2] We investigated the presence of rheumatoid factor (RF), anticitrullinated protein (ACPA) and antinuclear antibodies. In the clinically involved joints, ultrasonographic assessment was performed according to EULAR guidelines.[3]

We evaluated 72 patients (M/F 48/24, median age 66 years, IQR 13.0) affected by lung cancer (75.1%), renal cancer (15.3%), melanoma skin cancer (6.9%), or other neoplastic diseases (2.7%). Sixty-seven patients were treated with nivolumab and the remaining with pembrolizumab (median treatment duration 7 months, IQR 13.0). After 3 months of follow-up, the malignant disease had not progressed in 48 patients (66.7%), whereas an *exitus* was registered in 21 patients (29.2%). During the follow-up period, seven Caucasian patients (9.7%) developed clinically evident synovitis (absolute risk for IA 0.1, incidence rate 0.01). Table 1 reports the main demographic, oncologic and rheumatological features of these patients. Two patients could be classified as affected by rheumatoid arthritis (RA) according to ACR/EULAR 2010 criteria,[4] seropositive in one case (RF and ACPA). Autoantibodies assessment was negative in the remaining patients. Five patients (71.4%) were treated with prednisone (starting dosage 10–12.5 mg/daily, with 2.5 mg reduction every 2 weeks until drug stopping) and the remaining two with non-steroidal anti-inflammatory drugs (NSAIDs) (diclofenac 150 mg/daily for 15 consecutive days). The above-mentioned treatments induced a quick, complete and persistent response in all the patients, except for the seropositive RA subject, in which subcutaneous methotrexate (10 mg/weekly) was added after 4 weeks, achieving a remission status in 3 months. All the patients continued ICIs treatment.

Several differences could be identified by comparing our cohort with the one described by Braaten and colleagues. The previous study included patients developing IA after ICIs cessation, whereas in our cohort, IA appeared during treatment. Nonetheless, in the majority of our patients with IA, treatment with glucocorticoids or NSAIDs was able to induce a prompt and persistent remission. The only patient requiring a disease-modifying anti-rheumatic drug (DMARD) was affected by seropositive RA. Conversely, more than half of the patients evaluated in the Braaten's study showed an active disease at the last visit, as confirmed by the need to introduce synthetic and/or biological DMARDs. In our opinion, this is the most relevant difference between the two cohorts, and this could be explained by the different ICIs treatment. We selected patients treated by anti-PD1, in order to make the cohort homogeneous, whereas the other study included different ICIs. In conclusion, the high risk to develop IA in ICs inhibitors-treated patients confirms the need to include the rheumatologist in the management of these subjects, as recently underlined in the literature review conducted by Jamal and colleagues.[5] The longitudinal assessment of these patients could allow the identification of subjects at risk to develop this specific adverse event.

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Table 1 Demographic features, malignancy history, rheumatological, clinical and ultrasonographic manifestations, time to onset, autoantibody profile and treatment of the seven patients developing synovitis. Active synovitis was defined by the presence of power Doppler signal.

Pt	Sex	Age (years)	Malignancy (treatment)	Clinical manifestations	Interval (weeks)	Autoantibody assessment	US	Diagnosis	Treatment
1	F	55	RCC (nivolumab)	Simmetric polyarthritis	3	RF, ACPA, ANA neg	Active synovitis	RA	PDN 12.5 mg/daily
2	F	61	Melanoma (nivolumab)	Simmetric polyarthritis	3	RF 22 UI/mL, ACPA >300 UI/mL, ANA+ (sp), a-SSA+	Active synovitis	RA	PDN 10 mg/daily MTX 10 mg/weekly
3	M	68	NSCLC (nivolumab)	Monoartrhritis	8	RF, ACPA, ANA neg	Synovitis	UA	NSAIDs
4	F	72	NSCLC (nivolumab)	Polyarthritis	18	RF, ACPA, ANA neg	Synovitis	UA	PDN 12.5 mg/daily
5	M	77	NSCLC (nivolumab)	Oligoarthritis	4	RF, ACPA, ANA neg	Synovitis	UA	NSAIDs
6	M	70	NSCLC (nivolumab)	Simmetric polyarthritis	2	RF, ACPA, ANA neg	Active synovitis	UA	PDN 10 mg/daily
7	M	61	NSCLC (nivolumab)	Simmetric polyarthritis	36	RF, ACPA, ANA neg	Synovitis	UA	PDN 10 mg/daily

ACPA, anti-citrullinated protein antibodies; ANA, anti-nuclear antibodies; a-SSA, anti-SSA; MTX, Methotrexate; NSAIDs, non-steroidal anti-inflammatory drugs; NSCLC, non-small cell lung cancer; PDN, prednisone; RA, rheumatoid arthritis; RCC, renal cell carcinoma; RF, rheumatoid factor; sp, Speckled; UA, undifferentiated arthritis; US, ultrasonographic.

