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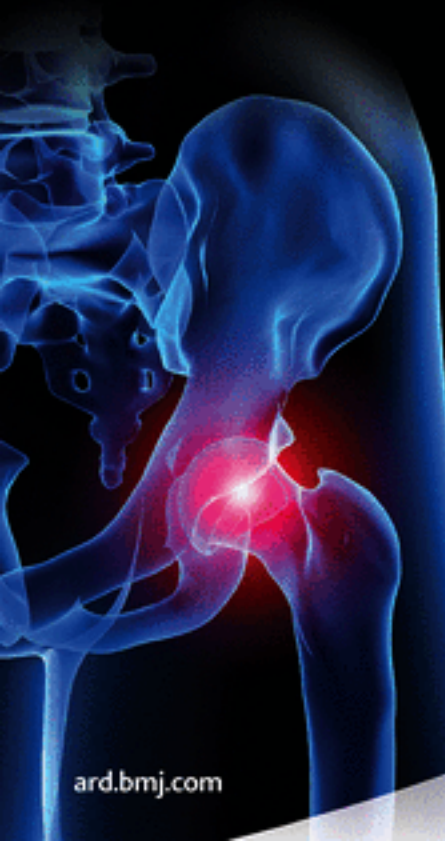

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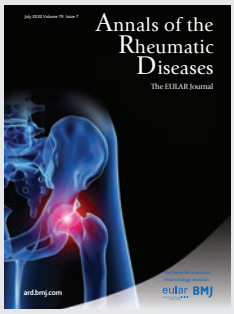




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# Hopefulness of 'Hope'

David S Pisetsky

'Hope' by Dr Kushboo Sheth is a profound and moving depiction of the inner life of a rheumatologist confronting the COVID-19 viral pandemic. The feelings that Sheth describes are as varied as they are powerful and range from fear to rage to distress. Burdened by the weight of these feelings, she vows to adapt, persevere and trudge onward. Most of all, she strives to get beyond the pandemic, aspiring to be a better person whatever battering and damage may come along the way.

The feelings that Sheth describes are inescapable at this time given the upheaval that the pandemic has caused in all aspects of life. Vulnerability is universal. Anyone can infect me and I can infect anyone. Those who can infect and be infected are the same: families, friends, patients, the checkout clerk at the grocery, a random passerby walking too close to you on the sidewalk.

In this calculus, infection is a matter of bad luck or, to use a word that has fallen out of fashion, fate. Even if infection does not lead to hospitalisation, it can lead to confinement at home, an isolation tantamount to imprisonment.

What I find striking about 'Hope' is not the range and depth of Sheth's feelings but rather the identity of the person who is experiencing them. Sheth is a physician. Physicians by training should be accustomed to sickness and death and, indeed, have developed coping mechanisms that make professional activity as well as ordinary living possible.

The transformation of an ordinary person into a physician is a remarkable process. It entails far more than the acquisition of knowledge or the mastery of technical skills. The transformation is also psychological and it is spiritual: to learn to confront misery on a daily and sometimes hourly basis without it becoming overwhelming and debilitating.

When the shift in the hospital or clinic session ends, ordinary life must resume. There are dinners to share with families and friends, children's sporting events to attend, interludes of delight with lovers. All must transpire without the residue of work—the disappointments, pressure and stress, the proximity to tragedy—impeding participation and enjoyment. Health professionals can acquire a shell so sturdy and effective that trying to achieve a life–work balance is not only possible but expected.

The shell that surrounds physicians is built gradually by accretion, with clinical training in medical school providing the first layer. I find it intriguing that the initial clinical experience of medical students in internal medicine occurs on the acute inpatient service of the hospital. There, the absolutely sickest patients receive their care. These are often older people with multiple comorbidities and, depending on the training environment, an array of psychosocial problems that defy solution. At present, many of these patients are infected with the virus.

Amazingly, the clinical clerk, armed with a snippet of knowledge in anatomy, biochemistry and pharmacology, is expected to dive into the thick of things—to serve on the front lines, to engage the patient as caregiver and to assume the role of physician.

While it might make more sense for medical students to begin learning medicine on an outpatient rotation to ease into the care of patients, I think that the goal of the first inpatient rotation is to start building a shell. I am not familiar with the training of nurses, respiratory therapists or social workers but I believe they all start in an acute hospital environment.

The training process that commences in medical school intensifies and accelerates during house staff years when responsibilities and duty hours increase and include nights on call in the hospital, often without sleep. (As an intern, I had rotations where I spent two straight nights on call in the hospital but, thankfully, those days are over.) While the process can be taxing and even harsh—sort of like 'boot camp' for a soldier—over time, the trainee learns to handle the exigencies of caring for the very sick and dying and develop defenses and coping strategies to compartmentalise their lives.

Is the shell built over the years impenetrable or can the exposure to too much sickness and too much death cause the shell to fissure and crack? Tragically, the disasters confronting healthcare professionals right now are so relentless and so extreme that the defence systems can fail; this is very evident in the big city hospitals where infections surge dangerously and colleagues and coworkers can also perish.

During catastrophes such as the COVID-19 pandemic, ordinary human feelings can surface in a physician as the protective shell crumbles. As Sheth elegantly recounts, a physician can break, cringe, rage and cry—even if not immediately on the front line. The demands on the hospitals, especially the intensive care units, are so massive that terror permeates the whole system and can afflict everyone. This is not burnout. This is a raging fire.

While physicians may be desperate to express feelings of dread, anxiety and uncertainty, resources and 'safe spaces' may not be available to accomplish this important function. Counselling services, wellness activities and 'heal the healer' initiatives may offer benefits, with those involving peer-to-peer interactions particularly valuable. I think that all institutions should have these programmes to help their staffs cope with the care of patients with the virus.

Among the many acts that Sheth describes, ultimately, hope may be the most saving. I am glad that she titled her article that way. Hope is something that everyone can do since it transcends philosophy, worldview and religion. To me, prayer is a vehicle for hope. I was intrigued to learn that one of my colleagues—a tough critical care physician and solid atheist—now begins each shift with his MICU team by leading them in prayer.

For the care of very sick patients like those who have the COVID-19 virus, hope is possible even if death looms inevitably. While hope for a miraculous cure—the magic silver bullet—never extinguishes, hope may simply seek for the patient to have a visit with the family, relief from pain or a death without suffering.

I agree with Sheth. As physicians and healthcare providers, we must adapt. We must persevere and, even if we can do no more than trudge onward, we must also hope. Hope is the foundation of all medical care. It is a source of strength, an essential act that affirms the meaning of life and defies the pull of loss and despair.

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# EULAR provisional recommendations for the management of rheumatic and musculoskeletal diseases in the context of SARS-CoV-2

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## ABSTRACT

The provisional EULAR recommendations address several aspects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus, and the disease caused by SARS-CoV-2, COVID-19 and are meant for patients with rheumatic and musculoskeletal diseases (RMD) and their caregivers. A task force of 20 members was convened by EULAR that met several times by videoconferencing in April 2020. The task force finally agreed on five overarching principles and 13 recommendations covering four generic themes: (1) General measures and prevention of SARS-CoV-2 infection. (2) The management of RMD when local measures of social distancing are in effect. (3) The management of COVID-19 in the context of RMD. (4) The prevention of infections other than SARS-CoV-2. EULAR considers this set of recommendations as a 'living document' and a starting point, which will be updated as soon as promising new developments with potential impact on the care of patients with RMD become available.

## INTRODUCTION

The provisional recommendations address several aspects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus, and the disease caused by SARS-CoV-2, COVID-19. They address the implications for patients with rheumatic and musculoskeletal diseases (RMD). They have been commissioned by EULAR, and developed under its auspices, in order to guide both rheumatologists and health professionals in rheumatology (HPR) who care for patients with RMD, COVID-19-treating physicians as well as patients with RMD themselves and their family members.

Many (inter)national professional organisations in rheumatology and beyond, as well as government bodies, have issued guidance documents pertaining to the prevention, diagnosis and treatment of SARS-CoV-2 infection and COVID-19. Since generic recommendations do not focus on patients with RMD and their circumstances, EULAR considered it essential to provide a set of recommendations that are applicable to all rheumatologists and HPRs and their patients with RMD in EULAR countries. Guidelines issued by national (professional) organisations can occasionally be more or less restrictive than EULAR recommendations. By no means EULAR intends to overrule existing guidelines at the country level of EULAR member states. EULAR only aims to provide a synthesis of the best available aggregated expert opinion to inform rheumatologists and HPR and patients with RMD about management decisions to be taken in the context of the SARS-CoV-19 epidemic.

SARS-CoV-2 is a new virus and COVID-19 a new disease. Scientific knowledge is rapidly accruing, but methodologically robust information from well-controlled trials and experiments is lacking to date. In contrast, we face a flood of unreliable largely uncontrolled studies and even *fake news*. It is to be expected that scientific knowledge of the calibre that EULAR usually requires to design and update their recommendations will be lacking for a while. Nevertheless, people with RMD appropriately confront their rheumatologists and HPR with questions about treatment implications and COVID-19-associated anxiety. In turn, rheumatologists and HPR may feel uncertain about how to advise in the best interest of their patients. Therefore, EULAR decided not to wait until robust scientific knowledge becomes available, but to deviate from their standard operating procedures[1] and to convene a task force of international experts to provide provisional guidance for rheumatologists, HPR and patients with RMD. Although the task force was hampered by restrictions of social distancing, preventing them to meet in person—it performed the complete process successfully by videoconferences.

EULAR is committed, in contrast to our usual procedures, to consider this set of recommendations as a 'living document' and a starting point, which will be updated as soon as promising new developments with potential impact on the care of patients with RMD become available. These developments will be monitored closely, their quality judged by a team of EULAR methodologists and, after further discussion in the task force, included in an updated version of the recommendations when appropriate.

## PROCEDURES

### Focus of recommendations

These recommendations pertain to the management of patients with RMD insofar as the current SARS-CoV-2 epidemic and its consequent COVID-19 disease may interfere with usual

management of patients with RMD. These recommendations are decidedly not focused on the diagnosis or treatment of COVID-19. There is some focus on so-called 'inflammatory' RMD, because of specific issues that patients with systemic autoimmune diseases, partly due to their treatments, may face or may have concerns about, without excluding all patients with other types of RMD.

### The task force composition

This EULAR task force consists of 22 experts from seven EULAR member states. Most experts are internationally recognised rheumatologists and immunologists with many years of clinical and scientific experience, who fulfil or have fulfilled official positions in the EULAR organisation. EULAR's current, past and incoming presidents (HWJB, GRB, IM, AI, JSS), as well as the current, past or incoming chairs of EULAR's standing committees on epidemiology and health services research (PMM, LC, LG), clinical affairs (UM-L, RBL) and investigative rheumatology (XM) are members of the task force, among others. The task force was supported by an expert on viral lung diseases (PO), an infectious disease specialist (MG), the EULAR vice-president representing HPR (TAS), the EULAR vice-president representing patients with arthritis and rheumatism (DW) and a clinical fellow (FK). The task force was presided by the past-president of EULAR (HWJB) and selected an overarching steering committee consisting of three clinically active rheumatologists (RBL, PMM, HS-K) and one fellow (FK). All task force members had ample experience with the development of EULAR recommendations according to EULAR's standard operating procedures (SOPs).[1]

### Handling potential conflict of interest

In accordance with EULAR's SOP, task force members are asked on an annual basis to provide and update their interactions with third parties (guideline committees, reimbursement bodies, pharmaceutical industries or other industries) that are not directly related to all day patient care but may give an impression to others of conflict of interest (*potential* COI). The EULAR office keeps record of these declared potential COIs.

### The steering committee's workflow

First, the steering committee collected, largely from official websites, existing guidance documents stemming from several European and non-European countries. Some of these focused on RMD and were prepared by national professional organisations of rheumatology (German,[2] French,[3 4] Spanish[5]) or general medical organisations (UK National Health Service,[6] National Institute for Health and Care Excellence[7]). Others were generic guidelines, not focusing on RMD (WHO).[8] During the process a set of recommendations by the American College of Rheumatology became available.[9]

Thereafter, the steering committee proposed five overarching principles (OPs). In EULAR's recommendation documents, OPs usually serve to underpin the content of the subsequent recommendations; OPs set the stage on which the body of guidance that follows is built.

Next, the steering committee distinguished four areas of interest for which recommendations seemed appropriate: (1) General measures and prevention of SARS-CoV-2 infection. (2) The management of RMD when local measures of social distancing are in effect. (3) The management of COVID-19 in the context of RMD. (4) The prevention of other infections than SARS-CoV-2.

In total, the steering committee conceived 114 recommendations (between 2 and 5 per area of interest) that were largely based on existing guidelines and recent personal clinical experience of steering committee members. Explanatory information accompanied each of the proposed OP and recommendations.

The steering committee met three times within a period of 10 days by videoconferencing.

### The task force's workflow

The task force members took notice of the draft recommendations by email and were given the opportunity to propose changes, new themes and recommendations. These suggestions were collected by the steering committee and discussed in a task force meeting by videoconference (14 April). The steering committee drafted a second proposal, including the proposed changes, and one new recommendation, which was discussed 1 week later in a second task force meeting (21 April). Consensus was reached on 21 April, and the steering committee was assigned the task to prepare the manuscript. All task force members commented on and agreed to the final version of the manuscript before submission.

### Target audience

In line with EULAR's SOP, the task force agreed to target their guidance primarily on rheumatologists, HPR and patients with RMD and their families. Secondarily, these recommendations target public health officials and public health experts by making them aware of particular problems pertaining to patients with RMD and their treatments, as well as policy makers, who decide about measures of social distancing, access to healthcare for patients with RMD and availability of drugs for patients with RMD.

### Systematic literature research

It was decided upfront that a systematic literature research to inform the process would not be performed. This is justified on the current absence of sufficient appropriately controlled clinical studies or relevant epidemiological reports as to inform a meaningful process.

### Formal decision making

A formal voting procedure was not performed. Each expert's level of agreement (from 0 (no agreement at all) to 10 (fully agree)) with the statement was solicited by email for each OP and recommendation on 23 April. The mean level of agreement, as well as the proportion of experts with a level of agreement of at least 8, was calculated.

## RESULTS

The task force finally agreed on 5 OPs and 13 recommendations. The bullet text of these OPs and recommendations can be read in table 1. Below, an item-by-item discussion is outlined, that clarifies the choice of themes and wording and sheds more light on the discussions that have taken place in the task force.

*OP 1. To date, there is no evidence that patients with RMD face more risk of contracting SARS-CoV-2 than individuals without RMD, nor that they have a worse prognosis when they contract it.*

This OP states that, according to current knowledge, patients with RMD should not be managed differently than individuals without RMD. It is currently unknown whether a specific RMD or treatment with a specific drug influences the risk (increase, decrease or no change in the risk) of developing COVID-19. While many advisories, including official government bodies

**Table 1** EULAR provisional recommendations for the management of rheumatic musculoskeletal diseases in the context of SARS-CoV-2—April 2020 version

| | | LoA | |
|---|---|---|---|
| | **Overarching principles** | **Mean±SD** | **≥8/10 (%)** |
| 1. | To date, there is no evidence that patients with RMD face more risk of contracting SARS-CoV-2 than individuals without RMD, nor that they have a worse prognosis when they contract it. | 9.1±1.2 | 84 |
| 2. | The diagnosis and treatment of COVID-19 in patients with RMD is the primary responsibility of an expert in treating COVID-19, such as a pulmonologist, an internist or a specialist in infectious diseases, dependent on local circumstances. | 9.3±1.3 | 84 |
| 3. | Rheumatologists are the leading experts for the immunosuppressive treatments of their patients and should be involved in the decision to maintain or discontinue them. | 9.2±2.4 | 89 |
| 4. | The knowledge about immunosuppressive treatments, including sDMARDs and bDMARDs, for the treatment of severe COVID-19 is rapidly evolving. In view of their expertise, rheumatologists should make themselves available for local-hospital, regional or national guideline committees for COVID-19. The use of immunosuppressive drugs for the treatment of COVID-19 should be a multidisciplinary decision. | 9.3±1.4 | 84 |
| 5. | Availability and distribution of, and access to, sDMARDs and bDMARDs for the treatment of patients with RMD as well as for patients with COVID-19 (but without RMD) is a delicate societal responsibility. Therefore, the off-label use of DMARDs in COVID-19 outside the context of clinical trials should be discouraged. | 8.9±1.2 | 89 |
| **Recommendations** | | | |
| 1. | Patients with RMD should be strongly advised to comply with all preventive and control measures prescribed by the health authorities in their countries. | 9.9±0.5 | 95 |
| 2. | Patients with RMD should in general be advised to comply with the same preventive and control measures as patients without RMD. | 9.3±1.0 | 89 |
| 3. | Patients with RMD who do not have suspected or confirmed COVID-19 should be advised to continue their treatment unchanged, namely NSAIDs, glucocorticoids, sDMARDs, bDMARDs, osteoporosis medications and analgesics, among others. | 9.6±0.6 | 94 |
| 4. | If the RMD and its drug treatment are stable, and signs or symptoms of drug toxicity are absent, regular blood monitoring and face-to-face rheumatology consultations can be postponed temporarily. If necessary, consultation can take place remotely. | 9.6±0.9 | 94 |
| 5. | If the RMD is active, if drug therapy has recently been started or needs adjustment, or if signs or symptoms of drug toxicity emerge, patient and rheumatologist should liaise, weigh the risks of a visit to the clinic against the limitations of remote advice and decide together. | 9.7±1.0 | 89 |
| 6. | If a patient with RMD is offered an outpatient, day care or other type of hospital appointment, patients and members of the rheumatology team should follow local guidance for infection prevention and control, including the use of personal protection equipment if indicated. | 9.9±0.2 | 94 |
| 7. | Patients with RMD without COVID-19 symptoms who have been in contact with a SARS-CoV-2-positive person should be tested for SARS-CoV-2 themselves. | 8.0±2.5 | 63 |
| 8. | If a patient with RMD and symptoms of COVID-19 is chronically treated with glucocorticoids, this treatment should be continued. | 8.8±1.6 | 79 |
| 9. | If patients with RMD experience mild* symptoms of COVID-19, potential treatment changes in DMARDs should be discussed on a case-by-case basis. | 8.9±1.4 | 84 |
| 10. | Patients with RMD and initially mild symptoms who experience worsening† of COVID-19 symptoms should immediately seek further healthcare advice of an expert in treating COVID-19, such as a pulmonologist, an internist or a specialist in infectious diseases, dependent on local circumstances. | 9.8±0.5 | 94 |
| 11. | Patients with RMD who are admitted to the hospital because of significant†, ‡ COVID-19 should follow local treatment recommendations for COVID-19 as applied by the treating expert. | 9.7±0.8 | 89 |
| 12. | Patients with RMD without symptoms of COVID-19 should be advised to update their vaccination status in accordance with the EULAR recommendations for the vaccination of patients with RMD, with a particular focus on pneumococci and influenza. | 9.4±1.0 | 89 |
| 13 | In patients with RMD treated with cyclophosphamide or glucocorticoids, *Pneumocystis Jiroveci* pneumonia prophylaxis should be considered. | 9.3±0.9 | 89 |

*See definition of mild symptoms in box 2.
†See definition of worsening in box 2.
‡See definition of significant COVID-19 in box 2.
bDMARD, biologic disease modifying antirheumatic drug; EULAR, European League Against Rheumatism; LoA, level of agreement; NSAID, non-steroidal anti-inflammatory drug; RMD, rheumatic and musculoskeletal diseases; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sDMARD, synthetic disease modifying antirheumatic drug.

in some countries,[6 10–12] postulate an increased risk for patients with inflammatory/autoimmune diseases or those using immunosuppressive drugs, since they extrapolate existing data stemming from registries that such patients have increased risk of some infections,[13–15] it should be stated clearly that such an association for SARS-CoV-2 and COVID-19 has not (yet) been established. From this OP follows that there is no current basis for preventive measures that are more or less restrictive than those issued for the general population (see recommendations 1 and 2). However, there is also no evidence that patients with RMD, irrespective of their treatment, have a better prognosis than other individuals.

Level of agreement: 9.1±1.2; 84% scored 8/10 or higher.

*OP 2. The diagnosis and treatment of COVID-19 in patients with RMD is the primary responsibility of an expert in treating COVID-19, such as a pulmonologist, an internist or a specialist in infectious diseases, dependent on local circumstances.*

**Box 1 Cytokine release syndrome**

- Cytokine release syndrome (CRS) (also described as cytokine storm, macrophage activation syndrome or secondary haemophagocytic lymphohistocytosis) is an emergency condition of systemic hyperinflammation that may occur in patients with COVID-19 pneumonia.[25] CRS should be suspected in patients with confirmed COVID-19 pneumonia (either by PCR testing or by CT scan) who rapidly deteriorate and experience respiratory failure. Potential biomarkers of CRS are very high levels of C reactive protein, D-dimer, ferritin and IL-6 or a high *H Score*, which computes a value based on the following components: temperature, organomegaly, number of cytopenias, triglycerides, fibrinogen, ferritin, aspartate aminotransferase, haemophagocytosis on bone marrow aspirate and known immunosuppression.[26]

This OP serves to make clear that the diagnosis and treatment of SARS-CoV-2-infection and COVID-19 does not and should not belong to the expertise and responsibility of the rheumatologist or the HPR working in the field of rheumatology. Dependent on local (national) circumstances, several different medical specialists take care of these patients.

Level of agreement: 9.3±1.3; 84% scored 8/10 or higher.

*OP 3. Rheumatologists are the leading experts for the immunosuppressive treatments of their patients and should be involved in the decision to maintain or discontinue them.*

This OP states that the rheumatologist is an important discussion partner in making decisions on drug treatment in patients with RMD, in particular patients that use synthetic or biologic disease modifying antirheumatic drugs (sDMARDs and bDMARDs, respectively) or other drugs that have an immunosuppressive connotation. This OP is important since recent information suggests that clinicians taking care of patients with COVID-19 are tempted to stop all treatments that are believed to be associated with impaired virus clearance, without considering the risk of a flare of the underlying RMD, leading to unwarranted situations and anxiety in patients. The treating rheumatologist is the pre-eminent discussion partner for experts in treating COVID-19 to decide if a drug for RMD can be paused safely or should be continued (see below). The rheumatologist's role should not be marginalised.

In the task force there was dissent about using the term 'immunosuppressive' versus the term 'immunomodulatory'. The task force finally decided to keep the term 'immunosuppressive', since it is the fear for and perception of inappropriate suppression of the immune system that leads to the discontinuation of these drugs in case of COVID-19. Still, some of them do not formally supress the immune system (eg, hydroxychloroquine (HCQ) and sulfasalazine) and bDMARDs that target cytokines specifically block one element of the immune system while leaving remaining components unmanipulated.

Level of agreement: 9.2±2.4; 89% scored 8/10 or higher.

*OP 4. The knowledge about immunosuppressive treatments, including sDMARDs and bDMARDs, for the treatment of severe COVID-19 is rapidly evolving. In view of their expertise, rheumatologists should make themselves available for local-hospital, regional or national guideline committees for COVID-19. The use of immunosuppressive drugs for the treatment of COVID-19 should be a multidisciplinary decision.*

This OP further elaborates on OP3, but addresses the matter from a different angle: it acknowledges the practice that some DMARDs (such as (hydroxy)chloroquine) are now, rightly or wrongly, propagated for the prevention or treatment of COVID-19. Several bDMARDs (such as interleukin (IL) 6 and IL-1 inhibitors) and janus kinase inhibitors (JAKi) are under investigation for treating severe COVID-19 and are sporadically used 'off-label', in particular in patients with COVID-19 with concomitant cytokine release syndrome (CRS; see box 1 2 for an explanation). The IL-6 receptor blocker tocilizumab has been approved by Food and Drug Admiistration (FDA) and European Medicines Agency (EMA) for patients with chimeric antigen receptor (CAR) T cell treatment associated CRS and recently by Chinese authorities for severe COVID-19.[16]

Rheumatologists may possess relevant knowledge about the indications, contraindications and toxicity of DMARDs and cytokine inhibitors and could be consulted by physicians treating patients with COVID-19 and by guideline committees. The term 'multidisciplinary' here refers to different medical specialists, but it is obvious that the decision to start, stop or continue treatment with DMARDs or cytokine inhibitors in the end should be a shared decision between patients and physician(s).

Level of agreement: 9.3±1.4; 84% scored 8/10 or higher.

*OP 5. Availability and distribution of, and access to, sDMARDs and bDMARDs for the treatment of patients with RMD as well as for patients with COVID-19 (but without RMD) is a delicate societal responsibility. Therefore, the off-label use of DMARDs in COVID-19 outside the context of clinical trials should be discouraged.*

This principle elaborates on the potential lack of drug availability for patients with RMD (with or without COVID-19) due to unproven overuse for patients with COVID-19. The best example is the shortage of HCQ for patients with systemic lupus erythematosus, that arose in some countries after rumours that this drug would be effective in COVID-19.[17] Similar concerns exist for particular bDMARDs (eg, tocilizumab). 'Delicate responsibility' refers to the following dilemma: in the absence of a proven treatment for COVID-19, clinicians will understandably try every drug with possible efficacy in critically ill patients, and publish their successes in case reports. However, by doing so they may unintentionally contribute to creating false hope and conveying wrong information. Since some DMARDs are potentially efficacious in COVID-19, patients with RMD can be affected disproportionally. It is because of this dilemma that off-label medication use outside the context of clinical trials should

**Box 2 Symptoms of COVID-19**

***Mild symptoms of COVID-19:**

- These include symptoms of common cold, such as sore throat, running nose, nasal congestion, anosmia or dysgeusia, fatigue, generalised or local myalgia, arthralgia without clinical swelling, anorexia, diarrhoea, as well as temperature elevation (<38°C).

****Worsening of mild COVID-19 symptoms:**

- This applies when a patient with formerly mild symptoms of COVID-19 gets fever ≥38°C or subjective shortness of breath or tachypnoea (>20/min) or hypoxia or cyanosis.

*****Significant symptoms of COVID-19:**

- These include all of the above, but accompanied by fever (≥38°C) or subjective shortness of breath or tachypnoea (>20/min) or hypoxia or cyanosis.

be discouraged. Physicians that nevertheless decide to treat patients with COVID-19 with DMARDs off-label have a responsibility to document their argumentation and the follow-up of these patients carefully.

Level of agreement: 8.9±1.2; 89% scored 8/10 or higher.

### General measures and prevention of SARS-CoV-2 infection

Recommendations 1–3 pertain to general public health measures and precautions. The scope is that of patients with RMD who have no signs of COVID-19 and have not been in contact with patients with COVID-19.

*RC 1. Patients with RMD should be strongly advised to comply with all preventive and control measures prescribed by the health authorities in their countries*.

In line with OP 1 to date there is no reason to assume that patients with RMD have a higher risk of being infected with SARS-CoV-2, or fare worse if they get COVID-19. Obviously, this means that currently known risk factors for severe COVID-19, including older age, male gender, comorbid cardiovascular disease and obesity, also pertain to patients with RMD.[18] This recommendation tells patients and their rheumatologist/HPR to behave like all other individuals in society in their attempts to avoid or control infection. We note that some RMDs share increased prevalence of some of these comorbidities especially metabolic syndrome, cardiovascular disease and obesity.

Level of agreement: 9.9±0.5; 95% scored 8/10 or higher.

RC 2. *Patients with RMD should in general be advised to comply with the same preventive and control measures as patients without RMD*.

This recommendation reiterates that there is also no reason for patients with RMD to take different measures, given there is no added risk for them. There is also no reason to believe that patients with RMD have more or less risk than others because of their DMARD use.

Level of agreement: 9.3±1.0; 95% scored 8/10 or higher.

*RC 3. Patients with RMD who do not have suspected or confirmed COVID-19 should be advised to continue their treatment unchanged, namely NSAIDs, glucocorticoids (GCs), sDMARDs, bDMARDs, osteoporosis medications and analgesics, among others*.

Based on the same rationale as OP 1, it is unadvisable to change chronic treatment for RMD in patients who are not suspected of COVID-19. This recommendation refers to patients with 'inflammatory' RMD, and to all patients with RMD, and serves to reassure those who are concerned about the safety of their drugs with respect to COVID-19.

Level of agreement: 9.6±0.6; 94% scored 8/10 or higher.

### Management of the RMD when local measures of social distancing are in effect

Recommendations 4–6 advise patients with RMD how to act during or in the aftermath of the SARS-CoV-2 epidemic, when official restrictions in the freedom of movement apply. They refer to all potential levels of existing social distancing, varying from, for example, keeping 1–1.5 m or 2 m distance for subpopulations to a complete country lockdown.

*RC 4. If the RMD and its drug treatment are stable, and signs or symptoms of drug toxicity are absent, regular blood monitoring and face-to-face rheumatology consultations can be postponed temporarily. If necessary, consultation can take place remotely*.

This recommendation tells patients with RMD and their caregivers that usual regular monitoring visits can safely be postponed once or twice (up to 6 months maximum) in patients with stable disease. Instead, patients may communicate with their rheumatologists and HPR via telephone or videoconference. Email communication is generally discouraged, because of issues with privacy protection, unless approved secure email transfer systems are used.

Level of agreement: 9.6±0.9; 94% scored 8/10 or higher.

*RC 5. If the RMD is active, if drug therapy has recently been started or needs adjustment, or if signs or symptoms of drug toxicity emerge, patient and rheumatologist should liaise, weigh the risks of a visit to the clinic against the limitations of remote advice, and decide together*.

This recommendation clarifies that a visit to a clinic or hospital implies a judgmental trade-off between the risk of advising the patient only remotely and the patient's and rheumatologist/HPR's risk of contracting SARS-CoV-2 in the hospital or care facility. A generic recommendation (*dos and don'ts*) cannot be formulated, since the outcome of this decision is situational and dependent on the needs of the patient and the appraisal of the physician/HPR. This may particularly be the case as COVID-safe areas of clinics and hospitals are increasingly created.

Level of agreement: 9.7±1.0; 89% scored 8/10 or higher.

*RC 6. If a patient with RMD is offered an outpatient, day care or other type of hospital appointment, patients and members of the rheumatology team should follow local guidance for infection prevention and control, including the use of personal protection equipment if indicated*.

If the decision is made to see the patient physically, then the patient as well as all members of the rheumatology team should do everything necessary to prevent SARS-CoV-2 infection during the visit. Since local guidance may differ, and supplies may be a limiting factor, a generic advice is given here. Personal protection equipment refers to masks, gloves, eye protection, safety footwear, gowns and hairnets, among others.

Level of agreement: 9.9±0.2; 94% scored 8/10 or higher.

### Management of COVID-19 in the context of RMD

Recommendations 7–10 refer to scenarios in which a patient with RMD has been in contact with a virus-positive patient or is virus-positive himself/herself. A focus is on the use of (potentially) immunosuppressive drugs, commonly used in patients with 'inflammatory' RMD.

*RC 7. Patients with RMD without COVID-19 symptoms who have been in contact with a SARS-CoV-2-positive person should be tested for SARS-CoV-2 themselves*

This recommendation raised a lot of dissent in the task force. The initially proposed version of this recommendation included more specific guidance about DMARD drug pausing (proposed to be done), the duration of such a drug pause (proposed for 6 days) and the recommencement of paused drugs (proposed when a virus test is negative and symptoms of COVID-19 do not occur). Task force members disagreed about the need to pause, the duration of a pause and the safety of recommencement, and therefore it was decided not to include these issues in the recommendation. Although testing is recommended for patients with RMD who were in contact with a virus-positive case, task force members acknowledge that test supplies may fall short or are not (yet) broadly available in many countries.

Level of agreement: 8.0±2.5; 63% scored 8/10 or higher.

*RC 8. If a patient with RMD and symptoms of COVID-19 is chronically treated with GCs, this treatment should be continued*.

GCs deserve a special mention in view of the fact that GCs cannot be stopped at once and should sometimes even be dose-increased in case of severe concomitant disease ('stress-scheme').

Members argued whether or not a 'lowest possible dose' should be recommended specifically, but agreed that the principle of 'lowest possible dose' as per existing EULAR recommendations for the management of GCs[19] is part of good clinical practice and valid under all circumstances.

Level of agreement: 8.8±1.6; 79% scored 8/10 or higher.

*RC 9. If patients with RMD experience mild* symptoms of COVID-19, potential treatment changes in DMARDs should be discussed on a case-by-case basis.*

It is currently assumed that at least 80% of patients with COVID-19 will experience a relatively mild course.[20] This recommendation reiterates that, currently, we have no reason to believe that patients with RMD and COVID-19 have an increased risk of a more severe disease course attributable to the use of DMARDs. The risks seem reasonably low and some DMARDs are less suspected than others. The opinions in the task force were divided on whether or not DMARDs should be paused and, if yes, which ones. Theoretically, some DMARDs may even be protective (eg, HCQ, IL-6 inhibitors, tumour necrosis factor inhibitors, JAKi), while for others (eg, methotrexate) pausing for a short period of time is futile due to their pharmacokinetic properties.

The task force finally agreed that, on balance, patient's fears and beliefs may be decisive. They finally agreed on a recommendation for a case-by-case judgement. That means: rheumatologists should not automatically advise a patient to stop DMARDs in case of mild symptoms of COVID-19 but, if the patient feels safer by pausing a DMARD for a while, and the rheumatologist believes that there is no increased risk of RMD complications, pausing the DMARD may be a defendable decision.

NSAIDs, under suspicion for a short while,[21] can as far as we know be used without additional risk and deserve no further specific mention in the recommendation.

In the risk assessment process, it should be borne in mind that many of these drugs including NSAIDs and cytokine inhibitors can potentially mask certain COVID-19 symptoms such as fever. Note also that IL-6 inhibitors and JAKi decrease the acute phase response irrespective of the clinical course.

Level of agreement: 8.9±1.4; 84% scored 8/10 or higher.

*RC 10. Patients with RMD and initially mild symptoms who experience worsening** of COVID-19 symptoms should immediately seek further healthcare advice of an expert in treating COVID-19, such as a pulmonologist, an internist or a specialist in infectious diseases, dependent on local circumstances.*

This recommendation emphasises the potential severity of COVID-19 infection in a minority of patients (we currently assume in less than 20% of those infected) after a course of relatively mild symptoms for 5–10 days. An unknown proportion of them will develop symptoms of CRS (see box 1). Patients with worsening should not hesitate to consult an expert in the treatment of COVID-19. Rheumatologists who have been contacted by their patients and have advised them in line with recommendations 8 and 9 should be alert on potential aggravation of initially mild disease and refer patients accordingly.

Level of agreement: 9.8±0.5; 94% scored 8/10 or higher.

*RC 11. Patients with RMD who are admitted to the hospital because of significant COVID-19 should follow local treatment recommendations for COVID-19 as applied by the treating expert.* This recommendation elaborates on OP 2 and states that, given the lack of proven effective treatments for COVID-19 and knowing that worldwide differences in hospital treatment protocols exist, it is in the best interest of patients with RMD and COVID-19 that local guidelines rather than individual (rheumatologists') beliefs are followed. Local treatment protocols for COVID-19 may among others include experimental treatment with (hydroxy)chloroquine, antibiotics, cytokine inhibitors or inhibitors of viral replication. The evidence base of these treatments is insufficient to allow specific recommendation for patients with RMD, but their use is not yet discouraged. Evidence is accruing rapidly, and the results of a very recent trial comparing low-dose and high-dose chloroquine, for instance, suggest that high-dose chloroquine, in use in many hospitals, may increase mortality rather than decrease it.[22]

The reasoning can also be turned around: if the rheumatologist truly believes that a particular drug may be effective but formal proof is still lacking (a situation that may arise in view of DMARDs which are now investigated for the treatment of COVID-19), he should first try to 'convince' the local hospital's protocol committee to adjust the existing local treatment protocol rather than acting on his own. Preferably, the management of patients with RMD with significant COVID-19 is a multidisciplinary matter; the consensual decision of a multidisciplinary team should be credited a higher weight than the opinion of one physician.

Level of agreement: 9.7±0.8; 89% scored 8/10 or higher.

### Prevention of other infections than SARS-CoV-2

Recommendations 12 and 13 remind the rheumatologist and HPR who care for patients with RMD of other important infectious diseases to consider in these patients. There are two reasons to focus on other infectious diseases: (1) Avoiding confusion between COVID-19 and phenotypical mimics. (2) Avoiding severe morbidity due to neglected coexisting infections. While these recommendations focus on three particular pathogens (pneumococci, influenza and *Pneumocystis jiroveci*), consideration of other infectious diseases should not be limited to these entities.

*RC 12. Patients with RMD without symptoms of COVID-19 should be advised to update their vaccination status in accordance with the EULAR recommendations for the vaccination of patients with RMD, with a particular focus on pneumococci and influenza.*

This recommendation is a generic one aimed at optimising public health adherence. The EULAR vaccination recommendations have recently been updated using the most contemporary evidence existing for other infections than SARS-CoV-2.[23] This recommendation particularly mentions pneumococcus and influenza since they may create clinical confusion with COVID-19.

Level of agreement: 9.4±1.0; 89% scored 8/10 or higher.

*RC 13. In patients with RMD treated with cyclophosphamide or GCs, Pneumocystis jiroveci pneumonia prophylaxis should be considered.* This recommendation pertains to patients with RMD with severe lupus, severe vasculitis or systemic sclerosis, among others. It reiterates a general recommendation[24] and is mentioned here since pneumocystis Jiriroveci pneumonia (PJP) may be clinically confused with COVID-19 pneumonia, and since PJP is an avoidable condition and it may be expected that the coexistence of PJP and COVID-19 pneumonia implies a worse prognosis.

Level of agreement: 9.3±0.9; 89% scored 8/10 or higher.

## DISCUSSION

These 5 OPs and 13 recommendations form the first EULAR set of recommendations for the management of patients with

RMD during the COVID-19 pandemic. While they provide the best possible consensual guidance according to international experts, it is self-evident that their scientific status is meagre. The level of evidence never exceeds that of 'expert opinion' and the strength of recommendation is therefore axiomatically low. The task force expects and hopes that the life span of several of these recommendations will be short, far shorter than usual, as a reflection of the accrual of solid scientific evidence that may fuel better recommendations and the advent of effective drugs for COVID-19 and its complications.

Comparing these EULAR recommendations with other recent recommendations, such as the American Society of Rheumatology (ACR) recommendations[9] and those from Germany[2] and the UK,[7] reveals, as expected, high levels of similarity, which is reassuring. Issues of controversy are sparse and of relatively minor importance. Formulated in a more negative tone, one may say that professional organisations are currently 'flying blind', due to the novelty and the impact of the pandemic and the lack of methodologically sound evidence. Such a situation is unprecedented for all professional medical organisations including ours. Providing meaningful guidance under such circumstances asks for creative solutions that are not prescribed by standard operating procedures. Many of these outstanding questions about COVID-19 in the field of RMD should be addressed in the near future. The task force hopes that the release of these expert-opinion-based recommendations meant for patients with RMD and their caregivers in 'COVID-time' will be a stimulus to initiate and conduct this research.

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CLINICAL SCIENCE

# Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry

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## ABSTRACT

**Objectives** COVID-19 outcomes in people with rheumatic diseases remain poorly understood. The aim was to examine demographic and clinical factors associated with COVID-19 hospitalisation status in people with rheumatic disease.

**Methods** Case series of individuals with rheumatic disease and COVID-19 from the COVID-19 Global Rheumatology Alliance registry: 24 March 2020 to 20 April 2020. Multivariable logistic regression was used to estimate ORs and 95% CIs of hospitalisation. Age, sex, smoking status, rheumatic disease diagnosis, comorbidities and rheumatic disease medications taken immediately prior to infection were analysed.

**Results** A total of 600 cases from 40 countries were included. Nearly half of the cases were hospitalised (277, 46%) and 55 (9%) died. In multivariable-adjusted models, prednisone dose ≥10 mg/day was associated with higher odds of hospitalisation (OR 2.05, 95% CI 1.06 to 3.96). Use of conventional disease-modifying antirheumatic drug (DMARD) alone or in combination with biologics/Janus Kinase inhibitors was not associated with hospitalisation (OR 1.23, 95% CI 0.70 to 2.17 and OR 0.74, 95% CI 0.37 to 1.46, respectively). Non-steroidal anti-inflammatory drug (NSAID) use was not associated with hospitalisation status (OR 0.64, 95% CI 0.39 to 1.06). Tumour necrosis factor inhibitor (anti-TNF) use was associated with a reduced odds of hospitalisation (OR 0.40, 95% CI 0.19 to 0.81), while no association with antimalarial use (OR 0.94, 95% CI 0.57 to 1.57) was observed.

**Conclusions** We found that glucocorticoid exposure of ≥10 mg/day is associated with a higher odds of hospitalisation and anti-TNF with a decreased odds of hospitalisation in patients with rheumatic disease. Neither exposure to DMARDs nor NSAIDs were associated with increased odds of hospitalisation.

## Key messages

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

- Data regarding outcomes for people with rheumatological disease and COVID-19 remain scarce and limited to small case series.
- Due to underlying immune system dysfunction and the common use of immunosuppressants, there is concern about poorer outcomes in this population and uncertainty about medication management during the pandemic.

**What does this study add?**

- Moderate to high dose glucocorticoids were associated with a higher risk of hospitalisation for COVID-19.
- Biologic therapies, NSAIDs and antimalarial drugs like hydroxychloroquine were not associated with a higher risk of hospitalisation for COVID-19.

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

- This study demonstrates that most individuals with rheumatological diseases or on immunosuppressive therapies recover from COVID-19, which should provide some reassurance to patients.

## INTRODUCTION

The COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus is of particular concern for people with rheumatic disease or those who are immunosuppressed. Whether having a rheumatic disease or receiving immunosuppressive treatment is associated with severe infection and subsequent poor outcomes is unknown. In general, immunosuppression and the presence of comorbidities are associated with an increased risk of serious infection in people with

rheumatic diseases[1] therefore, people with rheumatic disease may be at higher risk for a more severe course with COVID-19, including hospitalisation, complications and death. Importantly, some medications used to treat rheumatic diseases, such as hydroxychloroquine and interleukin-6 (IL-6) inhibitors, are being studied for the prevention and/or treatment of COVID-19 and its complications including cytokine-storm.[2–4] At present, the implications of COVID-19 for people living with rheumatic diseases remain poorly understood.

To address this knowledge gap, a global network of rheumatologists, scientists and patients developed a physician-reported case registry of people with rheumatic diseases diagnosed with COVID-19.[5 6] This report aims to (1) describe the demographic and clinical characteristics of the first 600 patients submitted to the COVID-19 Global Rheumatology Alliance (C19-GRA) physician registry and (2) identify factors associated with hospitalisation for COVID-19 in this population.

## METHODS

Details of the registry design have been described elsewhere.[5–7] Briefly, C19-GRA data regarding individuals with rheumatic diseases diagnosed with COVID-19 are captured from rheumatology physicians via two parallel international data entry portals for regulatory reasons: one limited to European countries (eular.org/eular_covid19_database.cfm; hosted by The University of Manchester, UK) and a second for all other sites (rheum-covid.org/provider-global/; hosted by the University of California, San Francisco, California, USA). Two patients sit on the C19-GRA steering committee and they contributed to the design of the registry, the questions being asked and the analysis of the results. The C19-GRA has a Patient Board, composed entirely of patients. These patients, and others, will be involved in disseminating the results of this analysis once published. No public were involved in the design or analysis of this project.

Physicians indicated whether the diagnosis of COVID-19 was based on PCR, antibody, metagenomic testing, CT scan, laboratory assay or a presumptive diagnosis based on symptoms only. Data elements for this analysis included physician city, state and country. Countries were assigned to the six WHO regions (www.who.int); the 'Americas' was further divided into north and south. Case information including age, sex, smoking status, rheumatic disease diagnosis, disease activity and comorbidities was collected. Medications prior to COVID-19 were categorised as: conventional synthetic disease-modifying antirheumatic drugs (csDMARDs; antimalarials (hydroxychloroquine, chloroquine), azathioprine, cyclophosphamide, cyclosporine, leflunomide, methotrexate, mycophenolate mofetil/mycophenolic acid, sulfasalazine, tacrolimus); biologic DMARDs (bDMARDs; abatacept, belimumab, CD-20 inhibitors, IL-1 inhibitors, IL-6 inhibitors, IL-12/IL-23 inhibitors, IL-17 inhibitors, tumour necrosis factor inhibitors (anti-TNF)) and targeted synthetic DMARDs (tsDMARDs) namely Janus Kinase (JAK) inhibitors. Physicians reported the approximate number of days from symptom onset to symptom resolution or to death. The primary outcome of interest was hospitalisation for COVID-19. As of 20 April 2020, a total of 604 cases were entered in the registry; hospitalisation status was unknown for four cases and these were excluded from analysis.

Continuous variables are reported as median (IQR). Categorical variables are reported as number and percentage (%). In univariable analyses, differences in demographic and rheumatic disease-specific features according to hospitalisation status were compared using $\chi^2$ tests for categorical variables and Mann-Whitney U tests for continuous variables. The independent associations between demographic and disease-specific features with the odds of COVID-19 hospitalisation were estimated using multivariable-adjusted logistic regression and reported as OR and 95% CIs; covariates included in the model were age group ($\leq$65 years vs >65 years), sex, rheumatic disease (rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), psoriatic arthritis (PsA), axial spondyloarthritis (axSpA) or other spondyloarthritis, vasculitis and other), key comorbidities (hypertension, lung disease, diabetes, cardiovascular disease and chronic renal insufficiency/end-stage renal disease), smoking status (ever vs never), physician-reported disease activity (remission, minimal/low disease activity, moderate disease activity or severe/high disease activity; or as a binary variable: remission and minimal/low disease activity vs moderate and severe/high disease activity), DMARD type (no DMARD, csDMARD only, b/tsDMARD only, csDMARD and b/tsDMARD combination therapy), non-steroidal anti-inflammatory drugs (NSAID) use (yes vs no) and prednisone-equivalent glucocorticoid use (0 mg/day, 1–9 mg/day, $\geq$10 mg/day). Categories with cell sizes <10 by hospitalisation status were collapsed to ensure sufficient power in the adjusted model. For univariable and multivariable models, patients with more than one of the following diseases recorded were classified as follows: SLE>RA>PsA>vasculitis>axSpA/other spondyloarthritis>other. Cardiovascular disease and hypertension were collapsed as a single comorbidity in the regression model due to significant collinearity between the two variables. Due to concerns regarding the possibility of confounding by indication, disease activity and prednisone-equivalent glucocorticoid use were analysed by including only one of the variables in the multivariable analysis at a time, and by including both variables in the multivariable analysis at the same time. Unknown/missing data (14% smoking status, 12% NSAIDs, 1% glucocorticoids) were treated as a separate category in multivariable models. In exploratory analyses, the independent association between antimalarials and specific b/tsDMARD therapies with hospitalisation status was estimated using multivariable logistic regression.

To assess the robustness of the results, sensitivity analyses were performed. First, we repeated the above analyses after excluding patients with a 'presumptive diagnosis', meaning that the patient's physician thought he/she had symptoms consistent with the disease, but there was no evidence of the patient having: a) a confirmatory COVID test; b) documentation of chest imaging showing bilateral infiltrates in keeping with COVID-19 pneumonia or c) close contact with a known COVID-19-positive patient. Second, we limited the analyses to patients whose COVID-19 outcome was known (resolved/died) or for whom at least $\geq$14 days from symptom onset (or diagnosis date if symptom onset was unknown) had elapsed, as it is unlikely that a patient would be hospitalised >2 weeks after onset. Third, we excluded cases with missing/unknown values within the covariate set included in the multivariable analyses. Data were considered statistically significant at $p<0.05$. Cell counts <5 are represented by 'n<5' in tables to protect patient anonymity. All analyses were conducted in Stata V.16.0 (StataCorp).

Data quality was assessed by two data quality teams (one at the University of Manchester, UK and the University of California, San Francisco) who also confirmed there were no duplicate entries. Due to the deidentified and non-interventional nature of the study, it was determined by the institutional review board that patient consent was not required. C19-GRA physician registry was determined 'not human subjects research' by the UK Health Research Authority and the University of Manchester, as

**Table 1** Demographic and clinical characteristics of patients with rheumatic disease with COVID-19 (n=600)

| | N (%) |
|---|---|
| Region | |
| Region of the Americas: North | 340 (57) |
| Region of the Americas: South | 16 (3) |
| European region | 218 (36) |
| African region | <5 (<1) |
| Eastern Mediterranean region | 11 (2) |
| South-East Asian region | <5 (<1) |
| Western Pacific region | 13 (2) |
| Female | 423 (71) |
| Age (years) | |
| 18–29 | 32 (5) |
| 30–49 | 169 (28) |
| 50–65 | 229 (38) |
| >65 | 170 (28) |
| Median (IQR) | 56 (45–67) |
| Most common rheumatic disease diagnoses* | |
| Rheumatoid arthritis | 230 (38) |
| Systemic lupus erythematosus | 85 (14) |
| Psoriatic arthritis | 74 (12) |
| Axial spondyloarthritis or other spondyloarthritis | 48 (8) |
| Vasculitis | 44 (7) |
| Sjögren's syndrome | 28 (5) |
| Other inflammatory arthritis | 21 (4) |
| Inflammatory myopathy | 20 (3) |
| Gout | 19 (3) |
| Systemic sclerosis | 16 (3) |
| Polymyalgia rheumatica | 12 (2) |
| Sarcoidosis | 10 (2) |
| Other | 28 (5) |
| Most common comorbidities | |
| Hypertension | 199 (33) |
| Lung disease† | 127 (21) |
| Diabetes | 69 (12) |
| Cardiovascular disease | 63 (11) |
| Chronic renal insufficiency/end-stage renal disease | 40 (7) |
| Disease activity (n=575) | |
| Remission | 173 (30) |
| Minimal or low disease activity | 286 (50) |
| Moderate disease activity | 102 (18) |
| Severe or high disease activity | 14 (2) |
| Smoking status (n=518) | |
| Ever | 129 (25) |
| Never | 389 (75) |
| Medication prior to COVID-19 diagnosis‡ | |
| No DMARD | 97 (16) |
| csDMARD only, including antimalarial therapy | 272 (45) |
| csDMARD only, excluding antimalarial therapy | 220 (37) |
| Antimalarial, with or without other DMARD | 130 (22) |
| Antimalarial only | 52 (9) |
| b/tsDMARDs only | 107 (18) |
| csDMARD+b/tsDMARD combination therapy | 124 (21) |
| NSAIDs (n=531) | 111 (21) |
| Prednisone-equivalent glucocorticoids (n=592) | |
| None | 403 (68) |
| 1–9 mg/day | 125 (21) |
| ≥10 mg/day | 64 (11) |
| Hospitalised | 277 (46) |
| Deceased | 55 (9) |
| Reported days from onset to resolution or death (n=275), median (IQR) | 13 (8–17) |

N (column %) for categorical variables unless otherwise noted.
Percentages may not sum to 100 due to rounding.
*Cases could have more than one disease diagnosis. 'Other' rheumatic disease category included (each n<10): undifferentiated connective tissue disease; ocular inflammation; autoinflammatory syndrome; mixed connective tissue disease; antiphospholipid antibody syndrome; calcium pyrophosphate deposition disease; systemic juvenile idiopathic arthritis; juvenile idiopathic arthritis, not systemic; IgG4-related disease.
†Chronic obstructive pulmonary disease, asthma, interstitial lung disease or other not specified.
‡csDMARD medications included: antimalarials (hydroxychloroquine, chloroquine), azathioprine, cyclophosphamide, cyclosporine, leflunomide, methotrexate, mycophenolate mofetil/mycophenolic acid, sulfasalazine, tacrolimus; b/tsDMARD included: abatacept, belimumab, CD-20 inhibitors, IL-1 inhibitors, IL-6 inhibitors, IL-12/IL-23 inhibitors, IL-17 inhibitors, anti-TNF and Janus Kinase inhibitors.
b/tsDMARD, biologic or targeted synthetic DMARD; csDMARD, conventional synthetic DMARD; DMARD, disease-modifying antirheumatic drug; IL, interleukin; NSAID, non-steroidal anti-inflammatory drug; TNF, tumour necrosis factor.

well as under United States Federal Guidelines assessed by the University of California, San Francisco and patient consent was not required. We did not systematically capture how cases were identified before being entered into the registry and therefore we cannot detail this. However, we are aware of a number of large institutions that are systematically collecting all cases in their health system/district and entering them into the registry.

## RESULTS

The demographic and clinical characteristics of the first 600 cases in the C19-GRA physician registry are shown in table 1. The majority of cases in the registry were from North America and Europe, female and in the 50–65 age range, the countries that the cases were reported from are shown in online supplementary table 1. The most common rheumatic disease was RA (230, 38%), followed by SLE (85, 14%) and PsA (74, 12%). The most common comorbidities were hypertension (199, 33%), lung disease (127, 21%), diabetes (69, 12%), cardiovascular disease (63, 11%) and chronic renal insufficiency/end-stage renal disease (40, 7%). Most cases were never smokers (389, 75%) and either in remission or had minimal/low disease activity (459, 80%). Five patients were pregnant (1%). Nearly half of the cases reported to the registry were hospitalised (277, 46%), and 9% (55) were deceased. COVID-19 diagnoses were predominately made through PCR testing (437, 73%), followed by laboratory assay of unknown type (58, 10%), CT scan (42, 7%) or other (31, 5%) (individuals could be tested using more than one method). Fifty-two (9%) cases had a presumptive diagnosis only (online supplementary table 2). The median number of days from COVID-19 symptom onset to resolution or death was 13 (IQR: 8–17). Demographic and clinical characteristics stratified by sex are presented in online supplementary table 3.

Demographic and clinical characteristics stratified by hospitalisation status are shown in table 2. Differences by age group in hospitalisation status were observed: most hospitalised patients were over age 65 (43%), compared with 16% of non-hospitalised cases ($p<0.01$). In unadjusted analyses, differences in hospitalisation status by disease revealed a higher percentage of people who were hospitalised had SLE and vasculitis (17% and 9%, respectively) versus those who were not hospitalised (11% and 5%, respectively), while a lower proportion of patients who were hospitalised had PsA and axSpA or other spondyloarthritis (8% and 6%, respectively) compared with those who were

**Table 2** Demographic and clinical factors of patients with rheumatic disease diagnosed with COVID-19 by hospitalisation status

| | Not hospitalised n=323 | Hospitalised n=277 | P value |
|---|---|---|---|
| Female | 238 (74%) | 185 (67%) | 0.10 |
| Age group (years) | | | <0.01 |
| <30 | 25 (8%) | 7 (3%) | |
| 30–49 | 113 (35%) | 56 (20%) | |
| 50–65 | 134 (41%) | 95 (34%) | |
| >65 | 51 (16%) | 119 (43%) | |
| Median (IQR), years | 52 (42–60) | 62 (51–71) | <0.01 |
| Most common rheumatic disease diagnoses† | | | <0.01 |
| Rheumatoid arthritis | 121 (37%) | 104 (38%) | |
| Systemic lupus erythematosus | 37 (11%) | 48 (17%) | |
| Psoriatic arthritis | 52 (16%) | 22 (8%) | |
| Axial spondyloarthritis or other spondyloarthritis | 32 (10%) | 16 (6%) | |
| Vasculitis | 15 (5%) | 24 (9%) | |
| Other | 66 (20%) | 63 (23%) | |
| Most common comorbidities | | | |
| Hypertension | 75 (23%) | 124 (45%) | <0.01 |
| Lung disease* | 44 (14%) | 83 (30%) | <0.01 |
| Diabetes | 21 (7%) | 48 (17%) | <0.01 |
| Cardiovascular disease | 23 (7%) | 40 (14%) | <0.01 |
| Chronic renal insufficiency/end-stage renal disease | 7 (2%) | 33 (12%) | <0.01 |
| Disease activity (n=575) | | | 0.49 |
| Remission | 88 (28) | 85 (32) | |
| Minimal or low disease activity | 157 (50) | 129 (49) | |
| Moderate disease activity | 60 (19) | 42 (16) | |
| Severe or high disease activity | 6 (2) | 8 (3) | |
| Ever smoker (n=518) | 61 (21%) | 68 (30%) | 0.03 |
| Rheumatic disease medication prior to COVID-19 diagnosis‡ | | | <0.01 |
| No DMARD | 45 (14%) | 52 (19%) | |
| csDMARD only | 123 (38%) | 149 (54%) | |
| b/tsDMARDs only | 76 (24%) | 31 (11%) | |
| csDMARD+b/tsDMARD combination therapy | 79 (24%) | 45 (16%) | |
| Any antimalarial therapy | 64 (20%) | 66 (24%) | 0.23 |
| Antimalarial only | 27 (8%) | 25 (9%) | 0.77 |
| NSAIDs (n=531) | 72 (25%) | 39 (16%) | 0.02 |
| Prednisone-equivalent glucocorticoids (n=592) | | | <0.01 |
| None | 241 (75%) | 162 (60%) | |
| 1–9 mg/day | 58 (18%) | 67 (25%) | |
| ≥10 mg/day | 21 (7%) | 43 (16%) | |
| Reported days from onset to resolution or death (n=275), median (IQR) | 14 (7–16) | 12 (8–17) | 0.72 |

N (column %) for categorical variables unless otherwise noted.
Percentages may not sum to 100 due to rounding.
P value calculated using $\chi^2$ tests for categorical variables and Mann-Whitney U tests for continuous variables.
*Chronic obstructive pulmonary disease, asthma, interstitial lung disease or other not specified.
†Patients with more than one disease within these five diagnoses were classified as follows: systemic lupus erythematosus>rheumatoid arthritis>psoriatic arthritis>vasculitis>axial/other spondyloarthritis>other. Other rheumatic disease category included (each n<10): undifferentiated connective tissue disease; ocular inflammation; autoinflammatory syndrome; mixed connective tissue disease; antiphospholipid antibody syndrome; calcium pyrophosphate deposition disease; systemic juvenile idiopathic arthritis; juvenile idiopathic arthritis, not systemic; IgG4-related disease.
‡csDMARD medications included: antimalarials (hydroxychloroquine, chloroquine), azathioprine, cyclophosphamide, ciclosporin, leflunomide, methotrexate, mycophenolate mofetil/mycophenolic acid, sulfasalazine, tacrolimus; b/tsDMARD included: abatacept, belimumab, CD-20 inhibitors, IL-1 inhibitors, IL-6 inhibitors, IL-12/IL-23 inhibitors, IL-17 inhibitors, anti-TNF and Janus Kinase inhibitors.
b/tsDMARD, biologic or targeted synthetic DMARDs; csDMARD, conventional synthetic DMARD; DMARD, disease-modifying antirheumatic drug; IL, interleukin; NSAID, non-steroidal anti-inflammatory drugs; TNF, tumour necrosis factor.

not (16% and 10%, respectively). There were more comorbidities among hospitalised cases, including hypertension (45% vs 23%), lung disease (30% vs 14%), diabetes (17% vs 7%), cardiovascular disease (14% vs 7%) and chronic renal insufficiency/end-stage renal disease (12% vs 2%) (all p<0.01). There was no association between disease activity and hospitalisation status (p=0.49). NSAID use was reported less frequently among hospitalised patients than non-hospitalised patients (16% vs 25%, p=0.02), while there was a higher proportion of patients receiving high doses of glucocorticoids among those who were hospitalised than not hospitalised (16% vs 7% for doses ≥10 mg/day, p=0.01). We found no significant difference in hospitalisation status by sex, antimalarial therapy (either monotherapy or in combination with other DMARDs) or reported days from symptom onset to symptom resolution or death.

In a multivariable model, age over 65 years (OR=2.56, 95% CI 1.62 to 4.04), hypertension/cardiovascular disease (OR=1.86, 95% CI 1.23 to 2.81), lung disease (OR=2.48, 95% CI 1.55 to 3.98), diabetes (OR=2.61, 95% CI 1.39 to 4.88) and chronic renal insufficiency/end-stage renal disease (OR=3.02, 95% CI 1.21 to 7.54) were associated with higher odds of hospitalisation (all p<0.05) (table 3). Treatment with b/tsDMARD monotherapy just prior to COVID-19 diagnosis was significantly associated with a lower odds of hospitalisation compared with no DMARD therapy (OR=0.46, 95% CI 0.22 to 0.93; p=0.03). Glucocorticoid therapy at prednisone-equivalent doses ≥10 mg/day, however, was associated with a higher odds of hospitalisation compared with no glucocorticoid therapy (OR=2.05, 95% CI 1.06 to 3.96; p=0.03). Neither adding disease activity to the model with glucocorticoids nor replacing glucocorticoids by disease activity changed the direction, strength or significance of the relationship between the various variables and hospitalisation status in a meaningful way (data not shown).

Further analyses were conducted to examine the independent association of antimalarials and specific b/tsDMARDs with hospitalisation. A total of 22% of cases were taking antimalarials before hospitalisation. The largest subgroup of b/tsDMARD therapies was anti-TNF medications (52%). We found no significant association between antimalarial therapy and hospitalisation (OR=0.94, 95% CI 0.57 to 1.57; p=0.82) after adjusting for sex, age over 65 years, rheumatic disease, smoking status, comorbidities, other csDMARD monotherapy, b/tsDMARD monotherapy, csDMARD-b/tsDMARD combination therapy (excluding antimalarials), NSAID use and glucocorticoid dose. A significant inverse association between any anti-TNF therapy and hospitalisation was found (OR=0.40, 95% CI 0.19 to 0.81; p=0.01), after controlling for sex, age over 65 years, rheumatic disease, smoking, comorbidities, csDMARD monotherapy, other b/tsDMARD monotherapy, csDMARD-b/tsDMARD combination therapy (excluding anti-TNF), NSAID use and glucocorticoid dose. Small numbers of non-anti-TNF b/tsDMARDs precluded analysing the association of these individual agents with hospitalisation (online supplementary table 4).

Our findings remained largely unchanged in sensitivity analyses excluding those with a presumptive diagnosis (n=52; online supplementary table 5), those with unknown outcomes (n=214; online supplementary table 6) and those with missing/unknown values (n=142; online supplementary table 7).

## DISCUSSION

This manuscript describes the largest collection of COVID-19 cases among patients with rheumatic diseases, with 600 cases from 40 countries. We identified factors associated with higher odds of COVID-19 hospitalisation, including older age, presence of comorbidities and higher doses of prednisone (≥10 mg/day). We did not see an association between prior NSAID use or antimalarials and hospitalisation for COVID-19. We did find b/tsDMARD monotherapy to be associated with a lower odds of hospitalisation, an effect that was largely driven by anti-TNF

**Table 3** Unadjusted and adjusted logistic regression models examining the association between demographic and clinical characteristics and COVID-19 hospitalisation status

| | No. hospitalised/ No. cases (%) | Unadjusted OR (95% CI) | Adjusted OR (95% CI) | P value* |
|---|---|---|---|---|
| Female | 185/423 (44) | 0.72 (0.51 to 1.02) | 0.83 (0.54 to 1.28) | 0.39 |
| Age >65 years | 119/170 (70) | 4.02 (2.74 to 5.89) | 2.56 (1.62 to 4.04) | <0.01 |
| Rheumatic disease diagnosis† | | | | |
| Rheumatoid arthritis | 104/225 (46) | *Ref* | *Ref* | -- |
| Systemic lupus erythematosus | 48/85 (56) | 1.51 (0.91 to 2.49) | 1.80 (0.99 to 3.29) | 0.06 |
| Psoriatic arthritis | 22/74 (30) | 0.49 (0.28 to 0.86) | 0.94 (0.48 to 1.83) | 0.85 |
| Axial spondyloarthritis or other spondyloarthritis | 16/48 (33) | 0.58 (0.30 to 1.12) | 1.11 (0.50 to 2.42) | 0.80 |
| Vasculitis | 24/39 (62) | 1.86 (0.93 to 3.73) | 1.56 (0.66 to 3.68) | 0.31 |
| Other | 63/129 (49) | 1.11 (0.72 to 1.71) | 0.94 (0.55 to 1.62) | 0.82 |
| Comorbidities (present vs not) | | | | |
| Hypertension or cardiovascular disease | 136/218 (62) | 2.83 (1.01 to 4.00) | 1.86 (1.23 to 2.81) | <0.01 |
| Lung disease‡ | 83/127 (65) | 2.71 (1.80 to 4.08) | 2.48 (1.55 to 3.98) | <0.01 |
| Diabetes | 48/69 (70) | 3.01 (1.76 to 5.18) | 2.61 (1.39 to 4.88) | <0.01 |
| Chronic renal insufficiency/end-stage renal disease | 33/40 (83) | 6.11 (2.66 to 14.04) | 3.02 (1.21 to 7.54) | 0.02 |
| Ever smoker (vs never smoker) | 68/129 (53) | 1.41 (1.13 to 1.77) | 1.18 (0.90 to 1.53) | 0.23 |
| Rheumatic disease medication prior to COVID-19 diagnosis§ | | | | |
| No DMARD | 52/97 (54) | *Ref* | *Ref* | -- |
| csDMARD only | 149/272 (55) | 1.05 (0.66 to 1.67) | 1.23 (0.70 to 2.17) | 0.48 |
| b/tsDMARDs only | 31/107 (29) | 0.35 (0.20 to 0.63) | 0.46 (0.22 to 0.93) | 0.03 |
| csDMARD+b/tsDMARD combination therapy | 45/124 (36) | 0.49 (0.29 to 0.85) | 0.74 (0.37 to 1.46) | 0.38 |
| NSAIDs | 39/111 (35) | 0.55 (0.35 to 0.84) | 0.64 (0.39 to 1.06) | 0.08 |
| Prednisone-equivalent glucocorticoids | | | | |
| None | 162/403 (40) | *Ref* | *Ref* | -- |
| 1–9 mg/day | 67/125 (54) | 1.72 (1.15 to 2.57) | 1.03 (0.64 to 1.66) | 0.91 |
| ≥10 mg/day | 43/64 (67) | 3.05 (1.74 to 5.32) | 2.05 (1.06 to 3.96) | 0.03 |

Adjusted ORs from models including all variables shown.