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Response to: Correspondence on "Immune checkpoint inhibitor-induced inflammatory arthritis persists after immunotherapy cessation" by Braaten *et al*

We were interested to read the letter by Ceccarelli *et al* regarding their experience with Immune checkpoint inhibitor (ICI)-induced inflammatory arthritis (IA) at Sapienza University.[1] Their findings support that ICI-induced IA is a heterogeneous disease with differing outcomes. The differences in the cohorts studied may also give us insight into the risk factors for persistence in ICI-induced IA. The authors point out one main difference between the cohorts, type of ICI therapy. Indeed, combination anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)/anti-programmed cell death protein-1 (PD-1) therapy was an independent risk factor for persistent IA in our cohort,[2] and their study included only patients on anti-PD-1 agents. There are several other relevant differences. First, the patients had a shorter duration of ICI use before IA was diagnosed and corticosteroids were started as compared with our study. Duration of ICI therapy was also an independent risk factor for IA persistence in our cohort.[2] Second, all patients in the study were evaluated deliberately for IA which likely led to earlier diagnosis and potentially milder disease. Disease activity is not specifically reported, but the higher incidence of IA (9.7%) than in any previously published studies suggests that milder disease was included.[1] The need for multicentre, international efforts to characterise longitudinal outcomes for ICI-induced IA is apparent.

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Do we need the PFAPA syndrome in adults with non-monogenic periodic fevers?

We read with great interest the article by Gattorno *et al* proposing a new set of criteria for the classification of autoinflammatory recurrent fevers.[1] This year's Paediatric Rheumatology INternational Trials Organisation(PRINTO) criteria are the third set of criteria for periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome in 3 years.[1–3] While these three different sets share common points, they also include distinct clinical features, thus resulting in discrepancies in the classification of patients.

To illustrate this issue, we report the clinical characteristics of a cohort of 34 consecutive adult patients (see table 1) followed in our centre between 2010 and 2018, and diagnosed with PFAPA based on the modified Marshall's criteria[4] (available as online supplementary material) with the exclusion of age at onset. Within this cohort, we sorted patients according to whether they did or did not meet one of the three sets of classification criteria (ie, Cantarini 2017, Vanoni 2018 and the PRINTO 2019 criteria available as online supplementary material). For Vanoni's criteria, we did not apply the age criterion. Regarding treatment response, we defined partial response as a clinically significant decrease in either the duration, the frequency or the intensity of flares as assessed by the treating physician. Complete response was defined as absence of flare. Of our 34 patients, 6 met Cantarini's criteria (designed specifically for adult-onset PFAPA), 17 met Vanoni's criteria, 13 met the PRINTO 2019 criteria and 13 did not meet any of the recent classification criteria for PFAPA (see online supplementary figure 1). Thirty-two (94%) patients had undergone genetic testing based on their specific characteristics, which all yielded inconclusive results. Regardless of the set of criteria fulfilled, our patients displayed globally similar therapeutic response and disease course. None developed AA amyloidosis. Most patients managed their flares using short courses of oral corticosteroids. Furthermore, long-term treatment with colchicine was successful in approximately 50% of patients (partial or complete response rate ranging from 43% to 76%). During follow-up, spontaneous remission or decrease in the duration, frequency or intensity of flares occurred in 50% of

Table 1 Patients' characteristics and therapeutic response according to the set of criteria fulfilled

	Overall (n=34)	Cantarini's criteria (n=6)	Vanoni's criteria (n=17)	PRINTO 2019 criteria (n=13)	Not classified* (n=13)
Female/male	23/11	4/2	12/5	8/5	9/4
Age at onset (years)	5.0 (2.4–15.8)	21.5 (15.8–25.3)	5.0 (1.5–9.0)	2.5 (0.65–5.0)	7.0 (4.5–16.0)
PFAPA duration (years)	16.4 (10.0–20.4)	4.5 (3.8–5.8)	14.9 (6.8–18.2)	16.4 (12.5–19.4)	18.6 (12.5–20.5)
Annual frequency of flares before start of therapy	12.0 (6.5–12.0)	10.0 (5.8–12.0)	12.0 (10.0–12.0)	12.0 (9.3–14.8)	8.3 (3.3–12.0)
Length of flares before start of therapy (days)	3.8 (3.0–4.6)	4.0 (3.9–4.8)	4.0 (3.3–4.6)	4.3 (3.3–5.1)	3.0 (2.6–3.5)
CRP during flares (mg/L)	113 (53–150)	152 (59–201)	104 (57–140)	111 (60–144)	67 (38–128)
Acute treatment of flares					
NSAID (n)	7	0	5	4	1
Corticosteroids (n)	24	5	13	11	8
Anakinra (n)	5	3	3	2	1
Long-term treatment					
Colchicine (n)	29	5	14	10	12
CR (%)	19	0	9	14	38
PR (%)	38	50	36	29	38
NR (%)	29	50	27	43	13
AE (%)	19	0	27	14	13
Anakinra (n)	1	0	1	0	0
CR (%)	100	–	100	–	–
PR (%)	0	–	0	–	–
NR (%)	0	–	0	–	–
Canakinumab (n)	1	1	0	0	0
CR (%)	0	0	–	–	–
PR (%)	0	0	–	–	–
NR (%)	100	100	–	–	–
Tonsillectomy/ adenoidectomy (n)	11	1	6	4	4
CR/PR (%)	27	0	17	25	50
NR (%)	73	100	83	75	50
Outcome at last follow-up					
CR/PR with no treatment (%)	50	40	56	67	40