*P value for multivariable logistic regression model (see 'Methods' section for details).

†Patients with more than one disease within these five diagnoses were classified as follows: systemic lupus erythematosus>rheumatoid arthritis>psoriatic arthritis>vasculitis>axial/other spondyloarthritis>other. Other rheumatic disease category included (each n<10): undifferentiated connective tissue disease; ocular inflammation; autoinflammatory syndrome; mixed connective tissue disease; antiphospholipid antibody syndrome; calcium pyrophosphate deposition disease; systemic juvenile idiopathic arthritis; juvenile idiopathic arthritis, not systemic; IgG4-related disease.

‡Chronic obstructive pulmonary disease, asthma, interstitial lung disease or other not specified.

§csDMARD medications included: antimalarials (hydroxychloroquine, chloroquine), azathioprine, cyclophosphamide, cyclosporine, leflunomide, methotrexate, mycophenolate mofetil/mycophenolic acid, sulfasalazine, tacrolimus; b/tsDMARD included: abatacept, belimumab, CD-20 inhibitors, IL-1 inhibitors, IL-6 inhibitors, IL-12/IL-23 inhibitors, IL-17 inhibitors, anti-TNF and Janus Kinase inhibitors.

b/tsDMARD, biologic or targeted synthetic DMARDs; csDMARD, conventional synthetic DMARD; DMARD, disease-modifying antirheumatic drug; IL, interleukin; NSAID, non-steroidal anti-inflammatory drug; TNF, tumour necrosis factor.

therapies. Over half of the reported cases did not require hospitalisation, including many patients receiving b/tsDMARDs. The rate of hospitalisation was higher than in cohorts of general patients with COVID-19 but this likely reflects the mechanism by which we collected the case information and should not be interpreted as the true rate of hospitalisation among patients with rheumatic disease infected with SARS-CoV-2.

Prior to this report, there had been several small case series of COVID-19 in patients with rheumatic disease reported from Europe.[8–11] With few exceptions,[12 13] prior large descriptive studies of patients with COVID-19 from China, Europe and the USA have not included rheumatic disease in their baseline comorbidities.[14–19] These studies have not allowed for further inference on the characteristics of patients with rheumatic disease and their associations with COVID-19 severity.

In accordance with previous studies of COVID-19 in different populations, we found that patients with comorbidities such as hypertension, cardiovascular disease and diabetes had higher odds of hospitalisation.[18–20] We also found that glucocorticoid use at a prednisone-equivalent dose ≥10 mg/day was associated with an increased odds of hospitalisation, which is in agreement with prior studies showing an increased risk of infection with higher dose of glucocorticoids.[21]

We did not find a significant association between antimalarial use and hospitalisation in adjusted analyses. The use of hydroxychloroquine for the treatment of COVID-19, which was based on in vitro studies, has had mixed results.[2 22] Studies from one group suggested a benefit on the surrogate outcome of viral clearance among hospitalised patients, but these studies either had inadequate or no comparator groups.[23 24] Two randomised controlled trials of hydroxychloroquine had conflicting findings.[25 26] A phase IIb randomised controlled trial comparing two doses of chloroquine among patients hospitalised with COVID-19 with historical controls from Wuhan detected a negative safety signal—QTc prolongation—but no clinical benefit.[27] Finally, two observational studies using propensity score matching to account for confounding by indication have found no significant benefit with either hydroxychloroquine alone or combined with azithromycin on clinical outcomes including mortality[28 29]; however,

these studies were limited by design issues and a high risk of bias due to unmeasured confounding.

We also did not detect a significant association between NSAID use and hospitalisation in adjusted analyses. Although no prior data in patients with COVID-19 have supported a deleterious effect of NSAIDs on clinical outcomes, early reports cautioned against the use of NSAIDs suggesting harm when used during the clinical course of COVID-19.[30] These observations, while anecdotal, may also relate to confounding by indication, since NSAIDs are also often sold over-the-counter and may not be documented in hospital records with the same accuracy as prescription medications, leading to a reporting bias.

We found a lower odds of hospitalisation with b/tsDMARDs monotherapy in our primary multivariable analysis, which was driven largely by anti-TNF therapies. The number of cases taking other biologic drugs or JAK inhibitors was small, and may have been insufficient to demonstrate other underlying effects if present. Although we caution against causal inference regarding drug effects given significant potential for residual confounding in our study, we also note that there is biological plausibility for the potential benefit of biologic medications in treating COVID-19, as evidenced by those with more severe disease having higher levels of cytokines, including IL-6 and TNF.[31 32] The use of IL-6 inhibitors is being investigated for COVID-19, particularly in cases complicated by aberrant inflammatory responses or 'cytokine storm'. This is based on two initial case series of fewer than 20 patients.[33 34] Anti-TNFs have also been suggested as a potential therapy in COVID-19, but this has been based solely on preclinical data.[35] Randomised, placebo-controlled trials are needed to clarify potential benefits or harms of biologic therapies in treating COVID-19.

Strengths of our study include the first large analysis of patients with rheumatic diseases and COVID-19. All case data were entered by rheumatology healthcare providers. The C19-GRA physician registry includes cases from 40 countries suggesting that our findings are more generalisable than single-centre or regional studies. The registry collects information on specific rheumatic disease diagnoses, which to date have not been captured in large, published case series of COVID-19.[15]

Despite these strengths, there are important limitations to these registry data. The C19-GRA registry is voluntary and does not capture all cases of COVID-19 in patients with rheumatic disease. This approach to data collection places limitations on causal conclusions and temporal relationships and therefore we can only make limited inferences based on our results. There is selection bias due to several factors, including geographic location, hospitalisation status and disease severity, with the more severe cases most likely to be captured. Therefore, the data cannot be used to comment on the incidence of COVID-19 in this patient population or its severity. Since the registry's inclusion criteria are restricted to those with rheumatic disease and COVID-19, this precludes the ability to make comparisons with those who do not have rheumatic disease, or those with rheumatic disease who do not have COVID-19. Although physicians may be contacted for follow-up information for unresolved cases, this is a cross-sectional analysis and there is the possibility that some patients may not have progressed to their maximum level of care prior to enrolment. In our dataset, 35% of cases were unresolved or had an unknown resolution status, although exclusion of these cases in sensitivity analyses did not change our conclusions. Furthermore, while we have collected information on medication use prior to COVID-19 diagnosis, we do not have specific data on the duration of treatment, medication dose, or additional historical treatments.

At the time of this report, the C19-GRA databases remain open for further case reports. With additional cases, we will be able to examine more detailed outcomes associated with specific rheumatic diseases and COVID-19 treatments, as well as the outcomes of COVID-19 in people with rheumatic diseases.

This series of cases demonstrates that the majority of patients with rheumatic diseases captured in our registry recover from COVID-19. In some cases, exposure to specific medication classes is associated with lower odds of hospitalisation; however, these findings should be interpreted with caution because of a high risk of bias. Results support the guidance issued by the American College of Rheumatology and the European League Against Rheumatism, which suggest continuing rheumatic medications in the absence of COVID-19 infection or SARS-CoV-2 exposure.[36 37]

In this series of people with rheumatic disease and COVID-19, use of DMARDs did not increase the odds of hospitalisation. As in the general population, people with rheumatic diseases who are older and/or have comorbidities have a higher odds of COVID-19-related hospitalisation. Anti-TNF treatment was associated with reduced odds of hospitalisation while prednisone use ≥10 mg/day was associated with a higher odds of hospitalisation. There was no difference in antimalarials, such as hydroxychloroquine, or NSAID use between those who were or were not hospitalised.

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**Correction notice** This article has been corrected since it published Online First. The 'csDMARD only' line in table 3 has been corrected.

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CLINICAL SCIENCE

# Therapeutic drug monitoring of adalimumab in RA: no predictive value of adalimumab serum levels and anti-adalimumab antibodies for prediction of response to the next bDMARD

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## ABSTRACT

**Background** After adalimumab treatment failure, tumour necrosis factor inhibition (TNFi) and non-TNFi biological disease-modifying anti-rheumatic drugs (bDMARDs) are equally viable options on a group level as subsequent treatment in rheumatoid arthritis (RA) based on the current best evidence synthesis. However, preliminary data suggest that anti-adalimumab antibodies (anti-drug antibodies, ADA) and adalimumab serum levels (ADL) during treatment predict response to a TNFi as subsequent treatment.

**Objective** To validate the association of presence of ADA and/or low ADL with response to a subsequent TNFi bDMARD or non-TNFi bDMARD. Sub-analyses were performed for primary and secondary non-responders.

**Methods** A diagnostic test accuracy retrospective cohort study was done in consenting RA patients who discontinued adalimumab after >3 months of treatment due to inefficacy and started another bDMARD. Inclusion criteria included the availability of (random timed) serum samples between ≥8 weeks after start and ≤2 weeks after discontinuation of adalimumab, and clinical outcome measurements Disease Activity Score in 28 joints - C-reactive protein (DAS28-CRP) between 3 to 6 months after treatment switch. Test characteristics for EULAR (European League Against Rheumatism) good response (DAS28-CRP based) after treatment with the next (non-)TNFi bDMARD were assessed using area under the receiver operating characteristic and sensitivity/specificity.

**Results** 137 patients were included. ADA presence was not predictive for response in switchers to a TNFi (sensitivity/specificity 18%/75%) or a non-TNFi (sensitivity/specificity 33%/70%). The same was true for ADL levels in patients that switched to a TNFi (sensitivity/specificity 50%/52%) and patients that switched to a non-TNFi (sensitivity/specificity 32%/69%). Predictive value of ADA and ADL were similar for both primary and secondary non-responders to adalimumab.

**Conclusions** In contrast to earlier research, we could not find predictive value for response to a second TNFi or non-TNFi for either ADA or random timed ADL.

## Key messages

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

- Anti-adalimumab antibody (anti-drug antibody, ADA) presence has been suggested to correlate with response to a second biological disease-modifying anti-rheumatic drug (bDMARD) after discontinuation of adalimumab use.

**What does this study add?**

- We investigated the predictive value of ADA and adalimumab serum levels (ADL) for EULAR (European League Against Rheumatism) clinical response to subsequent treatment with a second bDMARD (tumour necrosis factor inhibition (TNFi) or non-TNFi) after discontinuing adalimumab because of treatment failure.

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

- Neither ADA presence nor ADL had predictive value for clinical response to a subsequent TNFi or non-TNFi treatment after failure of adalimumab treatment.
- Combining these data with four earlier studies that did find some predictive value of adalimumab and etanercept (anti)drug levels, the next research step might be doing a well-dimensioned prospective trial.

## INTRODUCTION

Biological disease-modifying anti-rheumatic drugs (bDMARDs) are important in the treatment of rheumatoid arthritis (RA). bDMARDs with several modes of action are available, such as tumour necrosis factor inhibition (TNFi) (eg, adalimumab, etanercept, golimumab, infliximab, certolizumab) and non-TNFi (eg, rituximab, tocilizumab, abatacept). Adalimumab—a human monoclonal antibody TNFi—is one of the most frequently used bDMARDs, and is a safe and effective treatment for RA.

However, approximately 41% of RA patients do not achieve good response[1–3] after 6 months of treatment with adalimumab.[4 5] After non-response to adalimumab (or any bDMARD) treatment, current guidelines state that another TNFi or a non-TNFi bDMARD could be prescribed as subsequent

treatment with equal chance of response.[6] This is supported by current available evidence from four randomised controlled trials (RCT's),[7–10] and two systematic reviews on predictive factors for response to a bDMARD in RA.[11 12] Based on this, no preference should be given to starting either another TNFi, or a non-TNFi bDMARD after primary or secondary non-response to adalimumab.[13]

However, it has been suggested that measurement of adalimumab serum levels and/or anti-adalimumab antibodies (therapeutic drug monitoring, TDM) might be helpful for channelling the right patients to a TNFi or a non-TNFi thus increasing overall response chances.[14–16] The rationale for this is that approximately 20% to 50% of the RA patients treated with adalimumab develop antibodies against this drug (anti-drug antibodies, ADA) and this can result in primary or secondary non-response.[14–16] Another possible reason for non-response, however, is innate insensitivity to TNFi in a proportion of patients. It can be hypothesised that the first group of non-responders will have adequate response chances to a second TNFi, whereas in the second group of patients, TNFi response will be much lower. This is supported by several cohort studies and a recent systematic review in RA and axial spondylarthritis, for adalimumab and infliximab.[14–17]

Following this rationale, the optimal strategy after adalimumab non-response might be a second TNFi in patients with low adalimumab levels/ADA presence, and a non-TNFi in patients with adequate levels and no ADA presence. One could argue that just giving a non-TNFi in all adalimumab non-responders would negate the need for testing. However, many adalimumab non-responding patients experience secondary non-response rather than primary non-response, and patients in which secondary non-response occurred were indeed TNFi responding patients. Therefore, response rates to a second TNFi in these patients might be *higher* than response rates to a non-TNFi, resulting a better outcome for all patients after TDM.

The above-mentioned hypothesis has—in part—been tested in two studies with infliximab and adalimumab.[15 18] These studies showed that presence of ADA against either infliximab or adalimumab was associated with a larger decrease in disease activity after the next TNFi. Additionally, the same mechanism has been replicated using infliximab in RA, and adalimumab in axial spondyloarthritis.[14 16] However, these studies have some limitations; the number of patients was somewhat limited, and no differentiation was made between primary and secondary non-responders, a distinction that might be important for response chances to a second TNFi, as argued earlier. Also, these studies did not mention test characteristics (sensitivity/specificity), only difference in mean improvement, thus hampering judgement of test characteristics. In addition, the studies did not assess the predictive value of adalimumab TDM for response to non-TNFi after adalimumab, which is relevant to determine whether ADA presence is simply a marker of more refractory disease or able to differentially predict response to a second TNFi compared with a non-TNFi . Finally, testing with a newer competitive ELISA is now possible in order to quantify anti-drug antibodies even in the presence of large amounts of TNF inhibitor.[19] As this is a drug-tolerant assay, it is a more precise measure of ADA than conventional testing methods where ADA cannot be detected in the presence of large amounts of the drug.

Therefore, we set out to investigate this predictive value in a larger study population, estimating sensitivity and specificity of both presence of ADA and random timed adalimumab levels (ADL), and validate currently proposed thresholds, in both patients that switched to a TNFi (TNFi switchers) and patients that switched to a non-TNFi (non-TNFi switchers).

## METHODS

### Design

A retrospective diagnostic test accuracy cohort study to assess the predictive value of ADA and ADL for response to a subsequent TNFi or non-TNFi bDMARD in RA patients.

### Patients

All RA patients who received adalimumab and subsequently another TNFi (etanercept, golimumab, infliximab, certolizumab) or a non-TNFi bDMARD (rituximab, tocilizumab, abatacept) in the Sint Maartenskliniek or Radboud University Medical Centre (Radboudumc) between January 2012 and January 2018 were considered for inclusion in the current study. Potentially eligible participants were identified through the electronic patient records of the Sint Maartenskliniek and the Radboudumc. Patients included in this study had a diagnosis of RA according to American College of Rheumatology (ACR) (1987) or ACR/European League Against Rheumatism (EULAR) (2010) criteria,[20 21] or clinical diagnosis, and were ≥16 years of age. They had received adalimumab for at least 3 months (+/−2 weeks) in standard dosing (40 mg subcutaneously every other week). Acceptable reasons for stopping adalimumab were either inefficacy (primary or secondary, no formal disease activity cut-off) or toxicity, but not tapering because of remission. The next bDMARD should also have been administered in standard dosing (registered dose, exception being rituximab 1×1000/2×500 mg instead of 2×1000 mg) for at least 3 months (+/−2 weeks). Furthermore, a serum sample that is suitable for analysis should be available, being samples taken ≥8 weeks after start adalimumab and within 2 weeks after discontinuing adalimumab (for ADL) or within 12 weeks after discontinuation (for ADA).[22] Serum samples were derived from a period of biobanking at every visit of RA patients and an observational cohort study including consecutive bDMARD starters. Finally, Disease Activity Score in 28 joints - C-reactive protein/erythrocyte sedimentation rate (DAS28-CRP/ESR) scores had to be available to assess EULAR clinical response to subsequent bDMARDs, a baseline DAS at start and a follow-up DAS after 3 to 6 months of treatment (+/−8 weeks).

### Ethical approval, consent and funding

Approval from the local ethics committee (Commissie Mensgebonden Onderzoek (CMO) region Arnhem-Nijmegen) was obtained (CMO: 2019–5443). Patients had either previously consented to inclusion in several biobanking studies, including the Nijmegen RA protocollaire follow-up[23] (CMO-number: 2016–2281) and the BIOTOP study[24] (CMO region Arnhem-Nijmegen, NL47946.091.14) or were sent opt-out informed consent letters with information about the aims and methods of the study. Patients were given 4 weeks to read the information and respond in case they are not willing to participate (according to Dutch law: WGBO art 458 sub 2). This study received no external funding. The laboratory analyses of adalimumab and ADA levels and personnel costs were funded by the Sint Maartenskliniek.

The study was conducted according to the principles of the Declaration of Helsinki and in accordance to Dutch law: WMO, AVG, WGBO, code Goed Gedrag and NFU 'richtlijn kwaliteitsborging mensgebonden onderzoek'.

### Testing of serum adalimumab levels and anti-adalimumab antibodies

Blood samples were pseudonymised and stored at −80°C in the Sint Maartenskliniek or the Radboudumc biobank for collection. A drug-tolerant competitive enzyme-linked immunosorbent

assay (Sanquin, the Netherlands) was used to quantify ADA, enabling measurement of ADA in the presence of large amounts of TNF-inhibitor. In short, a high affinity adalimumab mutant (variant cb1-3, murine origin[25]) was used, which efficiently removes the TNFi from TNF due to increased affinity.

Thereafter, the adalimumab concentration was determined via an ELISA. Concentrations <0.004 µg/mL were deemed not detectable. Concentrations <5 µg/mL were considered as not effective.[26 27] ADA were quantified with the antigen binding test (radioimmunoassay), with a reference value of >12 AU/mL.[14 15]

Testing was performed by Sanquin, The Netherlands. The treating physician (who was responsible for the choice of subsequent bDMARD) was blinded to test results as sample analysis was done retrospectively.

### Assessment of clinical outcome

The primary outcome of this study was the association between ADA or ADL and EULAR good response to the bDMARD after adalimumab failure ('EULAR response'). Response was operationalised as EULAR good response to the subsequent bDMARD after adalimumab failure, measured between 3 to 6 months (+/−8 weeks) after start of the next and subsequent bDMARDs based on the DAS28-CRP/ESR, which is a valid, reliable and broadly accepted indicator of the clinical activity of RA.[2 3] When DAS28 response was unavailable/if glucocorticoid injection could have influenced the DAS28 score outcome, clinical assessment by a rheumatologist was used to assess response ('clinical response'). When DAS28 was low at baseline and remained low in follow-up, the clinical response assessment was also used. Of note, both DAS28-ESR-scores and DAS28-CRP scores were used during the study period, and slightly different cut-offs for response were used to change from baseline >=1.2 and current DAS28-ESR <3.2 and DAS28-CRP <2.9, respectively, to consistently assess response.[28]

Finally, a subanalysis was performed for primary and secondary failure on adalimumab. Non-response is classified as primary non-response if adalimumab was used for less than 12 months and patients had not shown clinical response at any point, and as secondary non-response if adalimumab is used for longer than 12 months or if patients had at any point shown clinical response. No fixed disease activity cut-off was used for this classification due to the retrospective nature of the study.

### Statistical analyses

Data management systems Castor EDC and Microsoft Power BI database were used to enter and store the data. Data was extracted to a Stata database and analysed (V.13.1).

Descriptive statistics are provided with mean (+/−SD), median (IQRs (p25 to p75)) or n (%) depending on data distribution. Baseline characteristics of the TNFi vs non-TNFi as second treatment groups were compared using a Student's t-test (or, if not normally distributed, Wilcoxon rank-sum) and $\chi^2$ test for continuous and categorical data, respectively.

Correlations between ADA presence and clinical variables (ie, age, gender, smoking, disease duration, rheumatoid factor, anti-citrullinated protein antibodies, DAS28-CRP/ESR and its' components, CRP and ESR) were first cross-sectionally explored by Spearman's correlation analysis.

Area under the receiver operating characteristic (AUROC) curves were generated to evaluate the predictive value of ADA presence and ADL for EULAR clinical response in respectively TNFi and non-TNFi as consecutive treatment. Sensitivity and specificity were calculated using the cut-offs suggested by earlier studies (ADL <5 mg/L[26 27] and ADA >12 AU/mL[14 15]), and precision is shown with a 95% CI. A p value <0.05 was considered statistically significant.

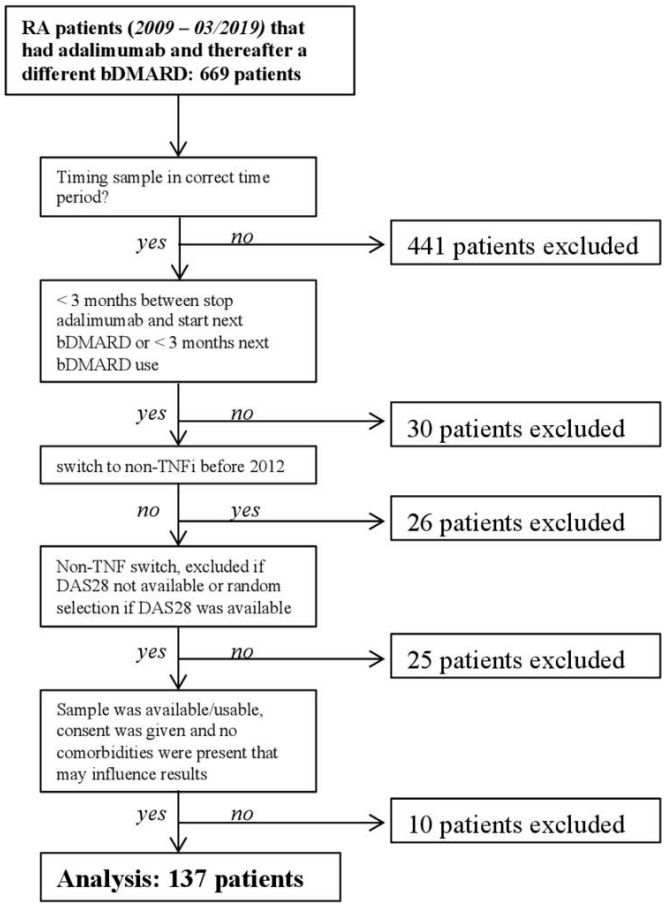


**Figure 1** Flow of participants. bDMARD, biological disease-modifying anti-rheumatic drug;DAS28, Disease Activity Score in 28 joints; RA, rheumatoidarthritis; TNFi, tumour necrosis factor inhibition.

## RESULTS

### Participants

One hundred and thirty-seven patients were included, of which 93.4% met the 1987 ACR or 2010 ACR/EULAR criteria (exclusion of the nine patients that did not meet either criteria did not significantly alter the results). Forty-seven of the 137 patients switched to a second TNFi and 90 to a bDMARD with another mode of action (figure 1). ADA were measured in all patients and ADL were measured in 92 patients due to timing of serum samples. Baseline characteristics and group differences are shown in table 1. The sample populations have comparable baseline values.

Twelve patients were assessed by means of DAS28-ESR. One hundred and two patients were assessed by means of DAS28-CRP. The remaining 23 patients were assessed by clinical response, in about one-fourth of these cases this was needed because baseline was DAS28-ESR and follow-up was DAS28-CRP or vice versa and no valid cut-off could be used to assess response. The remaining patients (three-fourth) were assessed by clinical response due to missing a DAS28 measure.

In patients receiving a second TNFi, 36% achieved good EULAR clinical response, while 23.4% achieved good EULAR clinical response in the non-TNFi group. ADA were present in 39 of 137 patients. ADL >5 µg/mL in 35 of 92 patients. It is

**Table 1** Baseline values and differences between groups

| | All patients (137) | TNFi switchers (47) | Non-TNFi switchers (90) | Difference between groups |
|---|---|---|---|---|
| | **Demographics** | | | |
| Age (mean±SD) | 64.4±13.2 | 64.7±12.9 | 64.2±13.4 | p=0.83 |
| Female (n %) | 94 (68.6) | 30 (63.8) | 64 (71.1) | p=0.38 |
| Adalimumab levels measured (n %) | 95 (67.4) | 38 (80.6) | 57 (63.3) | p=0.01 |
| | **Concomitant treatments at baseline (n%)** | | | |
| csDMARDs (any) | 105 (76.6) | 33 (70.2) | 72 (80) | $\chi 2$=1.65, p=0.20 |
| None | 32 (23.4) | 14 (29.8) | 18 (20) | |
| csDMARD (azathioprine) | 20 (14.6) | 4 (8.5) | 16 (17.8) | |
| csDMARD (methotrexate) | 60 (43.8) | 21 (44.7) | 39 (43.3) | |
| csDMARD (leflunomide) | 23 (16.8) | 5 (10.7) | 18 (20) | |
| csDMARD (hydroxychloroquine) | 13 (9.5) | 3 (6.4) | 10 (11.1) | |
| Glucocorticoid oral (prednisone/prednisolone) | 24 (17.5) | 10 (21.3) | 14 (15.6) | $\chi 2$=0.70 p=0.40 |
| None | 113 (82.5) | 37 (78.7) | 76 (84.4) | |
| | **bDMARD treatments** | | | |
| number of bDMARD used previous to adalimumab (median (IQR)) | 1 (1) | 0 (0) | 1 (0) | |
| Time until start bDMARD after adalimumab (days) (median (IQR)) | 1 (24) | 0 (24) | 2 (25) | p=0.36 |
| Duration of adalimumab use (years) (median (IQR)) | 0.75 (3.2) | 3.4 (4.7) | 0.53 (0.85) | |
| Difference (days) stop adalimumab and date serum sample (median (IQR)) | -7 (27) | -7 (81) | -8 (24) | |
| | **Disease status** | | | |
| Disease duration (years until sample) (median (IQR)) | 8.7 (12.7) | 9.1 (11.4) | 8.0 (14.1) | p=0.76 |
| Rheumatoid factor positive (n%) | 96 (70.1) | 35 (74.5) | 61 (67.7) | p=0.36 |
| anti-CCP positive (n%) | 83 (60.6) | 27 (57.4) | 56 (62.2) | p=0.79 |

anti-CCP, anti-cyclic citrullinated peptide; bDMARD, biological DMARD; csDMARD, conventional synthetic DMARD; DMARD, disease-modifying anti-rheumatic drug; IQR, interquartile range; TNFi, tumour necrosis factor inhibition.

often hypothesised that co-treatment with a csDMARD reduces antibody formation. In our study, the percentage of patients was identical in both csDMARD treated and non-csDMARD treated at 28%.

Results of adalimumab levels were also similar, with 38% and 39% of csDMARD treated and not csDMARD treated patients, respectively, having adalimumab levels >5 µg/mL.

### Correlations between ADA/ADL and patient characteristics

ADL showed a negative correlation with baseline DAS28 (Spearman's $\rho$=−0.68, p=0.00). However, ADA presence did not correlate significantly with baseline DAS28 ($\rho$=0.23, p=0.28) and both ADA and ADL did not correlate with follow-up DAS28 ($\rho$=−0.29, p=0.17, and $\rho$=0.10, p=0.65 respectively). Absolute numbers for DAS28 and ADA/ADL as well as baseline ESR and CRP are added as supplementary tables.

ADA correlates with baseline ESR ($\rho$=0.49, p=0.01) and ADL with baseline CRP ($\rho$=–0.67, p=0.00) and ESR ($\rho$=–0.546, p=0.006).

### Predictive value of ADA and ADL

No clear predictive value of ADA could be found in either TNFi or non-TNFi groups (figure 2). In the TNFi switchers, a sensitivity of 18% and specificity of 75% were found for presence of ADA predicting EULAR good response, with an AUROC value 0.46 (95% CI=0.32 to 0.59). For non-TNFi switchers, a sensitivity of 33% and specificity of 70% were found and the AUROC value was 0.52 (95% CI=0.42 to 0.63).

Additionally, in respect to ADL levels no predictive value was observed in the TNFi or non-TNFi group. In the TNFi switchers a sensitivity of 32% and specificity of 69% were found for ADL predicting EULAR clinical response, with an AUROC value of 0.50 (95% CI=0.29 to 0.71), whereas in the non-TNFi switchers a sensitivity of 50% and specificity of 52% were found, with an AUROC value of 0.50 (95% CI=0.34 to 0.65).

### Secondary outcomes

ROC analysis was conducted for patients with primary and secondary non-response as a mechanistic difference was expected between these groups. There were 53 patients with primary failure of which 45 had switched to a non-TNFi and 8 had switched to a TNFi. There were 84 patients with secondary failure of which 45 had switched to a non-TNFi and 39 had switched to a TNFi. Clinical response was not significantly different in the secondary failures than in the primary failures (32.1% vs 37.7% respectively, p=0.580). Additionally, there was no significant difference in ADA presence (26.2% vs 32.1%, p=0.560) or drug levels >5 µg/mL (32.5% vs 42.3% p=0.390) between the primary and secondary non-response groups.

ADA and ADL also did not show predictive value for response to either a second TNFi or a non-TNFi in subanalyses restricted to primary or secondary non-responders specifically (table 2).

## DISCUSSION

In this diagnostic test accuracy study, in contrast to other studies, no predictive value for response to a second (non-)TNFi was found for either ADA or random timed ADL.

This is due to the fact that sensitivity and specificity was assessed instead of mean DAS values. There were, however, some significant correlations were found as previously reported in other studies. Not only did the results of this study show no predictive values, in some analyses a prediction is found in the opposite direction of what was expected. The AUROC values

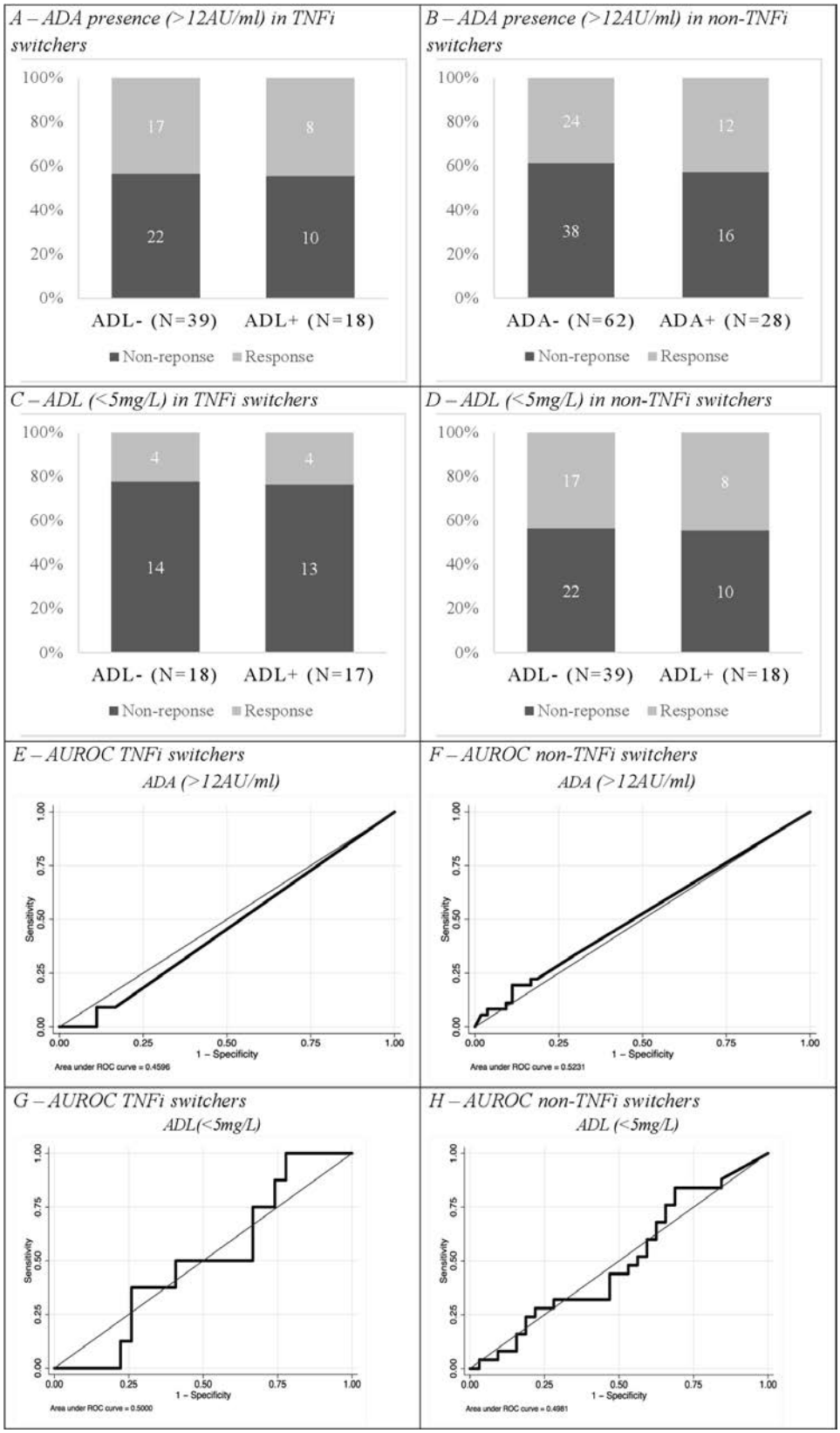


**Figure 2** Response and ADA presence in TNFi switchers (A) and non-TNFi switchers (B). Adalimumab levels <5 mg/L in TNFi switchers (C) and non-TNFi switchers (D). AUROC of ADA in TNFi switchers (E) and non-TNFi switchers (F). AUROC of ADL in TNFi switchers (G) and non-TNFi switchers (H). ADA, anti-drugantibodies; ADL, adalimumab; AUROC, area under thereceiver operating characteristic; TNFi, tumour necrosis factorinhibition.

were all close to 0.5 which shows that this is not likely due to lack of power.

This study has several strengths: First, the choice of treatment and outcome assessment were blinded for ADA/ADL as these had not been determined at time of treatment. Second, a larger patient sample was achieved than in previous studies, except for the 2019 L'Ami study.[29] Third, there was solely focussed on adalimumab. Fourth, selection bias is unlikely because the inclusion

**Table 2** Predictive values of ADA and ADL for primary and secondary non-responders in TNFi and non-TNFi switchers

| | Sensitivity (%) | Specificity (%) | AUC | CI |
|---|---|---|---|---|
| Primary non-responders | *TNFi switchers (n=8)** | | | |
| ADA presence (>12 AU/mL) | 0 | N/A | N/A | N/A |
| Low ADL (<5 mg/L) | 0 | 100 | N/A | N/A |
| | *non-TNFi switchers (n=45)* | | | |
| ADA presence (>12 AU/mL) | 30 | 28 | 0.52 | 0.38 to 0.66 |
| Low ADL (<5 mg/L) | 33.3 | 30 | 0.46 | 0.26 to 0.66 |
| Secondary non-responders | *TNFi switchers (n=39)* | | | |
| ADA presence (>12 AU/mL) | 18.1 | 17.9 | 0.49 | 0.35 to 0.63 |
| Low ADL (<5 mg/L) | 50 | 50 | 0.45 | 0.22 to 0.67 |
| | *non-TNFi switchers (n=45)* | | | |
| ADA presence (>12 AU/mL) | 60 | 45 | 0.53 | 0.37 to 0.69 |
| Low ADL (<5 mg/L) | 30 | 33.3 | 0.56 | 0.30 to 0.81 |

*unable to compute AUC as none of this subgroup were responders to the next bDMARD
ADA, anti-drug antibodies; ADL, adalimumab; AUC, area under the curve; TNFi, tumour necrosis factor inhibition.

criteria for the several studies in which patients were included when their sample was drawn were very broad. Finally, a control group was included with patients that switched to a non-TNFi treatment, to assess whether any predictive value of ADA/ADL was different for a second TNFi versus a non-TNFi bDMARD, as otherwise ADA/ADL might simply predict a more severe disease phenotype instead of differing chances of response to TNFi versus non-TNFi.

However, several limitations of this study should also be addressed. First, the samples were not taken at through level but rather timed at random related to adalimumab injection. This might have reduced the association between (anti)drug levels and response. However, it should be noted that random timed drug levels, and moreover ADA, are strongly correlated with trough level sampling.[30] So this should not have resulted in absence of any predictive value. In addition, random timed drug sampling is more feasible in clinical practice, thus increasing generalisability.

Second, as this was a retrospective study, both serum samples and clinical outcomes were not always available, and this might have resulted in selection bias.

Finally, misclassification of the outcome can occur, both by incorrectly classifying patients as responders (eg, by glucocorticoid injections resulting in spuriously low DAS28) or incorrectly classifying as non-responder (eg, a patient starting with a low baseline DAS28 that remains low during treatment). To correct this misclassification, the physician judgement of response was also assessed in a sensitivity analysis, which did not lead to a different result.

Further research should confirm if ADA presence and ADL are indeed not predictive for disease activity. This could be done in a prospective study with a large sample size in which DAS28 measurements and sample collection are done on the correct time points in all patients. Ideally, disease activity should always be assessed with use of validated scores, not physician judgement for disease activity. An RCT to address these is currently evaluating whether a switching strategy based on ADL is superior to usual care switching in RA patients failing adalimumab treatment.

Whilecounterintuitive, it is hard to find an explanation for the lack of a positive finding. It should be noted that this is also true for a very related other adalimumab TDM issue; low ADL/ADA presence are not predictive for being able to successfully stop adalimumab use.[17] The underlying reasoning is in fact the same; a drug should not work when levels are low/absent and/or ADA are present. This should be studied further, as it may offer new insights in the mode of action TNFi in general.

For now, a rheumatologists' decision to switch to a TNFi/non-TNFi treatment after adalimumab failure should not be led by the idea that one could be more effective than the other. Therefore, rheumatologists should let their decision be led by other important variables such as possible side effects, local protocol, economical aspects and patient preferences.

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**Contributors** EU, NdB, AdB, NvH and BvdB were involved in the conception and design of the study. EU, NdB, AB, LT and IM were involved in the data collection. EU, NdB and AdB contributed to the data analysis. EU, NdB, MW and AdB drafted the manuscript and all co-authors reviewed the manuscript critically and gave final approval for its submission.

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**Competing interests** None declared.

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

**Patient consent for publication** Not required.

**Ethics approval** Approval from the local ethics committee (Commissie Mensgebonden Onderzoek (CMO) region Arnhem-Nijmegen) was obtained (CMO: 2019–5443). Patients had either previously consented to inclusion in several biobanking studies, including the Nijmegen RA protocollaire follow-up[23] (CMO-number: 2016–2281) and the BIOTOP study[24] (CMO region Arnhem-Nijmegen, NL47946.091.14) or were sent opt-out informed consent letters with information about the aims and methods of the study. Patients were given 4 weeks to read the information and respond in case they are not willing to participate (according to Dutch law: WGBO art 458 sub 2). This study received no external funding. The laboratory analyses of adalimumab and ADA levels and personnel costs were funded by the Sint Maartenskliniek.The study was conducted according to the principles of the Declaration of Helsinki and in accordance to Dutch law: WMO, AVG, WGBO, code Goed Gedrag and NFU 'richtlijn kwaliteitsborging mensgebonden onderzoek'.

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**Data availability statement** Data are available upon reasonable request. Additional unpublished data can be obtained from the corresponding author upon reasonable request.

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CLINICAL SCIENCE

# Implication of baseline levels and early changes of C-reactive protein for subsequent clinical outcomes of patients with rheumatoid arthritis treated with tocilizumab

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## ABSTRACT

**Background** Rheumatoid arthritis (RA) is characterised by clinical joint swelling and elevation of acute phase reactant levels, typically measured by the C-reactive protein (CRP). Clinical and inflammatory responses are usually concordant, except for inhibition of IL-6, which often disproportionally reduces the CRP due to direct inhibition of its hepatic production. We investigated whether pre-treatment CRP is a useful marker that can guide a preferential treatment choice towards IL-6 inhibition.

**Methods** Data of 1126 treatment courses with tocilizumab (TCZ; early RA), 250 courses of rituximab (RTX; established RA) and 249 courses of methotrexate (MTX; established RA) were analysed. We compared clinical disease activity index (CDAI) values and change along 24 weeks' follow-up to CRP values at baseline or its early change. We validated the results using data from a separate TCZ trial in early RA.

**Results** CRP levels in the TCZ group on average dropped by 74% within 4 weeks. Patients who attained CDAI remission at 24 weeks on TCZ had the highest baseline CRP levels while patients in high disease activity had the lowest; this association was reverse in the RTX and MTX groups. TCZ patients who achieved remission at 24 weeks showed the largest reductions of CRP levels by week 4 compared with those reaching higher disease activity states. Early CRP non-response was indicative of a risk of not achieving clinical treatment goals (p=0.038).

**Conclusion** Baseline CRP appears to have a positive association with reaching the therapeutic target on TCZ treatment, but is a negative predictor for RTX and MTX. Patients on TCZ without an early CRP response have a lower chance of achieving remission. CRP and its early course may inform, to some extent, the estimation of potential therapeutic success in patients with RA.

## Key messages

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

- Effective inhibition of the inflammatory response by disease-modifying antirheumatic drugs (DMARDs) is key to the treatment of rheumatoid arthritis (RA) and is measured by a reduction of both swollen joint counts and acute phase reactant levels. Usually, these two markers of inflammation change in parallel, as can be seen in patients receiving conventional synthetic DMARDs or biological DMARDs. However, under IL-6 inhibition, there is often a disproportional reduction of C-reactive protein (CRP) compared with clinical measures of inflammation.
- It has not been sufficiently clarified whether patients who normalise or strongly improve their CRP levels on tocilizumab (TCZ) are also those more likely to exhibit a good clinical response, and whether (high) CRP levels before treatment could be a predictive marker for a preferential response to IL-6 inhibition.

**What does this study add?**

- We have shown that pre-treatment CRP is a positive predictor of reaching the therapeutic target on TCZ treatment, while being a negative predictor for rituximab (RTX) and methotrexate (MTX).
- Non-response of CRP to TCZ is afflicted with higher rates of patients not reaching the treatment target, which is in contrast to the respective analyses on RTX or MTX.

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

- Pre-treatment CRP and its early course may inform a preferential treatment choice in patients with RA.
- CRP reduction of <20% from baseline by 4 weeks of treatment on TCZ is a poor prognostic marker and could inform the clinical considerations for changing treatment to another agent.

Rheumatoid arthritis (RA) is a chronic inflammatory rheumatic disease leading to joint damage and disability.[1] The inflammatory response is characterised primarily by synovial joint swelling and elevation of acute phase reactant (APR) levels, both of which are a consequence of the pathogenetic pathways of RA, in particular the proinflammatory cytokines which trigger influx of inflammatory cells into the joint and hepatic APR production.[2 3] Aside from the frequent presence of autoantibodies, these two characteristics differentiate RA from non-inflammatory joint diseases, such as osteoarthritis, and are also hallmarks of the American College of Rheumatology (ACR)–European League Against Rheumatism classification criteria for RA.[4] Indeed, joint swelling in multiple joints and elevated APR together suffice to allow classification of a patient

as having RA, irrespective of other characteristics. Moreover, swollen joint counts (SJC) and APR levels are the major determinants of joint damage progression.[5 6]

For all these reasons, effective inhibition of the inflammatory response by disease-modifying antirheumatic drugs (DMARDs) is key and would be measured by a reduction, and ideally normalisation, of both SJC and APR levels. Usually, these two markers of inflammation change in parallel, as can be seen in patients receiving conventional synthetic (cs) DMARDs, such as methotrexate (MTX), or biological (b) DMARDs, such as abatacept or rituximab.[7–9] However, some therapies target specifically pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α) inhibitors or antibodies to the interleukin-6 receptor (IL-6R). IL-6 is a cytokine directly inducing hepatic APR production, and its inhibition, therefore, has an overly pronounced effect on measures of the APR and thus leads to their potentially disproportional reduction compared with clinical measures of inflammation.[10 11]

It has not been sufficiently clarified whether patients who normalise or clearly improve their C-reactive protein (CRP) levels on tocilizumab (TCZ) also exhibit a good clinical response or not, and whether (high) CRP levels before treatment could be a predictive marker for a preferential response to IL-6 inhibition. While a predictive value of CRP for TCZ responsiveness may be intuitive, based on the direct effects of IL-6 inhibition on the CRP production, previous work has failed to identify a significant association between baseline APR levels to clinical outcome for TCZ treatment.[12] However, in the absence of any biomarker that can serve this purpose, we explored here an additional aspect to this question, namely if the direction of the association (ie, positive or negative) of CRP with treatment response is different for TCZ as opposed to other compounds. For example, if patients with high CRP levels have a higher likelihood of reaching good targets with IL-6-inhibition, while they would indicate a lower likelihood of reaching these targets with other compounds, then the difference in the direction of the association (positive or negative) would be an important finding for the community. An exploration of such potential contrast has not previously been investigated, as most analyses were confined to the TCZ arms of clinical trials without comparison with control arms or other compounds.

## PATIENTS AND METHODS

### Patients

Data from four large, randomised controlled trials were kindly provided by the sponsor (Roche). For each trial, we received a random 80% cut of patient-level data. Three trials investigated TCZ in patients with RA who had remained active despite MTX therapy (the OPTION trial[10] and the LITHE trial[13]), or despite treatment with MTX or other csDMARDs (the TOWARD trial[14]). We only used data of patients receiving tocilizumab 8 mg/kg intravenously (TCZ) in combination with MTX or csDMARDs since this is the main licensed dose of TCZ.

The fourth trial was the IMAGE trial[15] in patients with early RA who were naive to MTX and received MTX monotherapy or a combination of MTX plus rituximab at the licensed dose of two 1000 mg infusions given 2 weeks apart. We used the rituximab plus MTX arm as well the placebo plus MTX (as here MTX was a newly introduced active comparator) for the analyses.

To validate the data derived for TCZ from trials of patients with established RA, we used data from the FUNCTION trial[16] which compared the results of de novo MTX with de novo TCZ in MTX-naive early RA patients with similar disease duration as seen in the IMAGE trial. All ethics statements were provided in the original publications which have been referred to.

### Outcome measures

We obtained tender joint counts (TJC) and SJC based on the assessment of 28 joints; the patient and evaluator global assessments (PGA and EGA) of disease activity (on a 100 mm visual analogue scale); the erythrocyte sedimentation rate (ESR); and the CRP level measured as milligrams per decilitre. From these core variables, we determined the Disease Activity Score with

**Table 1** Baseline characteristics of study patients

| | Tocilizumab (pooled 8 mg/kg, late disease) | Rituximab (early disease) | Methotrexate (early disease) | Tocilizumab (8 mg/kg, validation analysis—early disease) | Tocilizumab+Methotrexate (8 mg/kg, validation analysis—early disease) | Methotrexate (validation analysis—early disease) |
|---|---|---|---|---|---|---|
| n | 1126 | 250 | 249 | 292 | 290 | 285 |
| Age (years) at screening | 52.8±12.2 | 48.0±13.4 | 47.9±12.5 | 49.95±13.22 | 49.48±13.7 | 49.57±13.13 |
| % female | 82.30% | 84.80% | 77.10% | 75% | 78.6% | 80.0% |
| RA disease duration (years) | 9.41±8.5 | 0.9±1.2 | 0.9±1.1 | 0.46±0.48 | 0.5±0.53 | 0.43±0.48 |
| C-Reactive protein | 2.5±2.9 | 3.0±2.7 | 3.1±2.8 | 2.48±3.19 | 2.58±2.98 | 2.29±2.67 |
| Erythrocyte sedimentation rate | 48.2±27.1 | 57.4±29.9 | 62.0±28.6 | 51.32±28.39 | 52.81±30.15 | 50.45±26.9 |
| Evaluator's global score | 63.5±16.5 | 67.4±18.0 | 69.3±17.5 | 63.89±18.09 | 63.59±18.12 | 62.64±17.27 |
| Patient's global score | 65.1±22.3 | 67.3±22.8 | 68.3±21.0 | 67.54±22.39 | 66.46±21.46 | 63.7±21.52 |
| Global pain score | 57.8±22.2 | 62.8±22.6 | 63.6±23.2 | 62.53±21.82 | 61.64±22.1 | 59.64±22.03 |
| Swollen joint count of all 28 joints | 12.9±6.1 | 14.7±6.4 | 13.8±6.2 | 11.85 ±5.69 | 12.21 ±6.61 | 11.52 ±5.9 |
| Tender joint count of all 28 joints | 16.2±7.08 | 18.4±7.1 | 17.8±7.2 | 15.98 ±7.33 | 16.16 ±7.41 | 15.39 ±7.32 |
| HAQ-DI aggregate score | 1.5±0.6 | 1.7±0.7 | 1.8±0.6 | 1.58 ±.67 | 1.5 ±.62 | 1.48 ± .66 |
| SDAI | 44.5±14.1 | 49.5±14.8 | 48.6±14.8 | 43.33±14.13 | 44.03±15.56 | 41.99±14.45 |
| CDAI | 41.9±13.5 | 46.5±14.1 | 45.4±14.1 | 40.85±13.2 | 41.45±14.35 | 39.7±13.45 |
| DAS28 | 6.6±1.0 | 7.0±1.0 | 7.1±1.0 | 5.95±1.02 | 6.02±1.06 | 5.88±1.02 |

CDAI, clinical disease activity index; DAS28, Disease Activity Score with 28 joint counts; HAQ-DI, Health Assessment Questionnaire Disability Index; RA, rheumatoid arthritis; SDAI, simplified disease activity index.

28 joint counts (DAS28), the simplified and the clinical disease activity index (SDAI, CDAI), using the following equations: DAS28=0.56×√(TJC28)+0.28×√(SJC28)+0.70×ln(ESR)+0.014×GH; SDAI=SJC28+TJC28+PGA+EGA+CRP; and CDAI=SJC28+TJC28+PGA+EGA.[17] The global health (GH) was customarily replaced by the PGA and in SDAI/CDAI the global assessments were used in cm. We also determined the ACR 20, 50 and 70 response levels at each visit. For SDAI, CDAI and DAS28, established cutpoints for the disease activity states of remission (REM), low disease activity (LDA), moderate disease activity (MDA) and high disease activity (HDA) were applied to classify patients into the different disease activity states.[18] Since CDAI is the only composite score that does not use an APR in its formula, the analyses will be based on the CDAI metric.

### Analyses

Treatment outcomes were measured by CDAI values and CDAI states at 24 weeks. To reduce the effect of disease activity levels at baseline, we also calculated the CDAI change from baseline to 24 weeks, as an alternative endpoint. In these post hoc analyses, we assessed patients who had all necessary baseline and endpoint data available.

### Association of CRP with clinical outcomes

We identified patients in CDAI states (remission, low, moderate and high disease activity) at 24 weeks, and compared their mean baseline CRP values, as well as their 4-week changes, using the Kruskal-Wallis test. We again performed these analyses separately in the three patient groups treated with TCZ, rituximab (RTX) or MTX.

### Prediction of clinical outcomes by CRP non-response

Since TCZ has the potential to reduce CRP production independent of the clinical response, we also assessed if a non-response of CRP would carry predictive information (regarding a subsequent clinical non-response). We therefore defined CRP non-response as an observed change of <10% (and 20% in a sensitivity analysis following the concept of the ACR20 definition) from baseline to week 4. We identified the proportion of

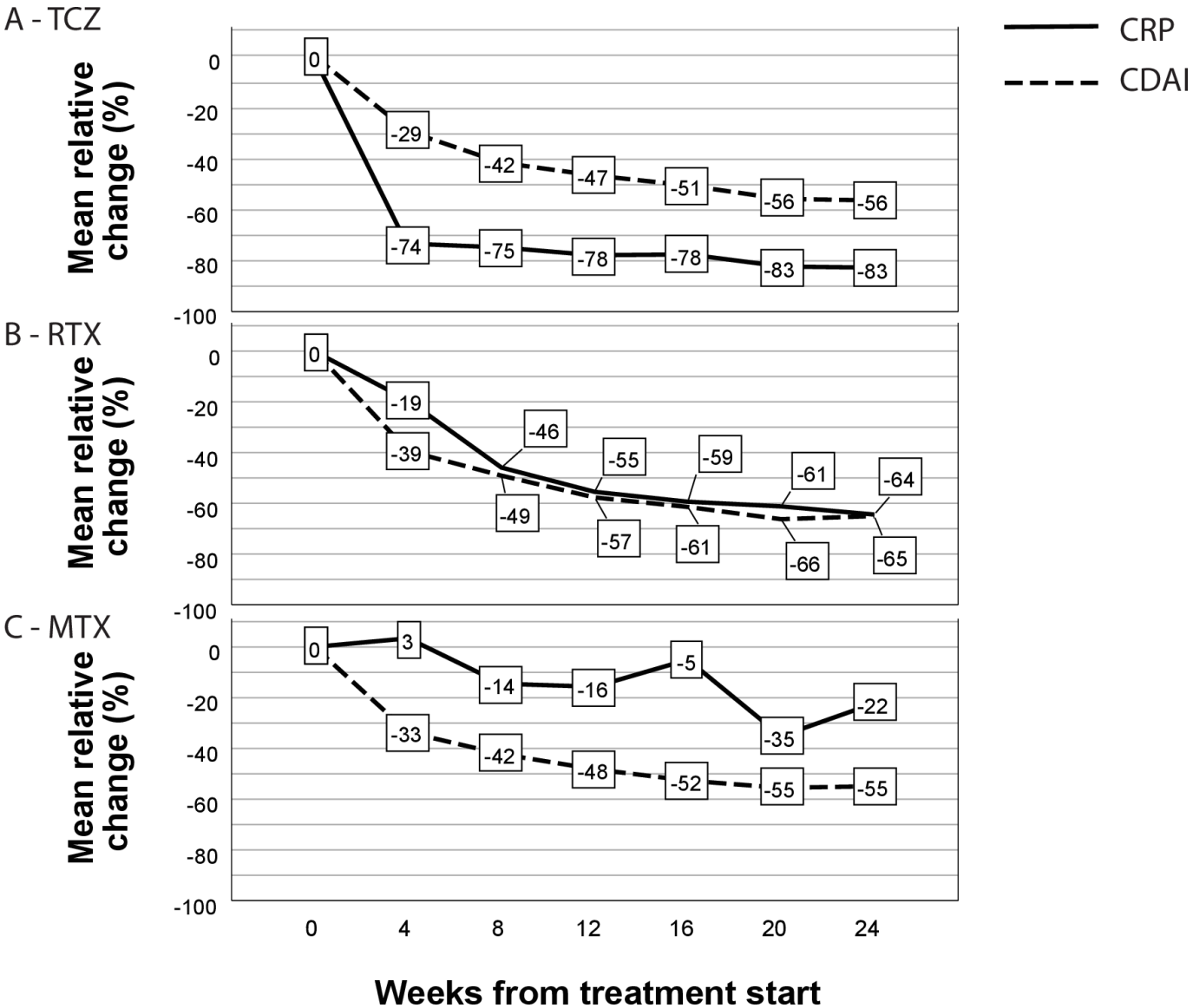


**Figure 1** Mean relative change for C-reactive protein (CRP) (continuous line) and clinical disease activity index (CDAI) (dashed line) by weeks from treatment start. (A) Tocilizumab (TCZ)–methotrexate (MTX)/conventional synthetic disease-modifying antirheumatic drug treatment; (B) rituximab (RTX)+MTX. (C) MTX.

CRP non-responders among patients achieving the four CDAI disease activity states at 24 weeks, and compared this to the CRP responders, across the three treatment regimens analysed here.

We finally calculated OR of response comparing patient groups with or without CRP elevations. In five calculations, we used CRP cutpoints to define 'elevation' as a CRP of greater than 0.5, 1.0, 2.0, 3.0 and 4.0 mg/dL. The OR for response with elevated versus non-elevated CRP was calculated for the three compounds.

### Statistical analysis

All analyses were performed using SPSS V.20 and SAS V.9.4.

## RESULTS

### Patient characteristics and overall responses in the trials

All data needed for our analyses were available in 1126 patients treated with 8 mg/kg TCZ plus MTX or other csDMARDs (TCZ8); 250 patients on rituximab and 249 on MTX. The baseline characteristics of the study patients were typical for the populations that were targeted in the respective trials (table 1).

At 24 weeks, patients treated with TCZ 8 mg, RTX or MTX exhibited CDAI HDA in 31%, 27% and 37%, respectively; MDA in 34%, 31% and 28%; LDA in 28%, 28% and 26%; and REM in 7%, 14% and 9%. Since these studies included different populations and were not head to head, the response rates are not meant for comparative interpretation. All clinical trials required patients to have active disease and a minimum joint count of 6–8 tender and swollen joints. The major difference between trials related to whether the patients were MTX-naive or MTX-insufficient responders. Baseline disease activity and differences between disease durations are shown in online supplementary table S2.

### Changes in clinical disease activity and CRP levels induced by different therapies over time

Aiming to understand the relationship between the treatment-induced changes of acute phase response and clinical disease activity, we examined mean CRP levels at each visit next to changes of CDAI over time in patients in the three treatment groups. As can be seen in figure 1, the CDAI response on the group level was gradual for all three compounds until the endpoint. In contrast, the CRP levels in the TCZ group dropped dramatically and by 74% from baseline values within 4 weeks, while only a small further reduction to about 85% was seen thereafter. This clearly differed from RTX-treated patients who experienced only about a 20% decline in CRP levels at week 4 and reached a total of 64% decline by week 24. MTX did not affect mean CRP levels by 4 weeks, while CRP values decreased by a mean 23% at 24 weeks.

### Association of early CRP measures with clinical outcomes at endpoint

Patients who attained CDAI remission at week 24 on TCZ had the highest mean baseline CRP levels, and patients still in HDA at week 24 had the lowest baseline CRP; this was exactly the opposite for RTX and for MTX (figure 2A, data in online supplementary table S1 in online appendix). At week 4, however, the lowest mean CRP values were consistently observed for the 24-week CDAI remitters in all three treatment populations; vice versa, for the 24-week HDA patients, mean 4-week CRP levels were highest (figure 2B). For TCZ, this implies that the largest reductions of CRP levels between baseline and week 4 occur in patients prone to reach REM at 24 weeks, and the smallest early reductions occur in patients who started with the lowest CRP (figure 2A), and who eventually continue to be in HDA; in fact, this is what is seen in the left cluster of figure 2C, where the change in CRP from baseline to week 4 is analysed for TCZ. In contrast, under RTX and MTX treatments, there was no difference in the CRP change between baseline and 4-week data, regardless of CDAI status at 24 weeks.

This difference between TCZ and the other two therapies was characteristic for CRP since no other variable showed a similar behaviour. As an example, we show the data for pain in figure 2D: patients who attained remission at week 24 had the lowest pain level at baseline, regardless of whether they were receiving RTX, MTX or TCZ, while those who had high disease activity at week 24 had the highest pain levels at baseline, irrespective of the treatment. This clearly contrasted the results presented earlier for CRP. We present pain here since this variable is not included in the CDAI.

Figure 3 shows the course of CRP and of CRP changes in the four outcomes groups across all visits until week 24. It can be seen that TCZ-treated patients who achieved remission after 24 weeks were those with the highest CRP levels at baseline and those who experienced the largest change from baseline (figure 3A,B), while for RTX and MTX, patients who attained remission had the lowest CRP on the group level from baseline to endpoint (figure 3C,E). The course of changes of CRP did not seem to differ across the four outcomes groups for RTX and MTX (figure 3D,F; data shown in online supplementary table S2).

When we assessed baseline CDAI values and CDAI changes over time in relation to outcomes at week 24, we found that—in contrast to the previously mentioned analyses of CRP courses—all three groups, TCZ, RTX and MTX, showed a similar pattern: patients achieving remission started with the lowest mean CDAI values at baseline and experienced the largest changes in CDAI already at early stages of the treatment and onward (figure 4), a finding that has been reported before.[19]

Evaluation of different CRP cutpoints at baseline to identify ORs of response when presenting with CRP levels above that cutpoint are shown in figure 5A (data provided in online supplementary table S3) for all three drugs. As can be seen, the presence of elevated baseline CRP had an impact on the response for TCZ, and this impact was different or even in contrast to the data obtained with MTX and RTX. The most discriminative cutpoint for baseline CRP levels was 4 mg/dL, but similar data were also seen at a cutpoint of 3 mg/dL (figure 5).

When calculating the odds of response only for patients above the respective CRP cutpoints, and comparing them between the compounds in a pairwise manner, then the results again indicate that the likelihood of benefit of TCZ over each of the other two compounds is greater in patient groups with higher levels of CRP (figure 5B). It is important to note that these comparisons are partly not head to head; therefore, only the trends across the CRP groups can be interpreted, which are the focus of interest, but no absolute/overall difference in response within each of the comparisons can be deduced.

Since the patients in the TCZ trials evaluated hitherto had established RA, while the patients in the RTX trial had early RA, validation of the results was deemed important. To this end, we assessed patients with early RA from a TCZ trial where MTX-naive patients with RA with a mean disease duration of 6 months were subjected to either MTX or TCZ therapy, head to head. When testing the patients reaching the different disease activity states at week 24 for their baseline CRP elevation (using the most discriminative cutpoint of 4 mg/dL as developed above), we found that among the remitters at 24 weeks, high CRP at baseline was present in only 6% of MTX-treated patients, but

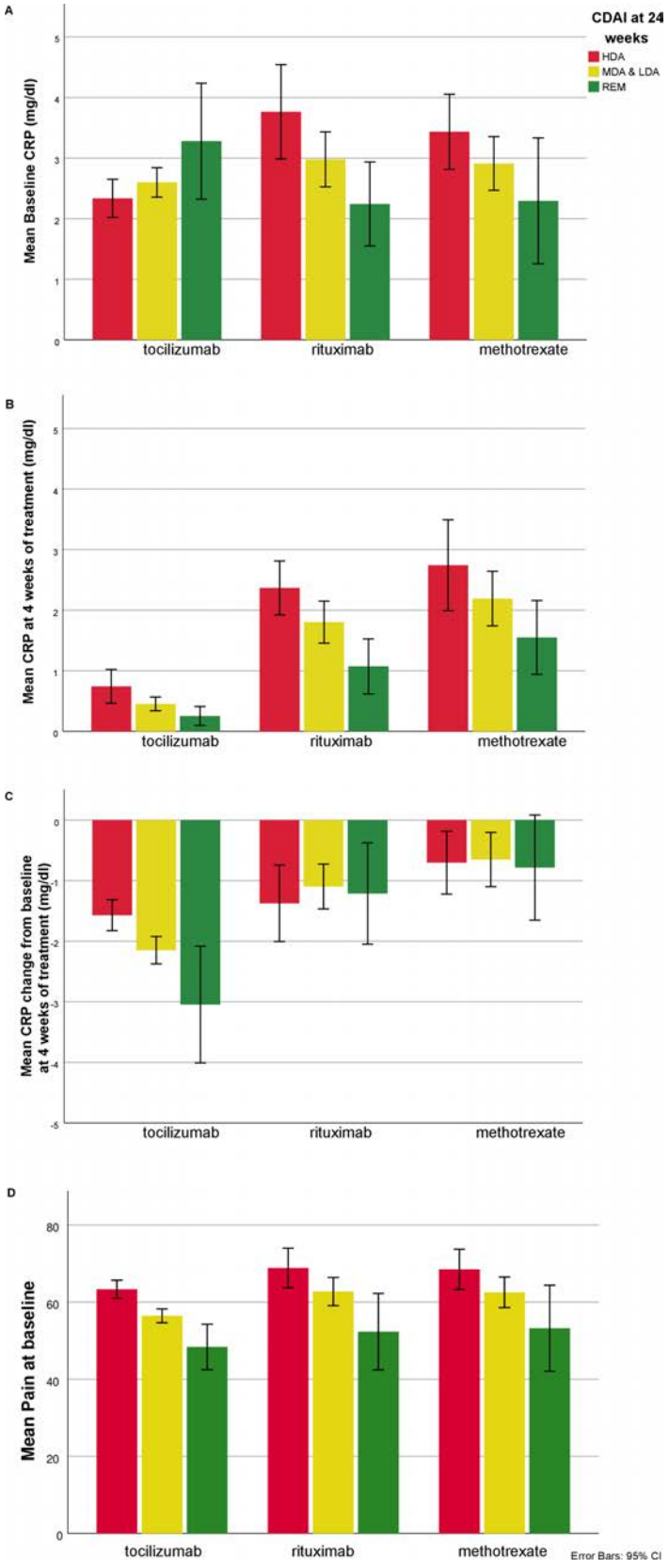


**Figure 2** Baseline C-reactive protein (CRP), its early changes and baseline pain for patients reaching clinical disease activity index (CDAI) high disease activity (HDA, red columns), moderate disease activity (MDA) and low disease activity (LDA) (yellow columns), and remission (REM, green columns) for tocilizumab (TCZ, left clusters), rituximab (RTX, middle clusters) and methotrexate (MTX, right clusters); (A) CRP at baseline (mg/dL): p values for TCZ, RTX and MTX: 0.07, 0.03, 0.14; (B) CRP at 4 weeks (mg/dL): p values for TCZ, RTX and MTX: 0.03, 0.01, 0.16 (C) CRP change at 4 weeks (mg/dL): p values for TCZ, RTX and MTX: <0.001, 0.73, 0.97; (D) pain scale at baseline—validation analysis (p values for TCZ, RTX and MTX: 0.00, 0.00, 0.02). While in (A) both TCZ and RTX are significant, but associations pointing in different directions, in (D) the directions of the significant associations are concordant.