Continuous variables are given in median (IQR).
*Patients fulfilling only modified Marshall's criteria.
AE, adverse effect leading to discontinuation; CR, complete response;CRP, C-reactive protein; NR, no response; NSAID, nonsteroidal anti-inflammatory drug;PFAPA, periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis; PR, partial response.

patients (partial or complete remission rate ranging from 40% to 67%). Of note, as previously reported in adult PFAPA, tonsillectomy and/or adenoidectomy was inefficient in the majority.[5] Although the size of the cohort was insufficient to perform statistical tests, it seems that regardless of the set of PFAPA criteria used, the disease course and therapeutic response were identical in the four criteria set groups. As a result, recent attempts to diagnose PFAPA more accurately may not translate into the identification of distinct patient profiles in terms of disease course and therapeutic management. Moreover, the recently described heterogeneous group of undefined systemic autoinflammatory diseases (USAID), defined as recurrent inflammation not corresponding to the clinical picture of any well-defined SAID or without pathogenic mutation causing a known hereditary SAID, seems to display similar characteristics.[6] Therefore, the relevance of positioning PFAPA as a distinct entity than USAID in adult patients with non-monogenic periodic fevers is questionable.

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Response to: 'Do we need the PFAPA syndrome in adults with non-monogenic periodic fevers?' by Fayand *et al*

We really thank Fayand and coworkers for their interest to the new Eurofever/PRINTO classification criteria for periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA)[1] and for their interesting exercise to apply the three most recent criteria to their population of adult patients with recurrent fever fulfilling the revised Marshall's criteria for PFAPA.[2]

Indeed, in the last few years, three new criteria for PFAPA have been proposed.[3–5] Of note, the methodology used for the development of the three set of criteria was rather different, as is for the process of their validation in an independent population.

Cantarini's criteria were exclusively developed for adult-onset PFAPA patients. Seventy-four adult patients fulfilling the modified Marshall's criteria were compared with 62 patients with fever of unknown origin (FUO). A multivariate analysis identified the set of variables with the highest accuracy in distinguishing PFAPA from FUO patients.[4] The criteria have not been validated in an independent population, so far.

Vanoni's criteria were created using the standard consensus procedures (Delphi survey and Nominal Group Technique in a Consensus Conference) among 22 paediatric experts in autoinflammation. The new proposed criteria were tested in 80 paediatric PFAPA patients followed in two centres for autoinflammatory diseases (Genoa and Lausanne). For this validation process, the diagnosis of PFAPA was not done on the basis of modified Marshall's criteria, but on the clinical judgement of the two centres. Notably, only 51% of the patients fulfilled the new criteria.[5]

The new Eurofever/PRINTO) criteria were developed with a multistep evidence-based approach. A Delphi questionnaire survey was proposed to 162 international experts in autoinflammatory diseases to identify the best possible candidate classification criteria for PFAPA syndrome. In the second step, 360 random paediatric and adult patients with recurrent fevers (familial mediterranean fever (FMF), TNF receptor 1 associated periodic syndrome (TRAPS), mevalonate kinase deficiency (MKD), cryopyrin associate periodic syndrome (CAPS), PFAPA and undifferentiated systemic autoinflammatory diseases (SAID)) from the Eurofever registry were evaluated by a panel of 25 clinicians and eight geneticists blinded to patients' diagnosis. Patients were classified on the consensus of at least 80% of the experts. In the third step, a multivariate statistical analysis performed on patients validated by the experts, identified for each condition a number of different set of variables able to discriminate them from the other confounding diseases. The best final classification criteria were chosen using the nominal group technique in a Consensus Conference that involved 33 international experts. Finally, all the new criteria, including those for PFAPA, were validated in an independent data set of 1018 patients extracted from the Eurofever registry not previously included in the statistical analysis.[3]

In our opinion, the strict methodological and evidence-based approach used for the development of the new Eurofever/PRINTO classification criteria should indicate them as the most reliable criteria available so far either for children or adults.