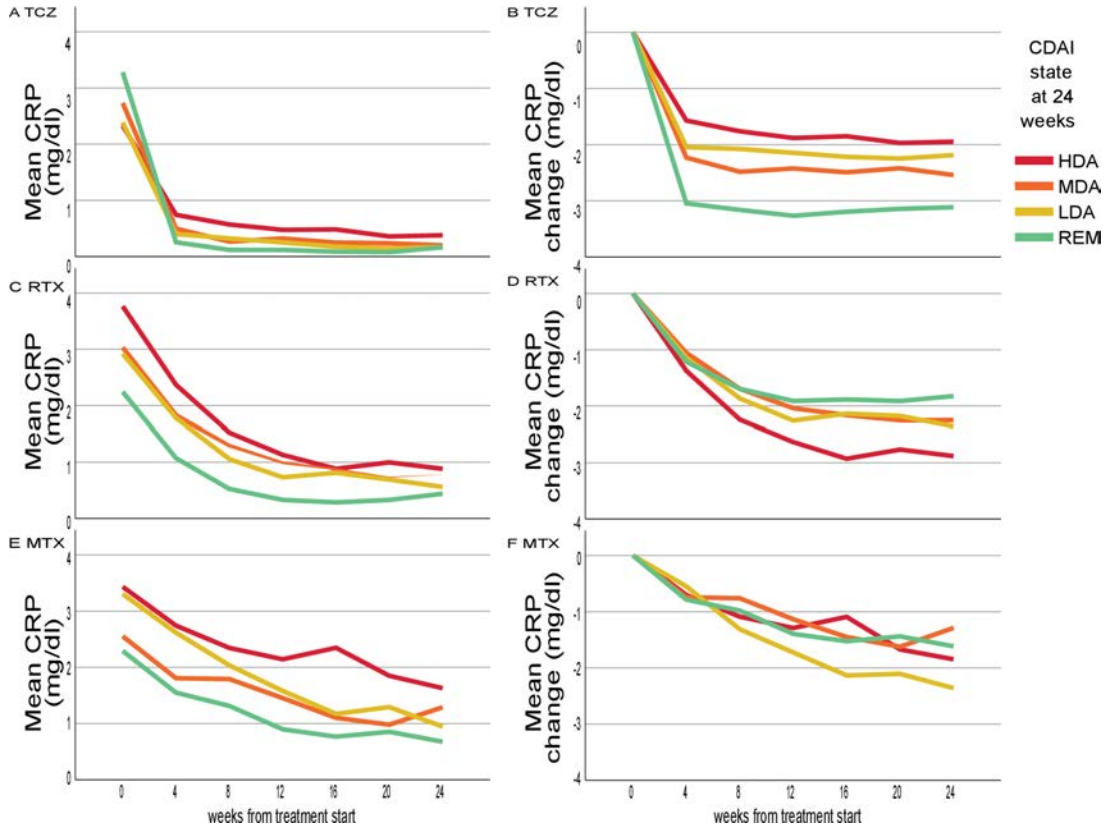


**Figure 3** CRP and CRP change from baseline by weeks from treatment start, for patients reaching CDAI HDA (red), MDA (orange), LDA (yellow) and REM (green) at 24 weeks, for TCZ patients: (A) CRP; (B) CRP change; RTX patients: (C) CRP; (D) CRP change; and MTX: (E) CRP; (F): CRP change. The detailed data and the statistical significances for each figure are detailed in online supplementary table S2. CDAI, clinical disease activity index; CRP, C-reactive protein; HDA, high disease activity; LDA, low disease activity; MDA, moderate disease activity; MTX, methotrexate; REM, remission; RTX, rituximab; TCZ, tocilizumab.

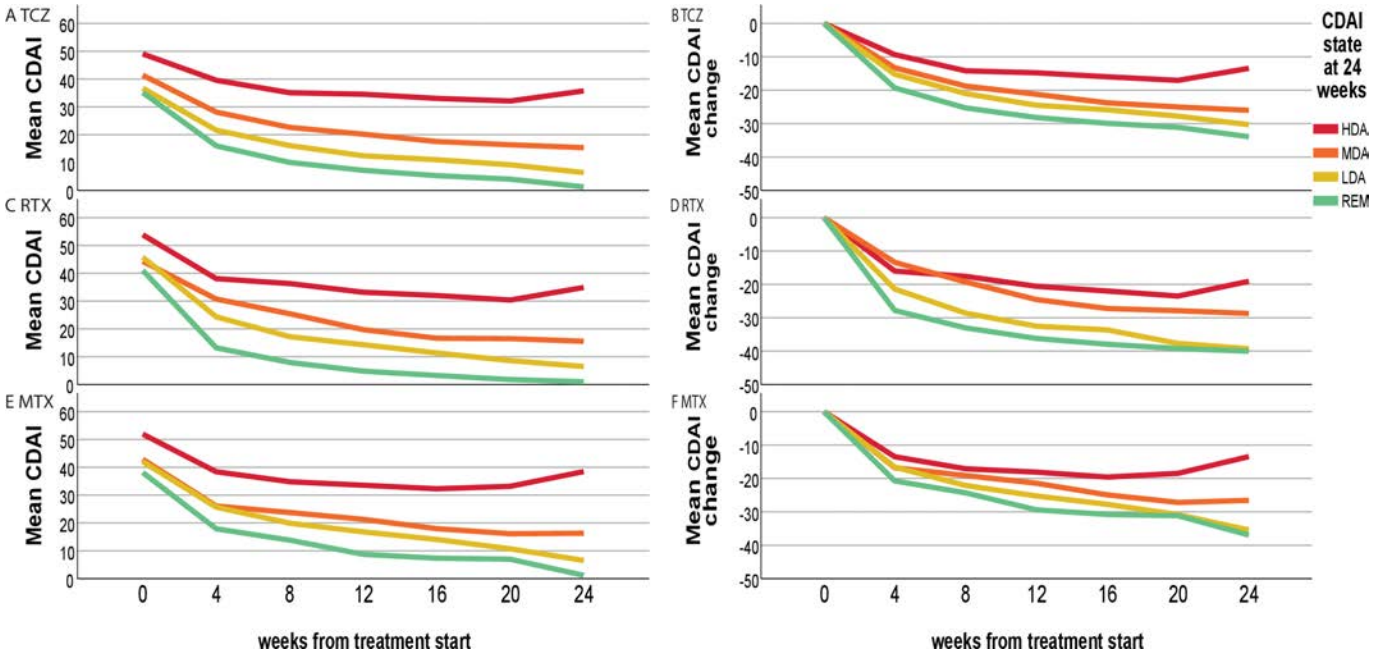


**Figure 4** CDAI and CDAI change from baseline by weeks from treatment start, for patients reaching CDAI HDA (red), MDA (orange), LDA (yellow) and REM (green) at 24 weeks, for TCZ patients: (A) CDAI; (B) CDAI change; RTX patients: (C) CDAI; (D) CDAI change; and MTX: (E) CDAI; (F) CDAI change. Detailed data and statistical significance for each figure are detailed in online supplementary table S2. CDAI, clinical disease activity index; HDA, high disease activity; LDA, low disease activity; MDA, moderate disease activity; MTX, methotrexate; REM, remission; RTX, rituximab; TCZ, tocilizumab.

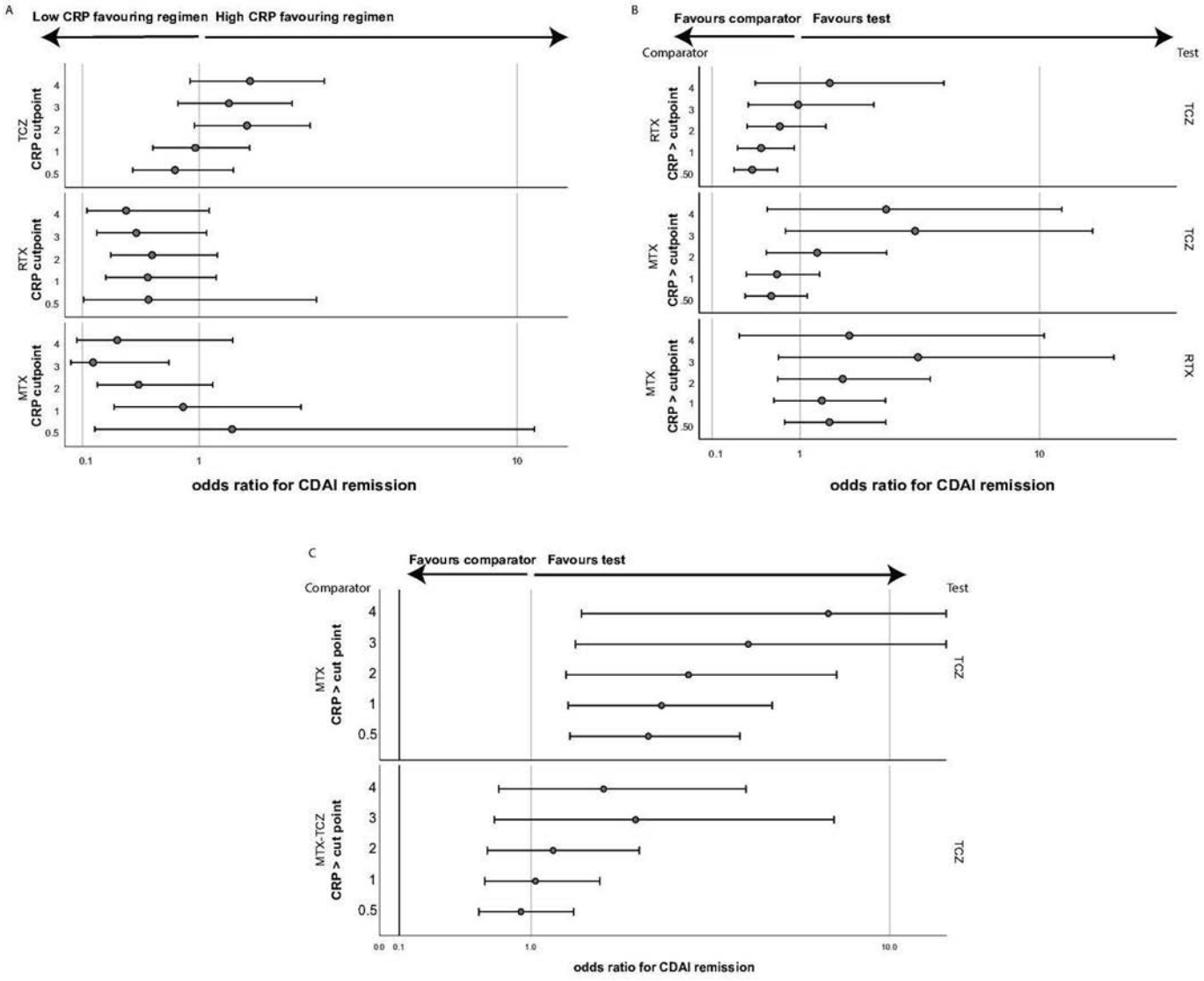


**Figure 5** OR for CDAI remission. (A) OR (on a logarithmic scale) for reaching CDAI remission at 24 weeks comparing patients with high vs low CRP at baseline using different CRP cutpoints (4 mg/dL top line, 3 mg/dL second line, 2 mg/dL third line, 1 mg/dL fourth line, 0.5 mg/dL bottom line) for TCZ (top panel), RTX (middle panel) and MTX (bottom panel); (B) comparative OR (on a logarithmic scale) for reaching CDAI remission at 24 weeks between different drugs, TCZ vs RTX (top panel), TCZ vs MTX (middle panel) and RTX vs MTX (bottom panel), for patients with baseline CRP above the respective cutpoint (4 mg/dL top line, 3 mg/dL second line, 2 mg/dL third line, 1 mg/dL fourth line, 0.5 mg/dL bottom line). (C) Validation of TCZ data in early rheumatoid arthritis: comparative OR (on a logarithmic scale) for reaching CDAI remission at 24 weeks between different drugs, TCZ vs MTX (top panel), TCZ vs TCZ+MTX (bottom panel), for patients with baseline CRP above the respective cutpoint (from top to bottom: 4 mg/dL, 3 mg/dL, 2 mg/dL, 1 mg/dL, 0.5 mg/dL). CDAI, clinical disease activity index; CRP, C-reactive protein; MTX, methotrexate; RTX, rituximab; TCZ, tocilizumab.

26% of TCZ monotherapy patients—a fourfold difference; in contrast, among patients remaining in high disease activity, 24% of MTX and only 17% of TCZ-treated patients had high baseline CRP levels (online supplementary figure S1). These findings substantiate the results seen in established RA. Similar data were obtained when a cutpoint of 3 mg/dL was used (data not shown). When we calculated the ORs of TCZ compared with MTX (this time in a head-to-head rather than an indirect comparison), we saw a very similar trend as before, with the ORs for achieving CDAI remission being about threefold higher for TCZ than MTX (figure 5C and online supplementary table S3C). Interestingly, patients with de novo introduction of the combination therapy resembled more the MTX group than the TCZ monotherapy group, with TCZ monotherapy having twice the remission odds with 4 mg/dL CRP at baseline than 0.5 or 1 mg/dL compared with TCZ+MTX (figure 5C and online supplementary table S3C).

## Relevance of early CRP non-response with regards to later clinical outcomes

We compared the proportions of CRP non-responders ($CRP_{NR}$; defined as <10% improvement of CRP at week 4) across patients reaching different CDAI states at 24 weeks. In TCZ-treated patients, there was a clear trend of a higher frequency of $CRP_{NR}$ patients continuing to be in HDA versus MDA versus LDA versus REM (46.8%, 29.0%, 19.4% and 4.8%, respectively), and a statistically significant difference was seen between the aforementioned frequencies in the $CRP_{NR}$ compared with the CRP responders ($p=0.038$ by $\chi^2$). In contrast, for RTX and MTX, there was no such trend in the proportions of $CRP_{NR}$

across the different CDAI states, and no difference was seen with respect to disease activity states reached comparing $CRP_{NR}$ and CRP responders (p=0.986 and p=0.670, respectively).

Under TCZ, mean CDAI at 24 weeks was 21.6±14.9 in the week 4 $CRP_{NR}$ group compared with 17.8±14.1 in the CRP responders (p=0.044); no statistically significant difference was found between $CRP_{NR}$ to CRP responders in mean CDAI values at 24 weeks in the RTX and MTX group (17.2±15.0 vs 15.9±13.1 for RTX, p=0.536; and 19.1±15.7 vs 21.3±16.9 for MTX, p=0.334).

As a sensitivity analysis, we examined the proportion of patients who did not achieve at least a 20% improvement in CRP: by this definition, 47.8% of $CRP_{NR}$ in the TCZ group were in CDAI-HDA state at 24 weeks, 27.5% were in MDA, 20.3% in LDA and 4.3% in remission at 24 weeks, which was essentially confirming the results obtained with the 10% definition. Also, similarly to the 10% cutpoint, using the 20% cutpoint there was a significant difference in the achieved CDAI states between CRP responders and non-responders in the TCZ group (p=0.014). No such difference was seen for RTX or MTX (p=0.391 and p=0.781, respectively).

## DISCUSSION

This comprehensive analysis of implications of CRP on treatment response to IL-6R inhibition by TCZ in comparison with RTX and MTX showed several key findings: first, patients who achieve remission on TCZ have higher levels of baseline CRP than those who reach worse clinical endpoints. For RTX and MTX, this is exactly inverse with those with the highest CRP at baseline having the worst clinical outcomes at week 24, using the CDAI score as the clinical outcome scale. This observation was made irrespective of the fact that disease activity was high at the start of all trials, and that clinical disease activity correlates well with the APR. However, in the absence of any useful markers for preferential treatment response to a specific mode of action in RA, the finding of an inverse relationship of CRP with response to TCZ (higher→good response more likely) as opposed to its relationship with other treatments (higher→good response less likely) constitutes an important information and may be used to inform clinical decision-making. Indeed, one of the most important finding relates to the CRP cutpoint: patients with baseline CRP levels ≥4 mg/dL fare much better on TCZ than on MTX or RTX.

Our data also imply that a non-response of CRP to TCZ is afflicted with higher rates of patients not reaching the treatment target, which also is in contrast to the respective analyses on RTX or MTX. Therefore, should a patient exposed to TCZ show <20% reduction of CRP by week 4, then this patient has a higher chance of not reaching the treatment target of low disease activity or remission compared with those who respond to TCZ by CRP decrease of 20% or more.

Ever since treatment with TCZ has been introduced, there was a specific interest in the observed effects on the APR. CRP and/or ESR have been integral parts of most composite disease activity measures used for RA, but a discussion emerged whether the directly normalising effects on the acute phase should not be disregarded when interpreting the clinical benefit conveyed from that treatment.[11 20] Indeed, that disconnect was proven and a claim was made, that—particularly in the comparison with other modes of action—the efficacy and clinical benefit should be evaluated with instruments that do not include an acute phase measure, which would typically be by the CDAI, which is the only composite instrument that does not include CRP or ESR, and is yet not a solely patient-reported outcome. In this way, using the CDAI, its changes and its states, as the main outcomes of our study provided a clear and unbiased perspective onto clinical disease activity. This approach differentiates our study from previous works aiming to clarify the relationship between CRP and outcome under TCZ treatment.[12] Moreover, we were able to confirm the importance of baseline CRP in a separate TCZ trial of patients with early RA, where the TCZ data and also the results obtained with MTX treatment could be validated; importantly, however, this time based on a true within trial comparison (head to head; the original evaluations were indirect comparisons). Interestingly, patients on combination therapy had results similar to MTX monotherapy; this is likely due to the fact that MTX alone conveys at least 50% of the effect that is seen when it is combined with a bDMARD in MTX-naive patients.[3] In patients with established RA, despite failure of MTX and ongoing treatment with MTX, TCZ is newly introduced—like monotherapy in early RA—and in fact, the informative value of baseline CRP is very much alike.

Since the extraordinary effects on reducing CRP levels elicited speculation about whether introduction of IL-6R inhibition in a patient with high or very high CRP levels would not also be clinically more beneficial than the use of other compounds, it was important to perform a study that—at least indirectly—compares the implications of pre-treatment CRP levels for TCZ success with those for success of other compounds. Here, we approached this aspect by choosing datasets that aside of TCZ included RTX as a non-cytokine targeted biologic with similar overall efficacy in clinical trials[21] and MTX as the most commonly used synthetic DMARD. Under usual circumstances, disease activity at baseline is correlated with disease activity at endpoint,[19] and this also includes CRP. However, as shown in the present study, this appears to be different for TCZ, when newly introduced, either as monotherapy or on top of existing (and failing) MTX. The distinct informative and predictive value of baseline CRP levels is further revealed by the fact that no other variable, such as pain, joint counts, and so on, showed this inverse relationship between baseline levels and 24-week outcome.

Our study has some limitations. One limitation relates to the use of trial data which are always afflicted with considerations about applicability in clinical practice; however, trial populations have the strong advantage that they include patients with active disease and best allow to differentiate predictors, such as CRP, for improving these active disease states. Also, the inclusion criteria regarding disease activity and acute phase measures across the different trial populations were quite comparable. Moreover, the 4-week data in the RTX trial may have been influenced by glucocorticoid use in the course of the RTX applications. Finally, irrespective of the associations described in this paper, it should be borne in mind that high CRP levels may be a consequence of an infection and then TCZ (but also other bDMARDs) should not be applied.

In summary, our findings indicate that although there is no linear and significant correlation between baseline CRP and clinical outcome, patients with very high CRP levels (≥4 mg/dL) at baseline have greater benefit from the use of TCZ than from RTX or MTX. The most relevant finding of our study is that in this population of clinically highly active trial patients, the response in those treated with TCZ and RTX is partly determined by baseline and early CRP levels, but that the association is positive for one (TCZ) and negative for the other (RTX) agent. Moreover, as another novel finding, a CRP reduction of <20% from baseline by 4 weeks of treatment on TCZ is a poor prognostic marker and should elicit considerations for changing treatment to another agent.

**Correction notice** This article has been corrected since it published Online First. The acknowledgement section has been added.

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EPIDEMIOLOGICAL SCIENCE

# Improving rheumatoid arthritis comparative effectiveness research through causal inference principles: systematic review using a target trial emulation framework

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## ABSTRACT

**Objectives** Target trial emulation is an intuitive design framework that encourages investigators to formulate their comparative effectiveness research (CER) question as a hypothetical randomised controlled trial (RCT). Our aim was to systematically review CER studies in rheumatoid arthritis (RA) to provide examples of design limitations that could be avoided using target trial emulation, and how these limitations might introduce bias.

**Methods** We searched for head-to-head CER studies of biologic disease modifying anti-rheumatic drugs (DMARDs) in RA. Study designs were reviewed for seven components of the target trial emulation framework: eligibility criteria, treatment strategies, assignment procedures, follow-up period, outcome, causal contrasts of interest (ie, intention-to-treat (ITT) or per-protocol effect) and analysis plan. Hypothetical trials corresponding to the reported methods were assessed to identify design limitations that would have been avoided with an explicit target trial protocol. Analysis of the primary *effectiveness outcome* was chosen where multiple analyses were performed.

**Results** We found 31 CER studies, of which 29 (94%) had at least one design limitation belonging to seven components. The most common limitations related to: (1) eligibility criteria: 19/31 (61%) studies used post-baseline information to define baseline eligibility; (2) causal contrasts: 25 (81%) did not define whether ITT or per-protocol effects were estimated and (3) assignment procedures: 13 (42%) studies did not account for confounding by indication or relied solely on statistical confounder selection.

**Conclusions** Design limitations were found in 94% of observational CER studies in RA. Target trial emulation is a structured approach for designing observational CER studies that helps to avoid potential sources of bias.

## Key messages

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

- Target trial emulation is an intuitive design approach that encourages researchers to formulate their question as a hypothetical randomised controlled trial (RCT). Using observational data to emulate such a target trial helps avoid common biases and has been shown to better align results with actual RCTs.

**What does this study add?**

- Most comparative effectiveness research (CER) studies in rheumatoid arthritis had at least one design limitation, such as using post-baseline information to define eligibility, not specifying whether interest is in intention-to-treat (ITT) or per-protocol effects, and using statistical selection of confounders. Each of these issues can introduce bias and affect data analysis and interpretation.

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

- The target trial emulation framework unifies and builds on existing good design practices to make robust observational designs intuitive. Improvement and standardisation of CER methodology is essential in rheumatology as more drugs become available, often without (timely) head-to-head RCTs to compare their effectiveness.
- Future studies should avoid using post-baseline information to define eligibility, clearly define the causal contrast pursued (ideally presenting both ITT and per-protocol effects) and consider confounding using prior knowledge (preferably using causal diagrams that make assumptions transparent).

## INTRODUCTION

There are a growing number of pharmacological treatment options in rheumatology, particularly high-cost biologic and targeted synthetic disease modifying anti-rheumatic drugs (bDMARDs and tsDMARDs). This enlarging armamentarium begs the question of how to choose the optimal treatment for a given condition. Head-to-head randomised controlled trials (RCTs) of these new and emerging therapies—the preferred evidence—are scarce and provide only limited guidance; for example, few have directly compared treatment options for rheumatoid arthritis (RA) patients who have failed one or more bDMARDs. When RCTs are not feasible, timely, or ethical, observational data can help fill the need for comparative effectiveness information.[1–3]

Observational comparative effectiveness research (CER) studies are common in rheumatology, as are

their critics. The target of much criticism lies in the sheer number of methodological approaches available to the analyst,[4] and the profound effect that nuanced differences in methods can have on results.[5 6] Large sample sizes in these studies may instil false confidence in the presence of critical design flaws. Improvement and standardisation of methodology guided by causal inference principles are paramount and increasingly demanded by many clinical journals.[7] One barrier, however, is that detailed theory based on 'potential outcomes'[8] can seem complex and unfamiliar.

A more intuitive approach is to ask: 'How would I answer my CER question as an *RCT*?' according to the recently popularised 'target trial emulation' framework of observational study design and analysis.[3] This way of thinking is rooted in the principles of causal inference, which were first conceived in the context of randomised experiments[9] and extended to observational studies.[2 10 11] Principled re-analyses of existing observational studies using the target trial emulation framework have repeatedly shown to reduce bias and better align results with actual RCTs.[6 12–16]

Through a systematic review of observational CER studies in RA, we provide examples of design limitations that might have been avoided by using target trial emulation, and how these limitations might introduce bias. Since the practice of explicitly writing down the target trial protocol is relatively new,[3] we did not expect the reviewed study designs to incorporate its terminology; rather, we retrospectively imposed the framework components as a structured way to appraise them.

## METHODS

### Target trial emulation framework

At the heart of the target trial emulation framework are two protocols—'target trial protocol' and 'emulation protocol'—for each observational CER study (figure 1). When prospectively *designing* an observational CER study, researchers first consider how the question can be answered and formulated as a hypothetical RCT—the 'target trial'.[3] Systematically specifying this protocol helps ensure that the question is clinically meaningful for decision-making (eg, 'among eligible patients, which treatment strategy maximises benefit?' rather than simply 'is exposure X associated with outcome Y?').[17 18] The 'emulation protocol' describes how available observational data might be used to obtain the best approximation of the 'target trial protocol'. The protocol is then reformulated when data limitations and feasibility of the ideal emulation are realised (figure 1).[10] This framework helps to avoid common methodological pitfalls[3 19] and better align results with RCTs.

The framework can also be applied retrospectively for *appraising* existing studies in a structured process.[3] The description of an observational CER study can be seen as the emulation protocol for the inferred target trial. After inferring the corresponding target trial protocol, it is possible to assess the clinical question[17 18] as well as subtle design limitations that fail to emulate a sound target trial.[19] This retrospective application is our approach in this review.

### Systematic literature review

We searched EMBASE, Medline and PubMed in August 2019. Search terms are shown in online supplementary materials. We only included *head-to-head effectiveness* comparisons to demonstrate the utility of target trial emulation beyond existing good design practice (online supplementary table S1).[4 20 21] We restricted to comparisons of *different classes* of bDMARDs, where RCTs are scarce but evidence is needed to guide clinical practice. Studies that did not report effectiveness (ie, only reported drug retention or adverse/comorbidity events) were excluded. Independent reviewers (SSZ, HL) assessed study eligibility and performed data extraction (table 1 and online supplementary table S2); discrepancies were resolved through discussion moderated by a third reviewer (KY). Where multiple analyses were presented in one study, analysis for the primary *effectiveness outcome* was chosen. We appraised each study's design against each of the target trial emulation components below[3]; the specific questions we asked of each study's design (ie, data extraction items) are listed in table 1 (left column).

### 1. Eligibility criteria

All RCTs are expected to have clear, predefined eligibility criteria before the study begins. Those with contraindications to any of the treatment strategies must be excluded. Obviously, RCT eligibility criteria can only consist of *baseline information* that are available to investigators at the time of prospective enrolment.

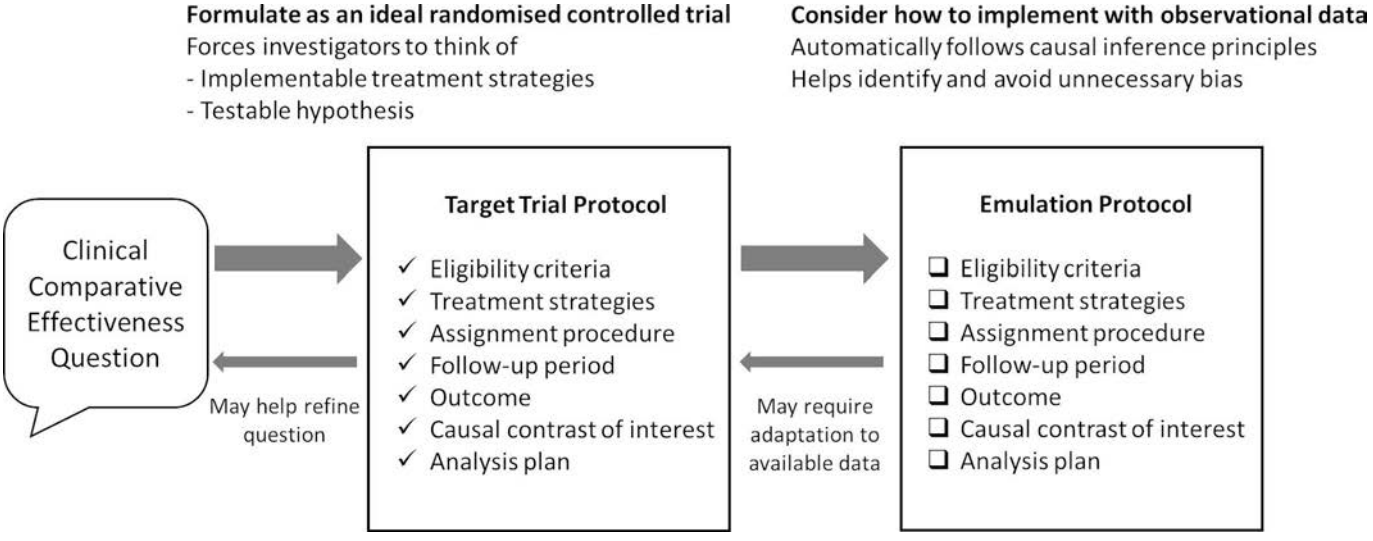


**Figure 1** The target trial protocol is used to guide observational comparative effectiveness research design. This idealised protocol may need to be reformulated once limitations of the data are realised. Divergence of the implemented observational study (emulation) from the target trial protocol should be addressed by sensitivity analyses or transparent reporting of limitations.

**Table 1** Items in the prespecified data extraction form and a summary of findings from 31 studies

| | Data extraction questions (see the Methods section for rationale) | Summary of findings from 31 studies* |
|---|---|---|
| 1. Eligibility criteria | What were the criteria? (eg, classification criteria RA with no prior exposure to bDMARDs) | All studies specified basic eligibility criteria such as RA definition, level of disease activity and number of prior bDMARDs. |
| | Was post-baseline information used to define eligibility? (eg, requiring ≥1 follow-up) | 19/31 (61%) studies explicitly required post-baseline information for eligibility.[29–43 50–53] |
| | How many bDMARDs had been used before? (eg, bDMARD naïve, ≥1 prior TNFi) | All studies described the number of prior bDMARDs. 5/31 (16%) studies combined response from both bDMARD-experienced and naïve patients.[40 52 54–56] |
| 2. Treatment strategies | What were the bDMARDs under comparison? | Drug name, dose and frequency were generally clearly defined, except when different TNFi were combined as one group. |
| | What were the treatment strategies? (eg, discontinuation due to remission or switch to biosimilar are permitted in the protocol) | One study clearly defined treatment strategies.[57] |
| 3. Assignment procedures | How did the study use statistics to emulate random assignment? Specifically, how were confounding factors selected? | 13/31 (42%) studies used only predefined confounders.[31–34 36–38 44–46 50 52 53] 5/31 (16%) studies used only statistical (eg, p value-based) variable selection.[29 30 41–43] 9/31 (29%) made no adjustments for confounding beyond active-comparator design.[39 40 43 47 51 54–56 58] |
| 4. Follow-up period | What was the specified duration of study? For studies using existing data from registries, duration of the implied trial was used. | Studies using binary response outcomes clearly defined follow-up times in all except one study.[50] 6 studies did not specify an end to follow-up at all.[30–32 40 46 50] |
| 5. Outcome | What was the primary effectiveness outcome measure and time frame/point? | Outcomes were clearly described, but time occasionally not (see above). |
| | Was sample size or statistical power discussed at the design stage? | 4/31 (13%) studies included sample size considerations.[29 31 41 44] |
| 6. Causal contrast of interest | Was a causal contrast of interest declared prior to analysis? | 6/31 (19%) studies clearly defined causal contrast.[31 43 45 55–57] |
| | What was the declared or inferred causal contrast? | 20/31 (97%) studies examined some version of the ITT effect[29 31 33–37 39 41 43 45 46 48–50 52–54 57 59]; analyses were compatible with traditional ITT effect definition in only one study.[45] 12/31 (42%) studies include per-protocol analysis[30–32 38 40 42 44 51 55–58] but did not apply any post-baseline adjustments. |
| 7. Analysis plan | What statistical model was used? (eg, linear regression) | 18/31 (42%) studies used regression-based methods. 8/31 used pairwise comparisons and 3/31 did not perform statistical comparison. |
| | How were missing data handled? (eg, complete-case analysis, imputation) | 17/31 (55%) studies used complete-case analysis.[29–33 39 41 43 44 47 49 50 52 54 55 58 59] 3/31 used multiple imputation for missing outcome data.[44 48 57] 9/31 use single imputation.[34–38 42 45 46 53] Reasons for discontinuation were rarely differentiated in analyses. |

Components of the target trial emulation framework are discussed in detail by Hernán and Robins[3] and Dickerman *et al*.[6]
*See the Results section for details; information extracted from individual studies are shown in online supplementary table S2.
bDMARDs, biologic disease modifying anti-rheumatic drugs; ITT, intention-to-treat; RA, rheumatoid arthritis.

To emulate such a target trial, observational cohorts first need to be defined using information up to the baseline (often called 'time zero'), but not beyond.[3] For example, some observational studies require at least one follow-up in the eligibility criteria. This practice does not have an RCT equivalent, since trial investigators cannot see into the future at the time of each patient's enrolment. Cheating the baseline criteria via such an oracle can bias results in either direction.[22]

Including key confounders in the emulation eligibility criteria may help comparability. One example is the number/type of prior bDMARD failure, which is an important predictor of response. This is intuitive when conceptualised as equivalent RCTs: a trial comparing bDMARD naïve patients, or a trial comparing switching of therapy after one or more TNF inhibitor (TNFi) failures. We examined each study's eligibility criteria for use of post-baseline information and specification of the prior bDMARD treatment history (table 1).

## 2. Treatment strategies

An RCT protocol specifies detailed treatment strategies beyond the drug name. The protocol also defines criteria for discontinuation or modification, and relevant concomitant care that are allowed or prohibited during the follow-up.[23] Each major treatment change should be defined as complying with or violating the protocol. For example, change of concomitant csDMARDs or discontinuation of bDMARD due to remission may be protocol-compliant, whereas switch to another bDMARD due to insufficient response may be considered a protocol violation. How treatment strategies are defined will have implications for the definition and analysis of the per-protocol effect (components 6 and 7). Observational CER studies also have to specify treatment strategies. Not all datasets have enough details to define granular treatment strategies, which may require the emulated target trial to be simplified (figure 1). We reviewed how treatment changes were defined as part of the treatment strategy.

## 3. Assignment procedures

In the simplest RCT design, participants have equal chance of being assigned to each treatment strategy, and each treatment group will have comparable distribution of prognostic factors. To emulate random treatment assignment in observational CER, all theoretical confounding factors—measured and unmeasured—need to be adjusted for. Inability to completely account for confounding is the the most common criticism for observational studies. While this may be the case, there are many ways to improve emulation of random assignment. Two considerations are the use of active-comparators[20] (which should be present by default in head-to-head comparisons) and methods for selecting confounders.[24] Confounding factors should not be selected *solely* based on their statistical association with the exposure or outcome. Selection should instead be based on subject knowledge and/or literature review, preferably supported by directed acyclic graphs (DAGs) that make assumptions transparent.[24] For the review, we focused on how confounding factors were selected.

## 4. Follow-up period

RCTs have a well-defined start, schedule and end of follow-up. In rheumatology trials, efficacy typically focuses either on

percentage of participants achieving a response definition at a certain time point or multiple repeated assessments over the study period. Each RCT participant is consented for a schedule of follow-ups and a finite study period (eg, 12 months after treatment initiation). By contrast, observational data often come from ad hoc clinic visits, where frequency may be associated with patient and disease characteristics. The data to be used in an observational analysis should ideally reflect the same (or similar) data that would have been collected in the target trial.[25] This reduces issues from differential health utilisation and provides greater structure for missing data assessment. We assessed whether duration to end of follow-up was defined.

### 5. Outcome

RCT outcomes typically include a response definition at a certain time point, for example, Disease Activity Score - 28 joints (DAS28) <2.6 at week 12. In observational CER, an appropriate outcome definition also needs to be accompanied by assessment time(s), which is why follow-up needs to be clearly prespecified. A common practice for binary response outcomes is to include assessments within a certain window (eg, 12±3 months), but this is less commonly considered for repeated continuous outcomes (eg, use all available follow-up DAS28 scores).

The choice of RCT outcome is often linked to power (sample size) calculations. This is not a component in the target trial emulation framework,[3] but an underpowered observational study can only emulate an underpowered RCT at best. In the majority of observational CER studies, sample size is not a decision. Investigators should estimate whether there is sufficient power to pursue their analysis at the design stage. Underpowered endeavours should be avoided or at least highlighted as a major limitation. Note that post-hoc power calculations, whether in trials or emulations, are not informative.[26 27] We reviewed whether outcomes were clearly defined and whether statistical power limitations were discussed.

### 6. Causal contrast of interest

Researchers should clearly define the answer they want—the intention-to-treat (ITT) or per-protocol effect—before thinking about the data or analysis,[28] in observational CER studies as it is the standard for RCTs. The two estimands require different analyses, have different interpretations and often have different effect sizes. In RCTs, ITT analyses estimate the effect of being assigned to treatment strategies, regardless of what happens thereafter (even if treatment is not initiated). The observational analogue of the ITT effect is the effect of *initiating* the treatment strategies; that is, ITT analysis will include outcomes from patients who remained on and those who discontinued the drug (or deviated from the protocol in any other manner). The per-protocol effect is the effect of the treatment strategy when fully adhered to, hence the importance of clearly defining it. Discontinuing treatment (for whatever reason) may be the only specified 'protocol deviation' in observational CER studies, in which case per-protocol analysis will include only the 'on-treatment' population. We assessed whether the authors defined their causal contrast of interest and what they were.

### 7. Analysis plan

Analyses for the ITT effect in RCTs do not require confounding adjustment and is the direct comparison of the average outcomes of treatment arms. However, in the presence of differential loss-to-follow-up, missing data handling that preserves the original randomised cohort is required for valid ITT analysis (eg, by imputing non-response). By contrast, analysis for the per-protocol effect occurs in a subset of data that artificially censor individuals at the time they deviated from the treatment strategies. Such non-random censoring likely introduces selection bias. Advanced statistical methods using post-baseline time-varying covariates are required to adjust this bias.[3] We reviewed analysis plans together with the declared or implied causal contrast as above.

The analysis plan in the emulation protocol should look identical to the target trial protocol except for the need to adjust for baseline confounding. Treatment strategy, causal contrast and analysis plan are dependent on each other and were reviewed together. Where causal contrasts were not declared, we assessed how censoring was defined in each study (ie, treatment strategies) to infer the authors' implied causal contrast. The chosen statistical method affects how missing data and censoring are handled; we therefore reviewed the statistical model and missing data handling in this section.

## RESULTS

A total of 31 studies met our inclusion criteria. The selection flowchart is shown in online supplementary figure S1. Twenty-one studies compared bDMARDs from two classes[29–49] and 10 compared three or more classes[50–59]; there were no studies of tsDMARDs. Information extracted from each study are detailed in online supplementary table S2. Only one study explicitly emulated a target trial.[45]

### 1. Eligibility criteria

Nineteen out of 31 (61%) studies explicitly included post-baseline information in their eligibility criteria by requiring at least one follow-up[29–42 50–52] and/or a minimal duration of follow-up.[43 49 53] The proportions of participants excluded without follow-up were typically large (up to 69%),[34] but frequently unreported. An additional seven studies[44 47 54–56 58 59] implicitly made these exclusions (up to 82%)[33] by using complete-case analyses.

Ten studies specified an exact number of prior bDMARDs, while 21 studies included varying numbers of prior bDMARD failure. Twelve studies combined bDMARD-experienced patients (ie, ≥1 prior bDMARD failures) or stratified analyses to that effect.[30–37 46 52 53 59] Eligibility criteria of nine studies included both bDMARD naïve and experienced patients, among which five did not stratify to separate treatment effect for these two groups.[40 52 54–56]

### 2. Treatment strategies

Treatments under comparison were well defined, but not treatment *strategies*. Only one study[57] defined whether treatment changes were protocol compliant (eg, biosimilar switch and discontinuation due to remission were permitted). Studies comparing bDMARD monotherapy[37 46 55 56] were unclear whether initiation of csDMARD (although rare in clinical practice) would be artificially censored. Due to limited descriptions of treatment strategies, we instead examined what investigators artificially censored in the analysis to infer what they considered protocol compliant (ie, whether discontinuation was censored; see online supplementary table S2). However, what the authors censored was not always clearly described.

### 3. Assignment procedures

Thirteen of the 31 (42 %) studies used only predefined confounders[31–34 36–38 44–46 50 52 53]; none explicitly cited

literature review or DAGs. Five (16%) studies used only statistical variable selection,[29 30 41–43] such as univariate or stepwise p value-based selection, or change-in-outcome selection. One study included post-baseline variables in the selection process.[29] Nine (29%) studies performed no adjustments for confounding[39 40 43 47 51 54–56 58] (one deliberately excluded baseline values of the outcome).[30] Active-comparator, new-user design was used in all except one study that included both new and prevalent users.[56]

### 4. Follow-up period

Fourteen of 31 (45 %) studies used binary outcomes with clear assessment time points akin to RCTs, thereby defining their end to follow-up.[35 42 45–51 53 54 56–58] Eighteen (58 %) studies included continuous outcomes (eg, DAS28 over time), among which six did not specify an end to the study period.[30–32 40 46 50] This was typically when linear mixed models were used with all available data. One extreme example used the last available follow-up before therapy switch, which could be any time point beyond 1 year.[50]

### 5. Outcome

Outcomes were clearly defined in all studies. Only four studies (two of which adopted RCT design) explicitly performed sample size calculations.[29 31 41 44] There was typically no discussion about power limitations, even when sample sizes were as small as <50 in each arm.[59] Four studies reported a joint outcome,[46 51 56 57] that is, the proportion achieving response *and* remaining on drug. When this was achieved using LUNDEX ('fraction of starters still in the study multiplied by the fraction responding'),[60] statistical comparisons and confidence intervals were not provided.

### 6. Causal contrast of interest

Only six studies defined their causal contrasts prior to describing analyses.[31 43 45 55–57] Inferring from analysis methods, 20 studies included some version of the ITT effect[29 31 33–37 39 41 43 45 46 48–50 52–54 57 59]; all except one[45] excluded patients without follow-up (through eligibility criteria or complete-case analysis) which is not compatible with the traditional ITT definition. Twelve studies declared or implied per-protocol effects in part of their analysis,[30–32 38 40 42 44 51 55–58] but none subsequently adjusted for post-baseline time-varying confounding. Causal contrasts could not be clearly determined in two studies, due to inclusion of prevalent users[56] and lack of clarity on whether discontinuation was defined as non-response.[47]

### 7. Analysis plan

Most studies either used (generalised) linear models for outcomes at a fixed time point,[35 36 41 45 48–50 52 57] or linear mixed models for repeated continuous outcome measures.[30–34 37 42 43 46] One study used generalised estimating equations.[53] Eight studies used pairwise comparisons (eg, t-test, $\chi^2$ test) or analysis of covariance (ANCOVA).[29 39 44 47 54 55 58 59] Three studies did not perform any statistical comparison, two of which due to the use of LUNDEX.[51 56]

Seventeen out of 31 studies used complete-case analyses.[29–33 39 41 43 44 47 49 50 52 54 55 58 59] Linear mixed models can handle missing (at random) data by default. Only three studies used multiple imputation for missing outcome data,[44 48 57] while nine studies used single imputation (eg, last observation carried forward, or non-response imputation) to obtain ITT effects.[34–38 42 45 46 53] It was often unclear whether outcomes of those who discontinued treatment were included or excluded from analysis. Reasons for discontinuation (eg, remission or switch to another bDMARD or biosimilar) were rarely differentiated in treatment strategies, which impacts definition of causal contrast and its analysis. The analyses of 10 studies artificially censored individuals discontinuing the initial bDMARD ('on-treatment' analysis).[31 37 38 40 42 44 51 55 56 58]

## DISCUSSION

We use the target trial emulation framework to identify design limitations in CER studies in RA. There was significant methodological variation despite restricting to a relatively narrow topic with simple designs. One study described the target trial[45] although not to the extent recommended.[3] Most (94%) had at least one design limitation with the potential to introduce bias; the most common were: (1) including post-baseline information in eligibility criteria, (2) not defining the causal contrasts (ie, ITT or per-protocol effects), and (3) inadequate emulation of random assignment with unadjusted comparisons and reliance on statistical confounder selection.

Excluding those without future follow-up in eligibility criteria cannot emulate an RCT. Beyond the conceptual conundrum, this practice can bias results in either direction when loss to follow-up differ across treatment arms and are associated with outcomes.[22] Many complete-case analyses also implicitly exclude those without follow-up. The underlying motive is to deal with missing data. Naïve methods of missing data handling, such as complete-case analysis or single imputation, are not recommended for RCTs.[61 62] This is still more relevant for observational studies where the proportions missing are much higher (the potential for bias increases as the proportion of missing data increases). Larger sample sizes seen in observational CER may instil greater confidence in the wrong result. Common alternative approaches include multiple imputation or likelihood-based methods depending on the pattern of missingness. Defining a follow-up duration and desired outcome assessment times can help assess missing data patterns and mechanisms. Note that the proportion of missing data does not dictate the validity of multiple imputation, rather it is the mechanisms of missingness and amount of information held by auxiliary variables (that inform generation of imputations).[63] Investigators might also attempt to reduce missing data by selecting participants with higher likelihood of follow-up without looking at post-baseline data (analogous to pre-randomisation run-in periods of RCTs).[64] A full discussion on how to handle missing data is beyond the scope of this review. We instead refer readers to Little *et al*[62] for an introduction and Molenberghs *et al* for comprehensive overview.[61 65] If analysis restricted to those with follow-up is unavoidable, comparison of included and excluded individuals should be clearly presented as a minimum. However, this would render causal contrasts difficult to define.

Choice of causal contrasts relates to a similar underlying missing data issue. ITT is appealing for its (perceived) simplicity in both trials and observational CER: analyse outcomes in all those assigned treatment strategies regardless of what happens thereafter (ie, adherence or protocol deviations). Unlike trials, observational data often have significant loss to follow-up. Imputing non-response to all missing cases may result in null ITT effect, even if one treatment is superior. ITT analyses also have limitations when studying harms of treatment and in non-inferiority comparisons.[66] Per-protocol effects have many advantages over ITT effects, but valid estimation can be challenging. One case study against per-protocol effects was the survival

difference between adherers and non-adherers to placebo in a cardiology trial, which could not be removed with simple statistical adjustment.[67] Successful adherence adjustment is possible with modern methods that adjust for post-baseline time-varying prognostic factors.[67] As is recommended for pragmatic trials, observational CER studies should present both ITT and per-protocol effects[66] or, as a minimum, declare the causal contrast before analysis. This was rarely done in the studies reviewed, partly because clear definitions were impossible when post-baseline information was used for eligibility criteria and/or because treatment strategies were not described. Further discussion of causal contrasts and related methods can be found in publications by Hernán and colleagues.[10 19 68]

Another common design issue was the emulation of random assignment in RCTs. Inclusion of prevalent users was uncommon because we reviewed head-to-head (ie, active-comparator) studies, but this practice and consequent time-dependent biases are common in the rheumatology literature (eg, immortal time bias resulting from a period of follow-up during which the study outcome cannot occur).[69] Applying intuition from the target trial protocol can prevent such 'self-inflected injuries'.[19] Selecting an active-comparator with similar indications also has several advantages for confounding adjustment.[20 21] Valid comparison requires individuals to have non-zero probability of receiving either treatment (ie, no absolute contraindications). Statistical adjustment for confounding also requires sufficient overlap in participant characteristics across treatment groups, which will be greater when treatments have similar indications; this is also true for unmeasured confounding (eg, frailty).[4]

Approaches for choosing confounders varied. Statistical approaches to covariate selection (eg, based on statistical associations that are widely used in studies of predictors) should generally be avoided for CER.[7 24] Selection should be based more on subject knowledge and/or literature review, preferably supported by DAGs that make assumptions transparent.[7] This helps avoid including variables that can introduce bias when adjusted, such as mediators (causal intermediate variables that are part of the treatment effect) and colliders (variable causally influenced by ≥2 variables which induces spurious associations if adjusted).[7] Adjusting for covariates that are not prognostic but strongly associate with treatment assignment (possible when selection is purely based on statistical associations with treatment group) does little to reduce bias but increases variance.[70] Declaring a priori confounders also improves transparency, which is another criticism of observational studies.[5] Propensity score methods—although not necessarily superior to traditional multivariable regression—provide an intuitive emulation of randomisation since it separates confounding adjustment from outcome analysis. They also help assess utility of the comparison; for example, analyses should be avoided or cautiously approached when propensity score overlap is poor (ie, violation of the positivity assumption required for valid causal inference).[4]

This review was limited to comparisons of effectiveness, but target trial emulation applies equally to safety and other types of time-to-event outcomes (existing target trial emulation examples are typically applications for the latter). Additional design considerations for studies of safety outcomes, such as infections, are discussed by Solomon *et al*.[71] A central concept in the emulation framework—clear definition of time zero—may seem obvious in the examples reviewed, but the principle is equally relevant for other observational designs such as case–control studies.[72 73] Our restriction in scope left out secondary analyses, which were also fraught with design issues; for example, comparing a bDMARD as first- versus second-line treatment[42 48] (try considering if this can be implemented as an RCT). Post-hoc adherence-adjusted response using the LUNDEX[60] was found in several papers, with the primary aim of accounting for missing outcome data. Assuming non-informative censoring, the LUNDEX estimates the 'proportion of patients who not only remain on a particular therapeutic regimen but also fulfil certain response criteria'.[60] Adherence issues are avoided at the cost of changing the outcome definition. Future work in this area should incorporate more modern approaches to adherence adjustment,[67] in emulation of more rigorous definitions and handling of missing data in RCT literature.[62]

In conclusion, target trial emulation builds on existing good design practices to make robust observational designs intuitive. It ensures that clear and clinically relevant questions are asked, incorporates causal inference principles, and has been shown to better align observational results with actual RCTs. Future CER studies should avoid using post-baseline information to define eligibility, clearly define the causal contrast pursued (ideally presenting both ITT and per-protocol effects) and consider confounding using prior knowledge (preferably using causal diagrams that make assumptions transparent). This framework is beginning to be adopted in the rheumatology literature,[45 74] but further improvement and standardisation of CER methodology are essential as more drugs become available, often without (timely) head-to-head RCTs to compare their effectiveness.

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TRANSLATIONAL SCIENCE

# Implication of the deacetylase sirtuin-1 on synovial angiogenesis and persistence of experimental arthritis

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Preliminary results from this study have been presented at the American College of Rheumatology congress in 2019: Leblond A, *et al*. Activation of the desacetylase sirtuin-1 counteracts the activated and proangiogenic profile of endothelial cells in rheumatoid arthritis and alleviates experimental arthritis. *Arthritis Rheumatol* 2019;71(suppl 10). Abstract no. 53.

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## ABSTRACT

**Objectives** To decipher the phenotype of endothelial cells (ECs) derived from circulating progenitors issued from patients with rheumatoid arthritis (RA).

**Methods** RA and control ECs were compared according to their proliferative capacities, apoptotic profile, response to tumour necrosis factor (TNF)-α stimulation and angiogenic properties. Microarray experiments were performed to identify gene candidates relevant to pathological angiogenesis. Identified candidates were detected by RT-PCR and western blot analysis in ECs and by immunohistochemistry in the synovium. Their functional relevance was then evaluated in vitro after gene invalidation by small interfering RNA and adenoviral gene overexpression, and in vivo in the mouse model of methyl-bovine serum albumin-(mBSA)-induced arthritis.

**Results** RA ECs displayed higher proliferation rate, greater sensitisation to TNF-α and enhanced in vitro and in vivo angiogenic capacities. Microarray analyses identified the NAD-dependent protein deacetylase sirtuin-1 (SIRT1) as a relevant gene candidate. Decreased SIRT1 expression was detected in RA ECs and synovial vessels. Deficient endothelial SIRT1 expression promoted a proliferative, proapoptotic and activated state of ECs through the acetylation of p53 and p65, and lead the development of proangiogenic capacities through the upregulation of the matricellular protein cysteine-rich angiogenic protein-61. Conditional deletion of SIRT1 in ECs delayed the resolution of experimental methyl-bovine serum albumin-(mBSA)-induced arthritis. Conversely, SIRT1 activation reversed the pathological phenotype of RA ECs and alleviates signs of experimental mBSA-induced arthritis.

**Conclusions** These results support a role of SIRT1 in RA and may have therapeutic implications, since targeting angiogenesis, and especially SIRT1, might be used as a complementary therapeutic approach in RA.

## Key messages

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

- Angiogenesis through the activation of endothelial cells (ECs) is a crucial event to promote the development of the pathological synovium in rheumatoid arthritis (RA).

**What does this study add?**

- This work provides the first experimental evidence of a proliferative, activated and proangiogenic profile of RA ECs.
- The deacetylase sirtuin-1 (SIRT1) was identified as a relevant actor involved in all the main pathological features of those cells, and SIRT1 expression is markedly reduced in ECs and synovial vessels of patients with RA.
- Endothelial SIRT1 invalidation reproduces the phenotype of RA ECs and exacerbates experimental arthritis, and these effects were reversed by SIRT1 overexpression.

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

- These results may have direct therapeutic implications, since targeting angiogenesis, and especially SIRT1, might be used as an adjuvant treatment of RA.

## INTRODUCTION

Rheumatoid arthritis (RA) is the most common chronic inflammatory arthritis.[1] The synovium is the primary site of the inflammatory process, which, if untreated, leads to irreversible damages to the adjacent cartilage and bone. One of the most noticeable features of rheumatoid synovitis is the amount of synovial vascularisation, which is critical for synovial proliferation and invasiveness. Increased vascular density in RA results from the pathological activation of angiogenesis and vasculogenesis by secreted mediators of tissue infiltrating inflammatory cells that lead to the unrestrained formation of new blood vessels.[2–5] However, recent evidence suggests a primary involvement of angiogenesis in the initiation of tissue inflammation, prior to infiltration of inflammatory cells.[6] These results add further data to the accumulating evidence on the relevance of endothelial cells (ECs) in the pathophysiology of inflammation.

Formation of new blood vessels consists of several complementary processes including activation, proliferation and migration of ECs. Our group has developed a non-invasive innovative method to obtain culture ECs derived from circulating progenitors, which represent valuable tools to study endothelial biology.[7–10]

To gain insights into the implication of angiogenesis and vasculogenesis in RA, our aims were to i) study the properties of circulating

progenitor-derived ECs issued from patients with RA, ii) decipher gene expression profiles of those cells to identify new potentially relevant angiogenic candidates and iii) study the consequences of angiogenic candidate invalidation/overexpression on EC functional properties and on experimental arthritis.

## PATIENTS AND METHODS

An extended 'Patients and methods' section is available in the online supplementary data.

### Patient samples and synovial tissue

This study involved 29 patients with RA fulfilling the 1987 American College of Rheumatology (ACR) or the 2010 ACR/European League Against Rheumatism classification for RA[11 12] and 18 age-matched and gender-matched controls (online supplementary tables S1 and S2).

### Microarray analysis

Microarray analysis was performed on 18 patients with RA and 11 controls. Affymetrix Microarray technology was used to analyse gene expression levels (Affymetrix GeneChip Human Exon 1.0 ST Arrays). Labelling and microarray processing were performed according to the manufacturer's protocol.[8 9]

### RNA interference assay and adenovirus transduction

ECs were seeded and transfected with deacetylase sirtuin-1 (SIRT1) small interfering (siRNA) (20 nM; Qiagen, Hilden, Germany) or control siRNA (20 nM). Adenovirus amplification (gift from Dr Christophe Lemaire) was performed using the Vivapure AdenoPACK 20 kit (Progen, Heidelberg, Germany).

### SIRT1 activity assay

The activity of SIRT1 from RA and control ECs was tested with a Biomol SIRT1 fluorescence assay kit (AK-555; Biomol, Farmingdale, New York, USA).

### Quantitative RT-PCR, western blot analysis, ELISA, immunohistochemistry, immunofluorescence, flow cytometry

These methods were performed with reagents and standard techniques described in online supplementary data (online supplementary tables S2 and S3).

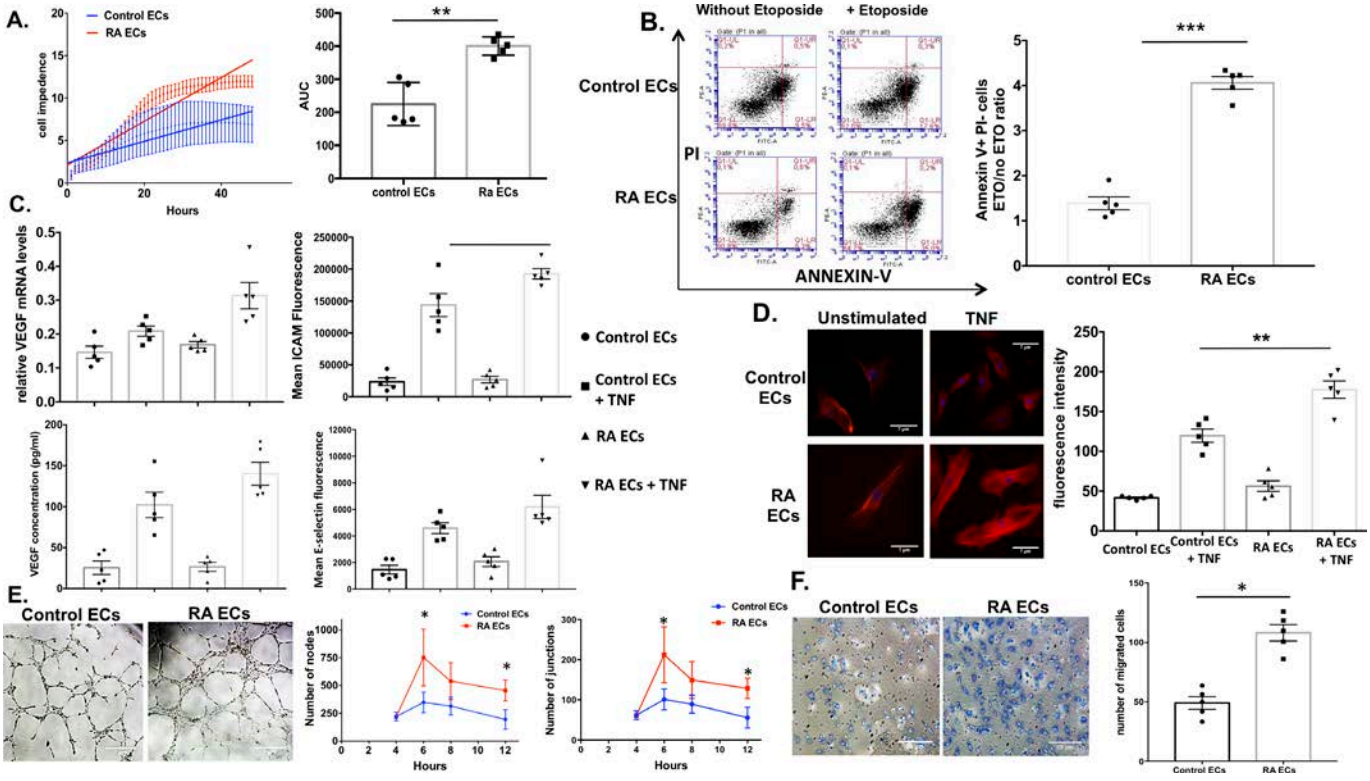


**Figure 1** Functional properties of rheumatoid arthritis (RA) and control endothelial cells (ECs). (A) Cell impedance measured by xCELLigence system in RA and control ECs. Y-axis shows the cell impedance and the area under the curve (AUC) of cell impedance in RA and control ECs. (B) Representative flow cytometry dot plots with double Annexin V-FITC/PI staining for RA and control ECs following etoposide-induced apoptosis (100 µM for 24 hours). Y-axis represents the x-fold change of Annexin V-FITC+/PI– cells after etoposide exposure (100 µM for 24 hours) in RA and control ECs. (C.) Relative vascular endothelial growth factor (VEGF) mRNA levels (qRT-PCR), VEGF concentration in culture cell supernatants (ELISA) and intercellular adhesion molecule (ICAM)-1/E-selectin expression (flow cytometry) in RA and control ECs following tumour necrosis factor (TNF)-α exposition (50 ng/mL for 5 hours). (D) Representative images of stress fibre formation on TNF-α stimulation (50 ng/mL for 5 hours) (scale bar=7 µm). Nuclei are stained with DAPI (blue). Y-axis shows fluorescence intensity quantified by ImageJ. (E) Representative images of tube formation at 6 hours in RA and control ECs (scale bar=7 µm). Y-axis shows the node and junction numbers at 4, 6, 8 and 12 hours. (F) Representative images of cell migration in modified Boyden chamber following VEGF activation (50 ng/mL for 6 hours) in RA and control ECs (scale bar=28 µm); Y-axis shows the number of migrated cells. ECs from five independent patients with RA and five independent controls were used in all experiments. All data are shown as the mean±SEM. *P<0.05, **p<0.01, ***p<0.001 determined by Student's t-test (A, B, E, F) or one-way analysis of variance with Tukey's post hoc test (C, D) for experiments including more than two groups in one experiment. Data are representative of two independent experiments.