It is important to note that in the validation phase, the accuracy of the new Eurofever/PRINTO PFAPA criteria was 81%, with an high specificity (98%) and a much lower sensitivity (66%).[3] This discrepancy between sensitivity and specificity was expected, since classification criteria are essentially done to identify patients with a very high probability to suffer from a given disease to be included in clinical trials or translational studies. Indeed, the classification criteria should not be used in the daily clinical practice and should not be considered as diagnostic criteria for which pathognomonic criteria are indeed necessary.

The different performances of the three classification criteria in the population analysed by Fayand *et al* could be merely due to patient selection methodology used in their study.[1] As stated above, patients were selected according the modified Marshall's criteria. However, previous studies have provided evidence of the low accuracy of these latter criteria when applied to a population of patients with different forms of recurrent fevers, including the monogenic ones.[6] Furthermore, the studied population seems to represent a mix between PFAPA patients with classical onset, as young child and adult-onset PFAPA patients. Since the phenotype may change with the time, it would be interesting to compare the classification obtained considering the initial clinical picture of the patients or that present at the time of the adult consultation. In table 1, we notice that the median age of onset was between 2.5 and 5 years in patients fulfilling the PRINTO and Vanoni criteria, 21.5 years for the Cantarini criteria and 7 years for the not classified patients.

On the basis of the high variability in the performance of the different PFAPA criteria observed in their study, Fayand and coworkers postulate the hypothesis that PFAPA syndrome should not be considered as a separate entity in adults presenting with recurrent fever episodes, that should be classified as undefined systemic autoinflammatory diseases (USAID). To support their thesis, the authors provide, in their study, the evidence that almost 50% of their patients had a complete or partial response to colchicine independently from the satisfaction on any of the three classification criteria. However, it should be noted that the higher rate of response to colchicine (72%) was observed in patients not classified by any of the three criteria, while the lower rate of response to colchicine (43%) was observed in those fulfilling the Eurofever/PRINTO criteria.

On the other hand, it is also clear that a relevant percentage of patients display the same response to steroids on demand observed in the paediatric PFAPA patients and that four out of 11 patients who underwent tonsillectomy had a complete response. These observations clearly show the extreme heterogeneity in these groups of patients with idiopathic, not-monogenic, recurrent fevers.

In two recent studies, the term of SURF (systemic undefined recurrent fevers) was proposed, with the actual aim to identify a more homogeneous group of patients with recurrent fevers characterised by a minor pharyngeal and lymph node involvement, an higher prevalence of arthritis and skin rash, and a good response to colchicine and anti-IL-1 blockers.[7 8] At least in children, this subgroup of patients should be clearly distinguished from the more common PFAPA patients.

It is also conceivable that a small percentage of adult patients with recurrent fevers could present the same clinical features of paediatric PFAPA deserving an appropriate treatment and follow-up.

In our opinion, the available classification criteria should be used to set up longitudinal studies enrolling paediatric and adult patients with recurrent fevers in order to verify the existence of different conditions (for clinical presentation, follow-up and response to treatment) in the heterogeneous group of USAID commonly observed in the daily clinical practice.

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Correction notice This article has been corrected since it published Online First. The PRINTO and Eurofever Registry author details were added.

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Correction: *Parsing multiomics landscape of activated synovial fibroblasts highlights drug targets linked to genetic risk of rheumatoid arthritis*

Tsuchiya H, Ota M, Sumitomo S, *et al*. Parsing multiomics landscape of activated synovial fibroblasts highlights drug targets linked to genetic risk of rheumatoid arthritis. *Ann Rheum Dis* 2021;80:440–50. doi.10.1136/annrheumdis-2020-218189

'Circus' should read 'Circos' in the legend of figure 5. The correct legend should be:

Enrichment of RA genetic risk in SFs SEs under eight cytokine stimulation. (A) Enrichment of RA risk loci in transcriptional regulatory regions of stimulated SFs and PBMCs. Active enhancers were classified into SEs and TEs following standard rose algorithms. The red solid lines and the black solid lines are the cutoffs for Bonferroni significance and p=0.05, respectively. (B) A Circos plot showing the overlap of SEs in SFs under different stimulatory conditions. only the regions unique to each condition or common to all of the conditions are depicted. (C) A Circos plot showing the overlap of RA risk loci and SEs in SFs under different stimulatory conditions.

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