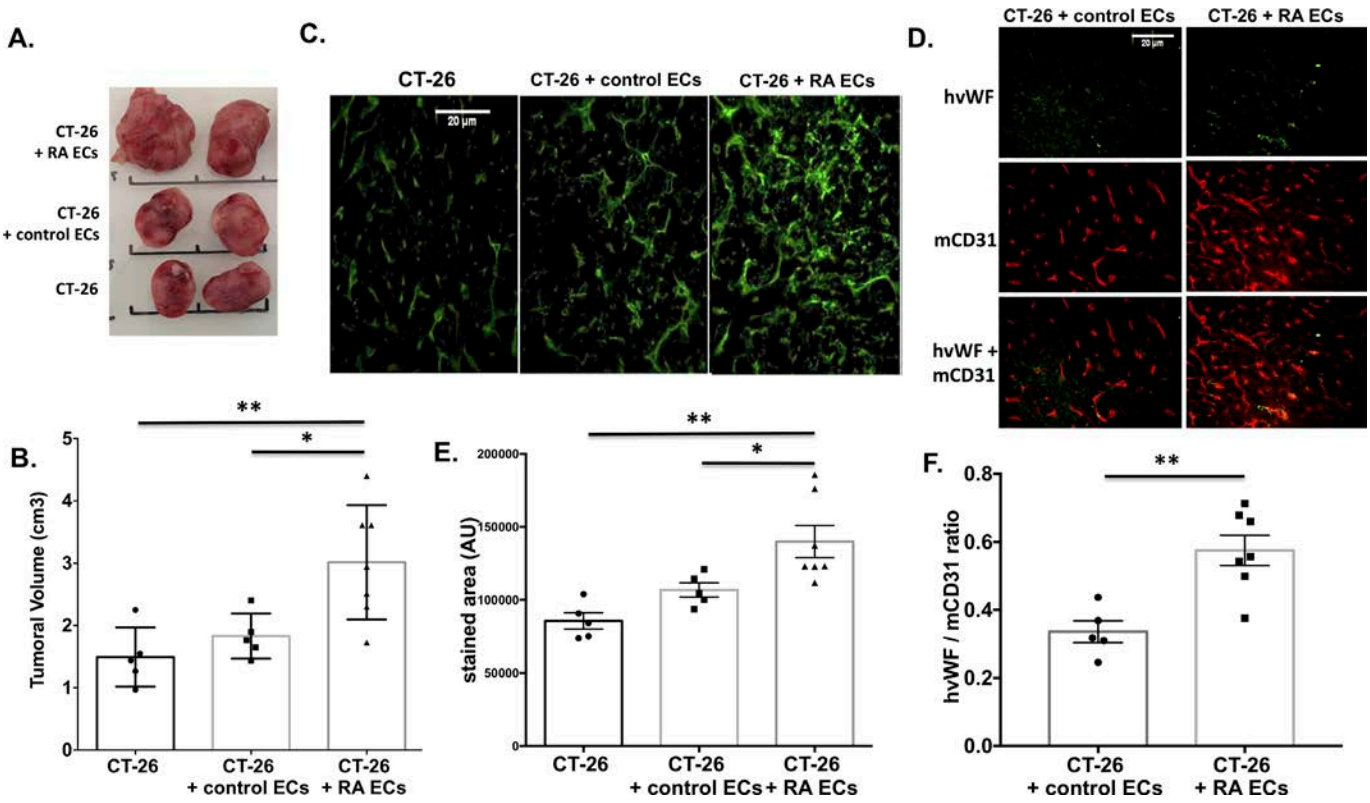


**Figure 2** Size and neovessel density in mice that received transplants of CT-26 cells alone or in combination with control or rheumatoid arthritis (RA) endothelial cells (ECs). (A, B) Representative subcutaneous tumours from mice that received transplants and the volume of tumours that developed. Each data point represents a single mouse (receiving a transplant from a single patient). (C) Representative images of intratumoural vessel density assessed by immunofluorescence for murine CD31 (green) (scale bar=20 µm). (D) Quantification of murine CD31 fluorescence intensity. (E) Representative images of the incorporation of human ECs in mouse vascular structures, assessed by double labelling for human von Willebrand factor (green) and murine CD31 (red) (scale bar=20 µm). (F) Quantification of the number of human von Willebrand factor positive cells reported to the number of murine CD31-positive cells. A total of 17 mice were used: 7 injected with CT26 cells and RA ECs, 5 with CT26 cells and control ECs and 5 with CT-26 cells alone. All data are shown as the mean±SEM of a single experiment. *P<0.05, **p<0.01 determined by Student's t-test (F) or one-way analysis of variance with Tukey's post hoc test (B, D) for experiments including more than two groups in one experiment.

### xCELLigence system

Cell proliferation was monitored using the xCELLigence RTCA MP (ACEA Biosciences, San Diego, California, USA), which measures cell impedance in real and continuous time.

### Angiogenic assays

These assays consisted of tube formation in matrigel matrix and migration in modified Boyden chambers.[7]

### Generation of conditional endothelial SIRT1 KO mice

To generate C57BL/6 mice carrying both the TEK-Cre-ER$^{T2}$ and the SIRT1$^{floxΔE4/floxΔE4}$ alleles, SIRT1$^{floxΔE4/floxΔE4}$ mice were crossed with TEK-Cre-ER$^{T2}$ mice. After two generations, homozygote SIRT1 Flox/Flox mice expressing the Cre recombinase were obtained. Excision of SIRT1 exon 4 was induced by tamoxifen diet (400 mg/kg) (Envigo, Gannat, France) (online supplementary figure S1).

### Mouse model of tumour neovascularisation

Syngeneic murine colon carcinoma CT-26 cells ($2.5\times10^5$ cells) (LGC standards, Molsheim, France) were transplanted subcutaneously into the backs of mice with severe combined immunodeficiency, alone (n=5) or in combination with control ECs ($5\times10^3$ cells) (n=7) or RA ECs ($5\times10^3$ cells) (n=7). The subcutaneous tumours were removed 15 days after tumour transplantation.

### Mouse model of antigen-induced arthritis

This model was induced on a total of 32 mice: 6 SIRT1 $^{Flox/Flox;\ WT/WT}$ mice, 7 SIRT1 $^{Flox/Flox;\ Cre/WT}$ mice and 19 C57BL/6 mice. An active group of 9 C57BL/6 mice received daily intraperitoneal injections of resveratrol (Sigma-Aldrich) (20 mg/kg/day in 100 µL of phosphate-buffered saline (PBS)) and a second control group of 10 C57BL/6 mice received daily intraperitoneal injections of 100 µL of PBS, starting the day of first injection of mBSA until mouse sacrifice.

### Statistics

All analysis was performed with GraphPad Prism 7.0 (GraphPad, San Diego, California, USA). All data are expressed as mean values±SEM. Multiple group comparisons were analysed by one-way analysis of variance with Tukey's post hoc test. Unpaired or paired t-test was used for a two-group comparison. P<0.05 (all two-sided) was considered to be statistically significant.

## RESULTS

### ECs issued from patients with RA display an activated and proangiogenic profile

RA ECs displayed higher proliferation rate compared with control ECs (figure 1A), with a significantly different slope of the curves for each dataset (best-fit values: 0.26±0.01 vs 0.19±0.01, p<0.001) and area under the proliferation curve (figure 1A). RA

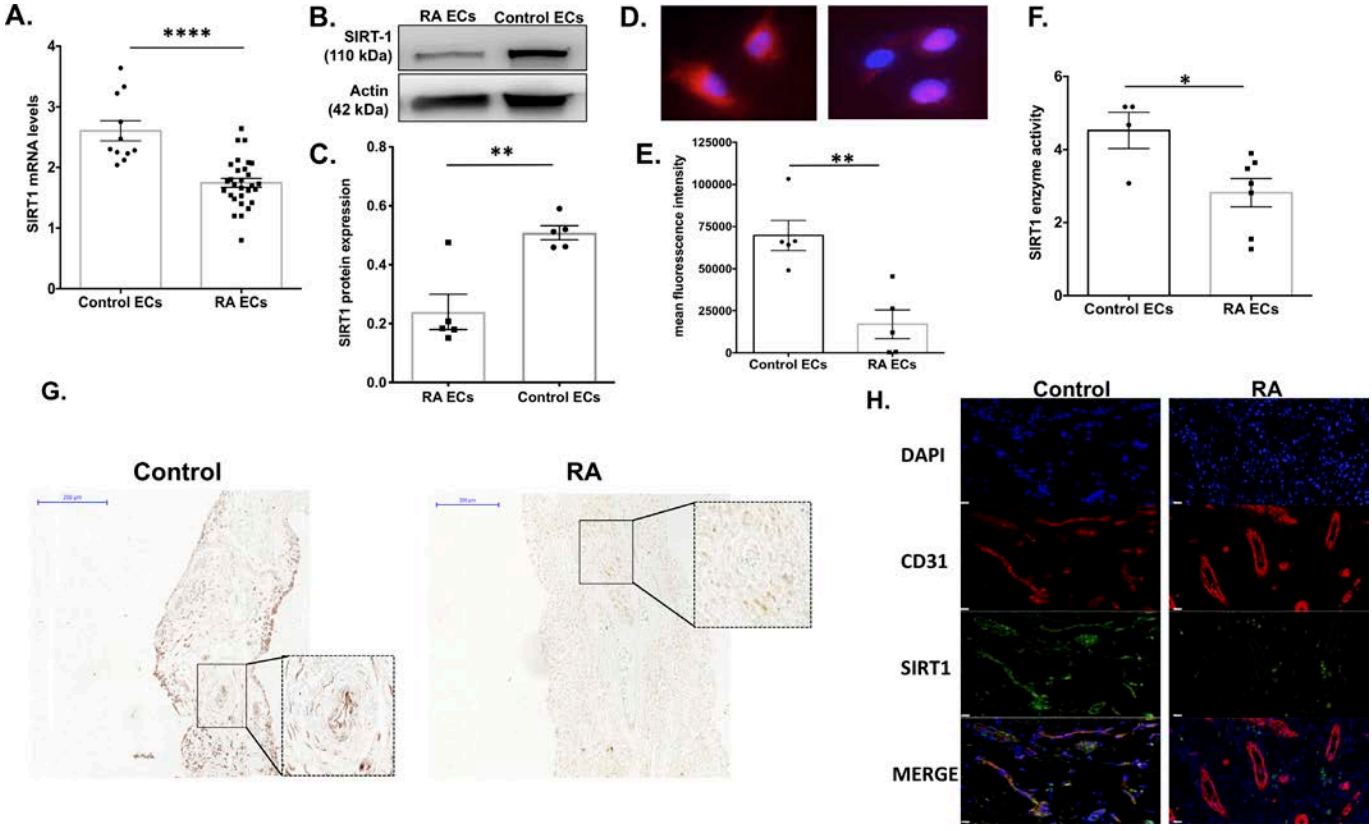


**Figure 3** Cellular and tissular sirtuin-1 (SIRT1) expression. (A) SIRT1 mRNA levels quantified by qRT-PCR in rheumatoid arthritis (RA) (n=29) and control (n=11) endothelial cells (ECs). (B) Cell extracts from cultured RA and control ECs were immunoblotted for SIRT1. (C) Quantification of anti-SIRT1 by western blot analysis. (D) Representative immunofluorescence staining for SIRT1 in RA and control ECs (scale bar=10 µm). Nuclei are stained with DAPI (blue). (E) Quantification of fluorescence intensity with ImageJ. (F) Quantification of SIRT1 activity in RA (n=7) and control (n=4) ECs. (G) Representative immunohistochemistry staining for SIRT1 in lesional synovial tissue issued from a patient with RA and a control (scale bar=200 µm). (H) Representative double labelling by immunofluorescence for SIRT1 (red) and CD31 in the synovial tissue taken from a patient with RA and a control (green). Nuclei are stained with DAPI (blue) (scale bar=20 µm). ECs from five independent patients with RA and five independent controls were used in all experiments, unless stated otherwise. Synovial tissue from five independent patients with RA and three independent controls was used. All data are shown as the mean±SEM of one experiment. *P<0.05, **p<0.01, ****p<0.0001 determined by Student's t-test. Data are representative of two independent experiments.

ECs exhibited a pro-apoptotic profile: the number of Aannexin V+/PI− cells was increased by 2.92-fold in RA compared with controls on exposure to etoposide (p<0.001) (figure 1B). RA ECs presented increased sensitisation to tumour necrosis factor (TNF)-α. On stimulation with rhTNF-α, vascular endothelial growth factor (VEGF) mRNA levels and concentrations released in cell culture supernatants increased in RA ECs compared with control ECs (1.50-fold, p=0.03 and 1.28-fold, p=0.160, respectively) (figure 1C). The expression of adhesion molecules and the formation of stress fibres on TNF-α stimulation were also strikingly more prominent in RA ECs (figure 1D). RA ECs also displayed greater angiogenic properties in vitro, with accelerated tube formation (figure 1E) and increased migration capacities (figure 1F). We next proceeded with the evaluation of proangiogenic capacities of RA ECs in experimental neoangiogenesis using the mouse model of tumour neovascularisation. When CT-26 cells were used transplanted with RA ECs, tumour growth was markedly stronger versus when they were transplanted with control ECs (mean±SD, $3.02\pm0.92\,cm^3$ vs $1.83\pm0.36\,cm^3$; p=0.005) (figure 2A–B). Neovessel density was significantly increased in tumours that developed when CT-26 cells were transplanted with RA ECs, as compared with those transplanted with control ECs (figure 2C–D), supporting the greater in vivo capacity of these cells to promote neovascularisation. We observed a correlation between neovessel density and tumour size (r=0.87, p<0.001), suggesting that neovessel formation is a potent contributor to tumour growth. In addition, a significantly higher proportion of transplanted human RA ECs expressing the human-specific mature-EC marker von Willebrand factor had been incorporated into the endothelium compared with control ECs (figure 2E–F).

### Decreased cellular and tissular expression and activity of the NAD-dependent deacetylase sirtuin-1 in RA

We next compared gene expression profiles of unstimulated RA and control ECs. Unsupervised analyses by hierarchical clustering allowed a correct segregation between patients with RA and controls (online supplementary figure S2A). Volcano plot illustrated fold-differences in individual gene expression and associated p values (negative log10) (online supplementary figure S2B). Supervised analyses identified 879 differentially expressed genes, with a significant enrichment in functional groups related to cell cycle (94 genes), cell death and survival (205 genes), cellular growth and proliferation (143 genes) and cell morphology (143 genes). A list of top genes and their upstream regulators, chosen according resulting p values (<0.05), fold change and biological relevance (online supplementary table S4), were then entered

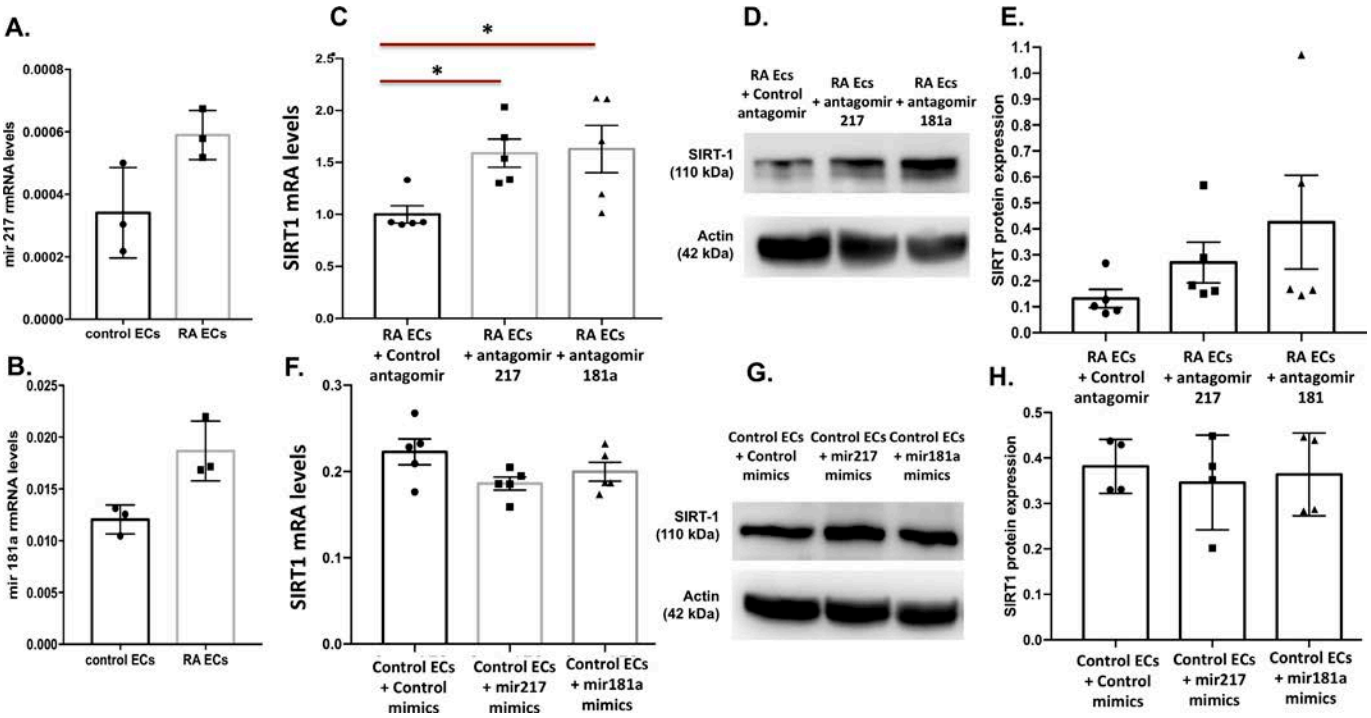


**Figure 4** Effects of miR-217 and miR-181a modulation on sirtuin-1 (SIRT1) expression in rheumatoid arthritis (RA) and control endothelial cells (ECs). (A, B) mRNA levels of miR-217 (A) and miR-181a (B) quantified by qRT-PCR in RA and control ECs (n=3 each). (C) SIRT1 mRNA levels quantified by qRT-PCR in RA ECs transfected with control antagomiR, antagomiR-217 or antagomiR-181a (n=5 each). (D) Cell extracts from cultured RA ECs transfected with control antagomiR, antagomiR-217 or antagomiR-181a (n=5 each) were immunoblotted for SIRT1. (E) Quantification of anti-SIRT1 by western blot analysis. (F) SIRT1 mRNA levels quantified by qRT-PCR in control ECs transfected with control mimics, miR-217 mimics or miR-181a mimics (n=5 each). (G) Cell extracts from cultured control ECs transfected with control mimics, miR-217 mimics or miR-181a mimics (n=5 each) were immunoblotted for SIRT1. (H) Quantification of anti-SIRT1 by western blot analysis. *P<0.05 determined by one-way analysis of variance with Tukey's post hoc test. Data are representative of two independent experiments.

into the biological database STRING to construct a functional protein association network. This analysis revealed an interaction network centred on the NAD-dependent SIRT1 (online supplementary figure S2C), implicated in cell proliferation and survival, inflammation and angiogenesis. SIRT1 mRNA and protein levels were decreased by 33% (p<0.001) and 53% (p=0.003), respectively (figure 3A–C). Immunocytofluorescence showed that SIRT1 expression was localised to the cytoplasm (figure 3D–E). Together with reduced expression, SIRT1 deacetylase activity was significantly decreased by 38% in RA ECs (p=0.039) (figure 3F) and the acetylation of the SIRT1-regulated transcription factors p53 and p65 was increased in RA ECs (online supplementary figure S3A-D). Epigenetic modifications by microRNAs are an important mechanism of SIRT1 expression and activity regulation.[13–15] The expression of miR-217 and miR-181a were increased in RA ECs (figure 4A–B). Moreover, SIRT1 expression was restored in RA ECs on transfection with antagomiR-217 and 181a (figure 4C–E). Conversely, SIRT1 levels did not markedly diminish in control ECs transfected with miR-217 or miR-181a mimics (figure 4F–H). Finally, SIRT1 expression was reduced in the synovial tissue of patients with RA (figure 3G). Double labelling with SIRT1 and CD31 revealed a markedly reduced SIRT1 expression in synovial vessels (figure 3H).

### SIRT1 silencing enhances control EC turnover, activation and proangiogenic properties

To assess whether decreased SIRT1 expression may contribute to the pathological profile of ECs, we transfected control ECs with SIRT1 siRNA (online supplementary figure S4A-B).

### SIRT1 silencing promotes control EC proliferation and mediates apoptosis

The proliferation rate of SIRT1 siRNA-transfected control ECs was significantly higher than mock-transfected cells (slope analysis with best-fit values: 0.14±0.01 vs 0.12±0.01, p=0.044) (figure 5A). Together with increased cell proliferation, SIRT1 invalidation was also associated with increased EC apoptosis. On etoposide exposure, the number of apoptotic Annexin V+/PI− cells increased by 1.84-fold in SIRT1 siRNA-transfected cells (p=0.038) (figure 5B).

### SIRT1 silencing leads to increased sensitisation of control ECs to TNF-α

On TNF-α stimulation, VEGF mRNA levels increased by 1.7-fold (p=0.009) in control ECs, and the release of VEGF in culture cell supernatants increased by 3.4-fold (p=0.021) (figure 5C). Transfection with SIRT1 siRNA strikingly enhanced the effects of TNF-α on VEGF synthesis at the mRNA and protein levels (figure 5C). Consistent with this finding, SIRT1 knockdown conducted to a more pronounced TNF-α-dependent expression of the adhesion molecules intercellular adhesion molecule-1 and E-selectin (figure 5C) and to the stimulation of actin stress fibre formation (figure 5D).

### SIRT1 silencing amplifies control EC proangiogenic properties

Transfection of control ECs with SIRT1 siRNA led to accelerated tube formation (figure 5E) and greater migration capacities on VEGF stimulation (figure 5F).

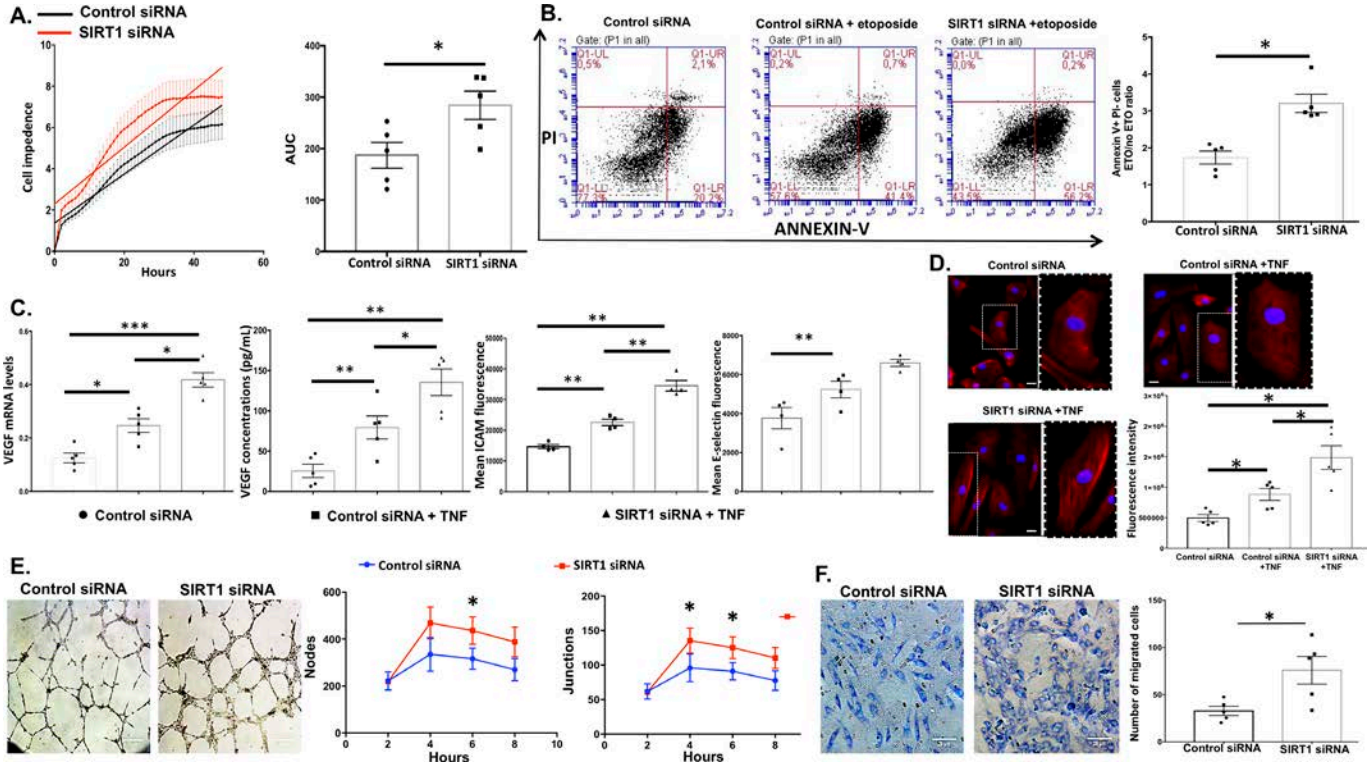


**Figure 5** Effects of sirtuin-1 (SIRT1) inhibition on cell proliferation, survival, activation and angiogenic properties in control endothelial cells (ECs) transfected with SIRT1 small interfering (siRNA). (A) Cell impedance measured by xCELLigence system. Y-axis shows the area under the curve of cell impedance in mock-transfected and SIRT1-transfected control ECs. (B) Representative flow cytometry dot plots with double Annexin V-FITC/PI staining for mock-transfected and SIRT1-transfected control ECs following etoposide-induced apoptosis (100 µM for 24 hours). Y-axis represents the x-fold change of Annexin V-FITC+/PI– cells after etoposide exposure (100 µM for 24 hours) in mock-transfected and SIRT1-transfected control ECs. (C) Relative vascular endothelial growth factor (VEGF) mRNA levels (qRT-PCR), VEGF concentration in culture cell supernatants (ELISA) in mock-transfected and SIRT1-transfected control ECs following tumour necrosis factor (TNF)-α exposition (50 ng/mL for 5 hours). Intercellular adhesion molecule (ICAM)-1 and E-selectin expression assessed by flow cytometry in mock-transfected (n=4) and SIRT1-transfected (n=4) control ECs following TNF-α exposition (50 ng/mL for 5 hours). (D) Representative images of stress fibre formation on TNF-α stimulation (50 ng/mL for 5 hours) (scale bar=20 µm). Nuclei are stained with DAPI (blue). Y-axis shows the fluorescence intensity quantified by ImageJ. (E) Representative images of tube formation at 6 hours in mock-transfected and SIRT1-transfected control ECs (scale bar=70 µm). Y-axis shows the node and junction numbers at 2, 4, 6 and 8 hours. (F) Representative images of cell migration in modified Boyden chamber following VEGF activation (50 ng/mL for 6 hours) in mock-transfected and SIRT1-transfected control ECs (scale bar=28 µm); Y-axis shows the number of migrated cells. ECs from five independent patients with RA and five independent controls were used in all experiments, unless stated otherwise. All data are shown as the mean±SEM. *P<0.05, **p<0.01, ***p<0.001 determined by Student's t-test (A, B, E, F) or one-way analysis of variance with Tukey's post hoc test (C, D) for experiments including more than two groups in one experiment. Data are representative of two independent experiments.

### Mechanism of action of SIRT1 in ECs

Given that p53 is required for etoposide-induced apoptosis in different cell types,[16] we aimed to determine whether the effects of SIRT1 knockdown on EC apoptosis were mediated by increased p53 acetylation. As expected, etoposide exposure led to increased acetylated (Ac)-p53/total p53 ratio in mock-transfected ECs. The transfection of control ECs with SIRT1 siRNA significantly enhanced p53 acetylation (figure 6A).

Since SIRT1 physically interacts with the p65 subunit of nuclear factor kappa B (NF-κB) and inhibits transcription by deacetylating RelA/p65 at lysine 310,[17] we aimed to determine whether the greater sensitisation of ECs invalidated for SIRT1 to TNF-α might be related to increased p65 acetylation. As expected, treatment with TNF-α stimulated the expression of total p65 in EC nuclear extracts (figure 6B). Transfection of TNF-α-stimulated control ECs with SIRT1 siRNA did not modify the expression of total p65, but led to a significant 4.9-fold increase in the Ac-p65/total p65 ratio (figure 6B).

The matricellular protein cysteine-rich angiogenic protein 61 (CYR61) is a strong regulator of angiogenesis, whose expression is regulated by SIRT1 in synovial and dermal fibroblasts.[18–20] In RA ECs, reduced expression of SIRT1 was associated with increased CYR61 expression. Indeed, The mRNA and protein expression of CYR61 were markedly increased in RA ECs (figure 6C–E) and CYR61 concentrations measured in EC culture supernatants were significantly higher in patients with RA compared with controls (figure 6F). Moreover, the transfection of control ECs with SIRT1 siRNA was associated with a significant increase of CYR61 protein (figure 6G).

### Upregulation of SIRT1 reverses the proliferative, activated and proangiogenic profile of RA ECs

We next aimed to evaluate whether the restoration SIRT1 expression and enzyme activity would reverse the pathological profile of RA ECs (online supplementary figure S4C-D). The restoration of SIRT1 expression in RA ECs using adenovirus transduction conducted to a significant reduction of their proliferation rate compared with mock-transduced cells (slope analysis with best-fit values: 0.29±0.01 vs 0.26±0.01, p=0.004)

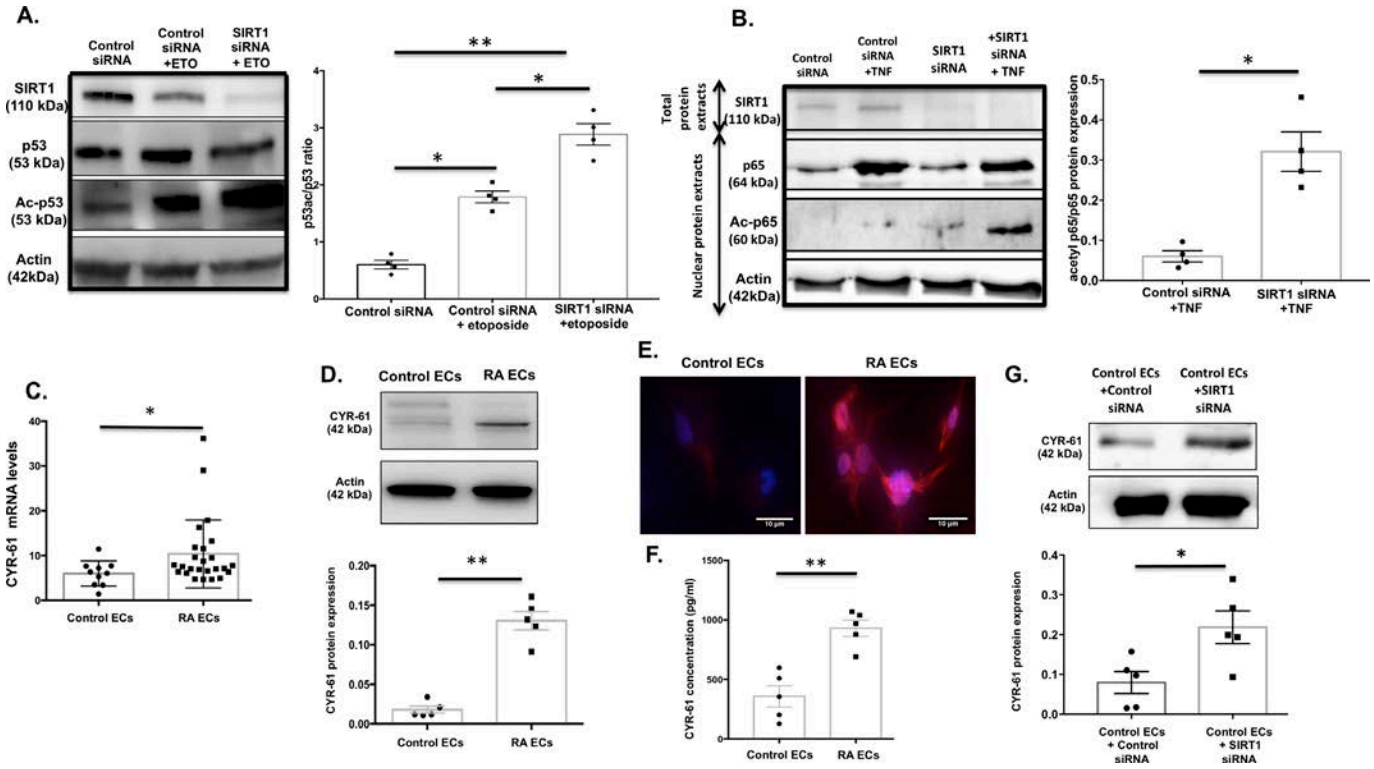


**Figure 6** Mechanism of action of sirtuin-1 (SIRT1) in control endothelial cells (ECs). (A) Cell extracts from mock-transfected and SIRT1-transfected control ECs were immunoblotted for acetylated (Ac)-p53 and p53 after etoposide exposure (100 µM for 24 hours). Y-axis represents the Ac-p53/p53 ratio after etoposide exposure (100 µM for 24 hours) in mock-transfected (n=4) and SIRT1-transfected (n=4) control ECs. (B) Cell extracts from mock-transfected and SIRT1-transfected control ECs were immunoblotted for Ac-p65 and p65 following tumour necrosis factor (TNF)-α stimulation (50 ng/mL for 5 hours). Y-axis shows the Ac-p65/p65 ratio on TNF-α stimulation (50 ng/mL for 5 hours) in mock-transfected (n=4) and SIRT1-transfected (n=4) control ECs. (C) Relative CYR61 mRNA levels quantified by qRT-PCR in RA ECs (n=25) and control ECs (n=9). (D) Cell extracts from cultured RA and control ECs were immunoblotted for CYR61. Y-axis shows the quantification of anti-CYR61 by western blot analysis. (E) Representative immunofluorescence staining for CYR61 in RA and control ECs (scale bar=10 µm). Nuclei are stained with DAPI (blue). (F) Quantification of fluorescence intensity with ImageJ. (G) Cell extracts from mock-transfected and SIRT1-transfected control ECs were immunoblotted for CYR61. Y-axis shows the quantification of anti-CYR61 by western blot analysis. ECs from five independent patients with RA and five independent controls were used in all experiments, unless stated otherwise. All data are shown as the mean±SEM. *P<0.05, **p<0.01 determined by Student's t-test (B, C, D, F, G) or one-way analysis of variance with Tukey's post hoc test (A) for experiments including more than two groups in one experiment. Data are representative of three independent experiments.

(figure 7A). Apoptosis of RA ECs was also significantly reduced on adenoviral SIRT1 overexpression, with a 40% decrease of the number of Annexin V+/PI− cells on exposure to etoposide (p=0.010) (figure 7B).

SIRT1 overexpression alleviated TNF-α-induced activation of RA ECs: RA ECs transduced with SIRT1 adenovirus displayed a significant reduction of VEGF mRNA expression, VEGF release in culture supernatants (44%, p=0.011), adhesion molecule expression (figure 7C) and stress fibre formation (figure 7D). Adenoviral overexpression of SIRT1 in RA ECs also reversed the proangiogenic properties of RA ECs (figure 7E–F).

## In vivo modulation of SIRT in experimental arthritis

### SIRT1 conditional deletion in ECs increases angiogenesis and delays the resolution of experimental arthritis

Conditional endothelial deletion of SIRT1 did not have major effects on the initiation phase of arthritis or in the maximal intensity of arthritis observed at days 2 and 3, but led to longer persistence of arthritis (figure 8A–B). Indeed, mice with conditional invalidation of SIRT1 in ECs showed persistent signs of arthritis at day 7, whereas their wild-type littermates had an almost complete regression of arthritis (figure 8A–D). Histological analysis performed at day 7 showed more synovial alteration and pannus formation in the paw of mice invalidated for SIRT1 in ECs (figure 8E–F). These mice also displayed a striking increase of synovial vessel density (figure 8G,H). Conditional endothelial SIRT1 invalidation was also associated with a substantial increase of Ac-p53 (figure 8G–I), illustrating the loss of SIRT1 activity in the target tissue, and CYR61 expression in synovial vessels (figure 8G–J).

### Upregulation of SIRT1 alleviates experimental arthritis

Resveratrol exerted anti-inflammatory effects during the initiation phase of arthritis and significantly reduced the maximal intensity of arthritis observed at day 2 (online supplementary figure S5A-D). Histological semi-quantitative score performed at day 6 was markedly reduced in the paw of resveratrol-treated mice (online supplementary figure S5E-F).

As expected, resveratrol led to the activation of SIRT1 activity, characterised by a striking reduction of p53 acetylation by 57% in the lesional synovial tissue (online supplementary figure S6A-C).

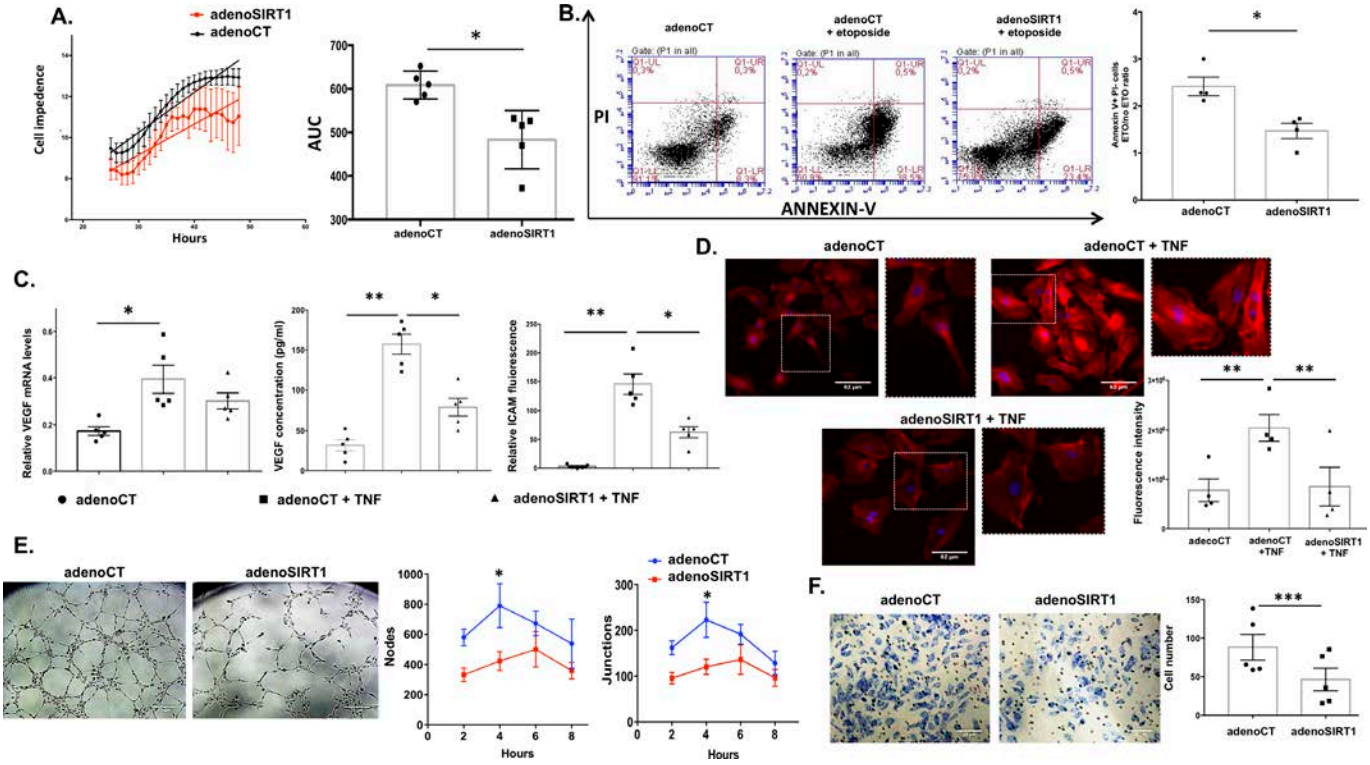


**Figure 7** Effect of sirtuin-1 (SIRT1) activation on functional properties in rheumatoid arthritis (RA) endothelial cells (ECs). (A) Cell impedance measured by xCELLigence system in SIRT1-overexpressing RA ECs and mock-transduced RA ECs. Control and SIRT1 adenovirus (adenoCT and adenoSIRT1, respectively) has been added at H24. Y-axis shows the area under the curve of cell impedance. (B) Representative flow cytometry dot plots with double Annexin V-FITC/PI staining following etoposide-induced apoptosis (100 µM for 24 hours). Y-axis shows the x-fold change of Annexin V-FITC+/PI− cells after etoposide exposure (100 µM for 24 hours) in SIRT1-overexpressing RA ECs (n=4) and mock-transduced RA ECs (n=4). (C) Relative vascular endothelial growth factor (VEGF) mRNA levels (qRT-PCR), VEGF concentration in culture cell supernatants (ELISA) and intercellular adhesion molecule (ICAM)-1 expression (flow cytometry) in SIRT1-overexpressing RA ECs and mock-transduced RA ECs following tumour necrosis factor (TNF)-α exposition (50 ng/mL for 5 hours). (D) Representative images of stress fibre formation on TNF-α stimulation (50 ng/mL for 5 hours) in SIRT1-overexpressing RA ECs (n=4) and mock-transduced RA ECs (n=4) (scale bar=62 µm). Nuclei are stained with DAPI (blue). Y-axis shows the fluorescence intensity quantified by ImageJ. (E) Representative images of tube formation at 4 hours in SIRT1-overexpressing RA ECs and mock-transduced RA ECs (scale bar=70 µm). Y-axis shows the analysis of node and junction numbers at 2, 4, 6 and 8 hours. (F) Representative images of cell migration in modified Boyden chamber following vascular endothelial growth factor (VEGF) activation (50 ng/mL for 6 hours) in SIRT1-overexpressing RA ECs and mock-transduced RA ECs (scale bar=28 µm). Y-axis shows the analysis of the number of migrated cells. ECs from five independent patients with RA and five independent controls were used in all experiments, unless stated otherwise. All data are shown as the mean±SEM. *P<0.05, **p<0.01, ***p<0.001 determined by Student's t-test (A, B, E, F) or one-way analysis of variance with Tukey's post hoc test (C, D) for experiments including more than two groups in one experiment. Data are representative of two independent experiments.

## DISCUSSION

Our results provide the experimental evidence of a major role of ECs derived from circulating progenitors in RA. Moreover, we identified in SIRT1 a relevant actor involved in all the main pathological features of those cells.

Although isolated from peripheral blood, and not directly from the synovium, ECs derived from circulating progenitors may be directly implicated in RA pathogenesis. Cells expressing progenitor markers have been detected in RA synovial tissue,[21] supporting their homing in the pathological synovium. Moreover, early passage progenitor-derived RA ECs display in vitro a proliferative, activated and proangiogenic profile, possibly triggered by RA local and systemic inflammatory microenvironment.[22] The number of circulating endothelial progenitor cell also correlate with RA disease activity indices.[23]

Gene expression analysis of RA ECs revealed a high number of differentially expressed genes involved in tumourigenesis. Moreover, SIRT1 was identified through a network constituted of highly differentially expressed genes implicated in cancer processes, possibly related to chronic exposition to metabolic stress signals, including hypoxia and inflammatory cytokines.[24] SIRT1 is a NAD-dependent protein deacetylase that links transcriptional regulation to a variety of metabolic signals.[25] Decreased endothelial SIRT1 expression is consistent with SIRT1 underexpression previously reported in RA fibroblast-like synoviocytes and peripheral blood mononuclear cells.[24 26] Together with decreased SIRT1 expression, diminished lysyl deacetylase activity was detected in RA ECs. This was not related to decreased substrate availability, given the increased p53 and p65 acetylation in RA ECs, but rather to post-translational modifications. Indeed, miR-217 and miR-181a were able to specifically target SIRT1 in RA ECs.[27] miR-181a is known to inhibit SIRT1 expression by directly binding to the 3' untranslated region of SIRT1 mRNA and miR-217 was shown to be important in senescence by inhibiting SIRT1, reducing nitric oxide availability and deacetylating Forkhead Box O1.[13]

SIRT1 silencing in control ECs reproduced the proliferative, pro-apoptotic, activated and proangiogenic profile of RA ECs (online supplementary figure S7), and these effects were reversed by adenoviral SIRT1 overexpression. The regulation of cell

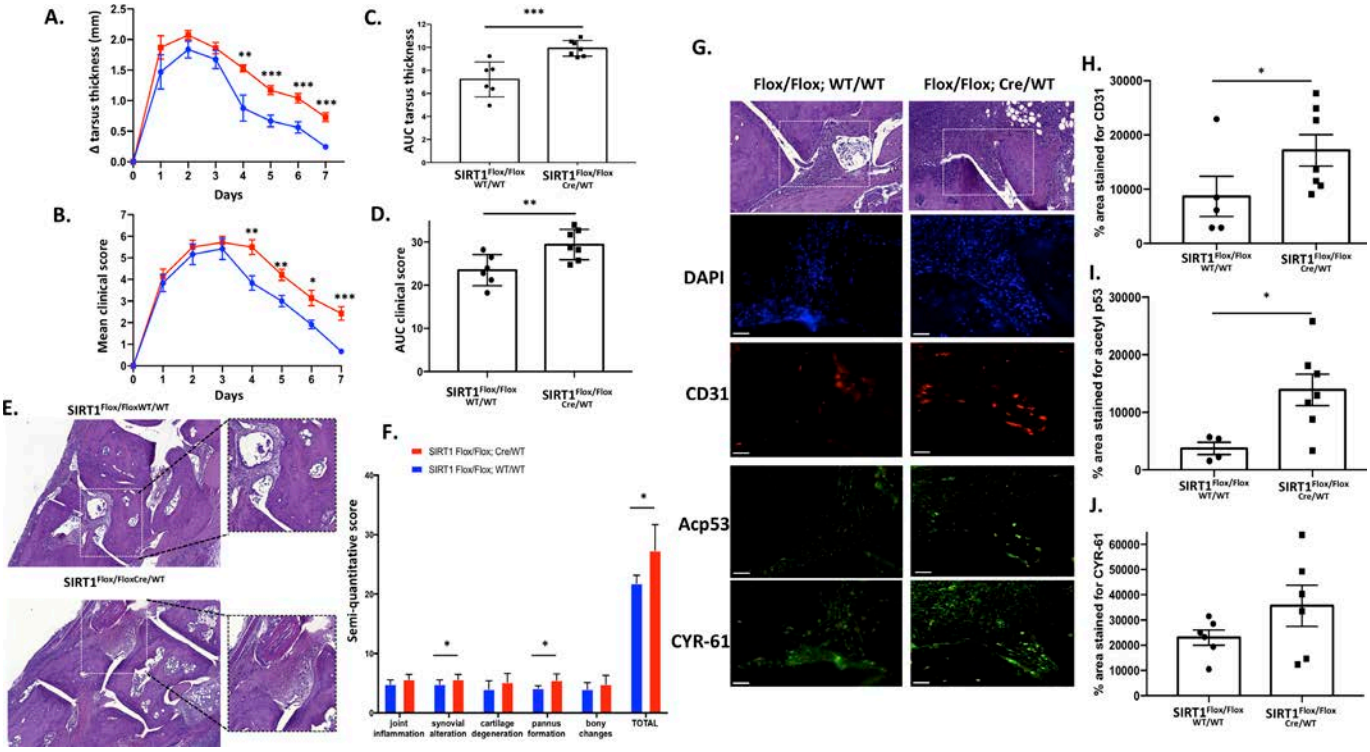


**Figure 8** Effect of sirtuin-1 (SIRT1) endothelial invalidation on experimental arthritis. (A–D) Methyl-bovine serum albumin-(mBSA)-induced arthritis in littermates of SIRT1 $^{Flox/Flox;WT/WT}$ mice (n=6) and SIRT1 $^{Flox/Flox;Cre/WT}$ mice (n=7); Y-axis shows tarsus thickness (A) and clinical score (B), as well as the area under the curve (AUC) of tarsus thickness (C) and the clinical score (D). (E) Sections of ankle and tarsus joints from SIRT1$^{Flox/Flox;WT/WT}$ mice and SIRT1$^{Flox/Flox;Cre/WT}$ mice stained with H&E at day 7 of arthritis (scale bar=100 µm). (F) Histomorphometric analysis of the area of synovitis and bone destruction. (G) Sections of ankle and tarsus joints from SIRT1$^{Flox/Flox;WT/WT}$ mice and SIRT1$^{Flox/Flox;Cre/WT}$ mice stained by immunofluorescence for CD31, acetylated (Ac)-p53 and CYR61 (scale bar=50 µm). Nuclei are stained with DAPI (blue). (H–J) Quantification of CD31 (H), Ac-p53 (I) and CYR61 (J) fluorescence intensity with ImageJ. All data are shown as the mean±SEM. *P<0.05, **p<0.01, ***p<0.001 determined by Student's t-test. Data are representative of a single (A–F) or two independent experiments (G–J).

proliferation is a key downstream effect of SIRT1, with various cell type-specific effects.[28–31] Invalidation of SIRT1 was associated with increased EC apoptosis mediated by the upregulation of p53 acetylation, which is indispensable for p53 transcriptional activity.[32] SIRT1 effects on apoptosis remain elusive,[33 34] and discrepancies may be related to SIRT1 subcellular localisation.[35 36]

Endothelial SIRT1 invalidation markedly increased EC sensitisation to the proinflammatory cytokine TNF-α, through the acetylation of the NF-κB family protein p65. NF-κB is constitutively activated in RA and maintains a damaging phenotype of several cell types in RA.[37] The transcriptional activity of p65 could be further regulated by phosphorylation and acetylation. Endothelial invalidation of SIRT1 did not inhibit the induction of p65 by TNF-α, but was associated with increased p65 acetylation, which is required for p65 full transcriptional activity,[38] and led to amplification of TNF-α-induced response and EC activation. Thus, SIRT1 may serve as a regulator of the NF-κB pathway in ECs, coordinating multiple downstream signals that may interact to reduce synovial inflammation.

Invalidation of SIRT1 in control ECs was also associated with increased proangiogenic properties and increased expression of the matricellular protein CYR61, which is essential for the control of angiogenesis. Moreover, CYR61 was upregulated in RA ECs and arthritic mice with conditional endothelial invalidation of SIRT1 displayed increased vessel density and higher CYR61 expression. In line with our results, inhibition of SIRT1/FoxO3a signalling has been shown to be crucial to induce CYR61 in RA synovial fibroblasts, since forced SIRT1 expression in RA synovial fibroblasts led to decreased CYR61 levels.[18] Interestingly, serum CYR61 levels were significantly increased in patients with RA and its concentrations were inversely correlated with RA disease activity and upregulated in those therapeutic responders.[39]

Recent evidence has suggested primary involvement of angiogenesis in the initiation of tissue inflammation prior to infiltration of inflammatory cells. Indeed, angiogenesis may precede leucocyte infiltration during inflammation in experimental models of inflammatory diseases.[6] However, the effect of increased angiogenesis through conditional endothelial invalidation of SIRT1 did not modify the initial phase of arthritis, but resulted in a longer resolution phase of experimental arthritis. This is likely due to the features of mBSA model, in which the initial phase of arthritis is characterised by a potent inflammatory response even in control mice, which may have masked an early effect of SIRT1 invalidation. SIRT1 activation by Resveratrol reduced the maximal intensity of arthritis in the initial phase experimental arthritis, as previously described in complementary preclinical models,[40–42] which may have direct therapeutic implications. Indeed, targeting angiogenesis, and especially SIRT1, might be used as a complementary therapeutic approach in RA.

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CLINICAL SCIENCE

# Ultrasound erosions in the feet best predict progression to inflammatory arthritis in anti-CCP positive at-risk individuals without clinical synovitis

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## ABSTRACT

**Objectives** To investigate, in anti-cyclic citrullinated peptide antibody positive (CCP+) at-risk individuals without clinical synovitis, the prevalence and distribution of ultrasound (US) bone erosions (BE), their correlation with subclinical synovitis and their association with the development of inflammatory arthritis (IA).

**Methods** Baseline US scans of 419 CCP+ at-risk individuals were analysed. BE were evaluated in the classical sites for rheumatoid arthritis damage: the second and fifth metacarpophalangeal (MCP2 and MCP5) joints, and the fifth metatarsophalangeal (MTP5) joints. US synovitis was defined as synovial hypertrophy (SH) ≥2 or SH ≥1+power Doppler signal ≥1. Subjects with ≥1 follow-up visit were included in the progression analysis (n=400).

**Results** BE were found in ≥1 joint in 41/419 subjects (9.8%), and in 55/2514 joints (2.2%). The prevalence of BE was significantly higher in the MTP5 joints than in the MCP joints (p<0.01). A significant correlation between BE and US synovitis in the MTP5 joints was detected (Cramer's V=0.37, p<0.01). The OR for the development of IA (ever) was highest for the following: BE in >1 joint 10.6 (95% CI 1.9 to 60.4, p<0.01) and BE and synovitis in ≥1 MTP5 joint 5.1 (95% CI 1.4 to 18.9, p=0.02). In high titre CCP+ at-risk individuals, with positive rheumatoid factor and BE in ≥1 joint, the OR increased to 16.9 (95% CI 2.1–132.8, p<0.01).

**Conclusions** In CCP+ at-risk individuals, BE in the feet appear to precede the onset of clinical synovitis. BE in >1 joint, and BE in combination with US synovitis in the MTP5 joints, are the most predictive for the development of clinical arthritis.

## Key messages

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

- Very few studies have demonstrated the potential role of ultrasound (US) for the prediction of clinical arthritis in individuals at-risk of rheumatoid arthritis (RA).
- These studies have focused on subclinical synovitis, rather than the role of bone erosions.

**What does this study add?**

- Our study provides new insights into the prevalence, pattern and relationship with subclinical synovitis of US-detected bone erosions in anti-cyclic citrullinated peptide antibody positive (CCP+) individuals without clinical synovitis.
- The detection of US bone erosions in the classic sites for RA damage, especially in the fifth metatarsophalangeal (MTP5) joints, significantly improves prediction of inflammatory arthritis in CCP+ at-risk individuals.

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

- In CCP+ at-risk individuals without clinical synovitis, the detection of bone erosions on US, especially at the MTP5 joints, may improve risk-stratification and therefore inform management of these individuals.

## INTRODUCTION

Bone erosions are cardinal features of rheumatoid arthritis (RA) and their central role in the pathogenesis, diagnosis and prognosis of the disease is widely recognised.[1 2] They have traditionally been considered as late-stage lesions, developing as a consequence of persistent synovitis. However, several studies have showed that bone erosions might occur very early in the course of RA.[3] Moreover, recent studies have demonstrated that bone loss can occur in the preclinical phases of the disease,[4] and long before the onset of clinical synovitis in some subjects with positive anti-cyclic citrullinated peptide (CCP+) antibodies (Ab).[5]

Bone erosions represent joint damage in RA, and as such are important biomarkers for disease severity. Indeed, their presence has been associated with poor functional outcome and irreversible loss of function.[6 7] Since most patients with RA develop bone erosions within 12–24 months of disease onset (some patients a few weeks after disease onset), their early detection and recognition is of critical importance for guiding management,[8 9] with potential implications for treatment approaches aimed at preventing further joint damage and disability.[10]

Conventional radiography remains the imaging tool most commonly used for the detection of bone erosions in RA.[11] However, in recent years, the use of musculoskeletal ultrasound (US) in the assessment of patients with RA has increased significantly.[12] US has been shown to be more

sensitive than conventional radiography for the detection of bone erosions, especially in the early phase of the disease.[13 14]

While the central role of bone erosions in patients with RA is widely recognised, their prevalence, pattern and relationship with subclinical synovitis in individuals at-risk of RA (eg, anti-CCP+ with musculoskeletal symptoms but without clinical arthritis) is not well understood. To the best of our knowledge, among the few studies that have evaluated the role of US in individuals at-risk of RA,[15–19] only one has explored the predictive role of bone erosions for the development of clinical arthritis.[17] Nam *et al* showed that the presence of US-detected bone erosions, in addition to grey scale and power Doppler (PD) synovitis, could predict progression to IA in 136 CCP+ individuals with musculoskeletal symptoms but without clinical arthritis, raising implications for the risk stratification of individuals at-risk of RA.[17]

The detection of reliable biomarkers, which help to identify individuals at-risk for future arthritis, is a critical prerequisite for RA prevention trials. It is also important that such biomarkers are readily available to rheumatologists who are now routinely being referred at-risk individuals in clinical practice.[20] As such, a focused US examination, which enabled risk stratification in the clinic setting, would be invaluable for managing these patients.

We hypothesised that a targeted US examination, evaluating the areas that have been reported as most specific for the identification of US bone erosions in RA,[21] could be used for risk prediction in individuals at-risk of RA. Based on these considerations, the objectives of this study were twofold:

- To determine, in CCP+ at-risk individuals without clinical synovitis (CCP+ at-risk), the prevalence and distribution of US bone erosions, and their correlation with subclinical synovitis, in the classical sites for RA damage: the second and fifth metacarpophalangeal (MCP2 and MCP5) joints, and the fifth metatarsophalangeal (MTP5) joints.
- To study the association between US-detected bone erosions and the development of clinical arthritis.

## MATERIALS AND METHODS

The baseline US scans (from June 2008 to December 2019) of CCP+ at-risk individuals, with musculoskeletal symptoms but without clinical synovitis, from 'The CCP Study: Coordinated Programme to Prevent Arthritis—Can We Identify Arthritis at a Pre-clinical Stage?', were analysed. Full details of the Leeds CCP study have been published previously.[22] Briefly, in this national study, individuals with new musculoskeletal joint symptoms presenting to their primary care physician (or other healthcare professional) are tested for anti-CCP Ab. Those who test positive for anti-CCP Ab are invited to a dedicated research clinic in Leeds, UK, as part of a prospective observational study.

The US evaluations were carried out by rheumatologists experienced in sonography and sonographers, blinded to the individuals' clinical data. The US and clinical examinations were conducted by different physicians. All the US operators had a training session on the scanning protocol. The US scans were initially carried out using a Philips (ATL HDI 5000) machine working with 5–12 MHz and 8–15 MHz transducers. A small number of US scans were then performed using a General Electric (GE) S7 machine, employing a 6–15 MHz transducer. Due to the change in the US machine during the course of the study, sensitivity analyses between the first two US machines (Philips ATL HDI 5000 and GES7) were performed.[17] Subsequently (from 2014), a GE Logiq E9 machine, employing a 6–15 MHz transducer, was used. PD was set as follows: pulse repetition frequency (PRF) 700–1000 Hz, Doppler frequency 6 MHz for the Philips (ATL HDI 5000), 10 MHz for the GE S7 and GE Logic E9.

The presence of bone erosions and synovitis was explored in the MCP2 joints, MCP5 joints and MTP5 joints. These have been reported as the most specific joints for the detection of US bone erosions in RA.[21] Bone erosions were identified as intra-articular discontinuities of the bone surface that are visible in two perpendicular planes, according to the Outcome Measure in Rheumatology (OMERACT) definitions.[23] The size of bone erosions (diameter of the cortical break) was evaluated according to a semi-quantitative scoring system (from 0 to 3), where 0: no definite erosion, 1: erosion <2 mm, 2: erosion 2–4 mm and 3: erosion >4 mm.[14 24] The dorsal, lateral and palmar aspects of the joints were assessed for the presence of bone erosions. Synovitis was defined as synovial hypertrophy ≥2, or synovial hypertrophy ≥1+PD signal ≥1, according to the OMERACT definitions.[25]

For each individual, the following data were collected: age, sex, smoking exposure, X-rays of the hands and feet, second-generation anti-CCP (CCP2) Ab titre (BioPlex 2200 CCP2, BioRad, USA) and rheumatoid factor (RF) status (positivity/negativity). Anti-CCP2 test positivity threshold was set at >2.99 IU/mL, according to manufacturer's cut-offs. Anti-CCP2 titre was considered low or high when it was <three or ≥three times the positivity threshold, respectively, according to the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010 criteria.[2] RF positivity was set at ≥20 IU/mL. Moreover, for each individual, tenderness on physical examination in the small joints of the hands and feet (MCP2, MCP5 and MTP5 joints) was also registered. According to the study protocol, the CCP+ at-risk individuals were assessed at baseline, at 3-monthly intervals for the first year and then yearly or until they developed IA. The US scans were repeated at 6 and 12 months visits and then yearly (unless the individuals developed IA). Anti-CCP Ab, RF and X-rays of the hands and feet were performed at baseline and then annually, or when they developed IA.

Only CCP+ at-risk individuals with ≥1 follow-up visit were included in the progression analysis (n=400). Individuals who withdrew from the study were excluded from this analysis. Progression to IA was defined as the development of clinical synovitis (tenderness and swelling) in ≥1 joint. RA was defined according to the 2010 ACR/EULAR RA classification criteria.[2]

## PATIENT AND PUBLIC INVOLVEMENT

The design of the Leeds CCP study including biomarkers measured and data collected has been informed by several patients and public involvement (PPI) meetings, hosted by the Leeds Biomedical Research Centre PPI group, in which patients and public partners were actively involved. Within these PPI groups, different potential biomarkers were discussed, which could help identify risk factors for the development of RA. The PPI group placed significant importance on the use of routinely available clinical biomarkers, such as blood tests (ie, autoAb, inflammatory markers) and imaging exams (ie, musculoskeletal US), in risk-stratifying individuals at-risk of RA. PPI members were involved at different stages of the study and their preferences and priorities informed the development of the study.

## STATISTICAL ANALYSIS

Results are expressed as mean and SD for the quantitative variables with a normal distribution, as median and IQR for those without a normal distribution (Kolmogorov-Smirnov test), and

**Table 1** Baseline demographic and clinical characteristics of the CCP+ at-risk individuals

| | |
|---|---|
| **Age, years (mean±SD)** | **50.9±13.4** |
| Sex | |
| Female | 302 (72.1%) |
| Male | 117 (27.9%) |
| Anti-CCP2 Ab | |
| High titre (≥9 IU/mL) | 290 (69.2%) |
| Low titre (<9 IU/mL) | 129 (30.8%) |
| Rheumatoid factor positivity (≥20 IU/mL) | 160 (38.2%) |
| Smoking exposure | |
| Never smoker | 181 (43.2%) |
| Previous smoker | 143 (34.1%) |
| Current smoker | 95 (22.7%) |

Percentages refer to the total number of individuals (n=419).
Ab, antibody; Anti-CCP2, second generation anti-cyclic citrullinated peptide.

as absolute frequency with corresponding percentage for the qualitative variables. The Student's t-test was used for comparing quantitative variables with a normal distribution, the Mann-Whitney U test for those without a normal distribution and $\chi^2$ test for the qualitative variables. To test the hypothesis that bone erosion and synovitis coexist in the same joint, we performed a $\chi^2$ test evaluating a 2×2 contingency table (presence/absence of synovitis and presence/absence of bone erosions). The strength of the relationship between US findings was measured using Cramer's V. Multiple logistic regression analysis was used to define predictive values of US findings for the development of clinical arthritis (at 1 year, at 3 years and ever). All regression analyses were adjusted for age, gender, smoking exposure, anti-CCP2 titre and RF status. Significance-based backward stepwise selection of variables was used for the final multivariable model. All covariates with a p<0.10 in the univariable models were included in the multivariable models. Kaplan-Meier analysis and log-rank test were performed to analyse and visualise the IA-free survival time for the US findings. These analyses were adjusted by the same parameters as the regression analysis. Statistical analysis was performed using Statistical Package for the Social Sciences software V.24.0 for windows (Chicago, Illinois, USA). The level of significance was set at 5%.

## RESULTS

### Demographic and clinical characteristics of the CCP+ at-risk individuals

A total of 2514 joints, in 419 CCP+ at-risk individuals, were evaluated. The median follow-up was 497 days (IQR: 256–1111.5 days). The demographic and clinical characteristics of the CCP+ at-risk individuals are reported in table 1.

### US bone erosions: prevalence, distribution, association with subclinical synovitis, tenderness on physical examination and X-rays findings

Bone erosions were found in ≥1 joint in 41 out 419 (9.8%) individuals, and in 55 out of the 2514 (2.2%) joints scanned. Bilateral and symmetrical erosions were identified in 11 out of 41 (26.8%) individuals. The prevalence of bone erosions was significantly higher in the MTP5 joints than in the MCP2 joints and MCP5 joints (p<0.01). In particular, bone erosions were detected in 42 MTP5 joints (31 individuals; 7.4%), in 10 MCP2 joints (10 individuals; 2.4%), and in 3 MCP5 joints (3 individuals; 0.7%).

**Table 2** Distribution and size of US bone erosions

| | MCP2 joints | MCP5 joints | MTP5 joints | Total |
|---|---|---|---|---|
| Bone erosions | 10 (18.2%) | 3 (5.5%) | 42 (76.4%) | 55 |
| Grade 1 | 9 (16.4%) | 3 (5.5%) | 29 (52.7%) | 41 (74.5%) |
| Grade 2 | 0 | 0 | 11 (20%) | 11 (20%) |
| Grade 3 | 1 (1.8%) | 0 | 2 (3.6%) | 3 (5.5%) |

Percentage refer to the total number of joints with bone erosions (n=55).
MCP2 and MCP5, second and fifth metacarpophalangeal joints; MTP5, fifth metatarsophalangeal.

Bone erosions in ≥1 MTP5 joint were found in 12 out of 13 (92.3%) individuals with multiple (>1 joint) bone erosions. The distribution and size of the US-detected bone erosions are reported in table 2.

A significant correlation between bone erosion and synovitis in the same joint was detected for MTP5 joints (Cramer's V=0.37, p<0.01), whereas it was not significant for MCP2 joints (Cramer's V=0.02, p=1.0), and for MCP5 joints (Cramer's V=0.02, p=0.41), likely due to the low number of bone erosion at these levels. US synovitis was detected in 145 (5.8%) joints, in 96 (22.9%) individuals. US synovitis was found in 22 out of 55 (40%) joints with bone erosions, in 17 out of 41 (41.5%) individuals. In particular, US synovitis was found in 20 out of 42 (47.6%) MTP5 joints, in 2 out of 10 (20%) MCP2 joints and in none of 3 MCP5 joints showing bone erosions. Synovitis was found in 13 out of the 41 (31.7%) joints showing grade 1 bone erosions. No significant difference in the size of bone erosions in the joints with concomitant synovitis in comparison with those without synovitis was found (p=0.114). On the other hand, US bone erosions were found in 22 out of 145 (15.2%) joints with US synovitis. In particular, US bone erosions were detected in 20 out the 55 (36.4%) MTP5 joints, in 2 out of the 66 (3%) MCP2 joints and in none of the 24 MCP5 joints with synovitis.

Tenderness on physical examination was detected in 7 out of the 55 (12.7%) joints with bone erosions, in 5 (12.2%) individuals. In particular, joint tenderness was found in 6 out of 42 (14.3%) MTP5 joints, in 1 out of 10 (10%) MCP2 joints and in none of the 3 MCP5 joints with bone erosions. Bone erosions were detected in combination with US synovitis in 3 out of the 7 (42.8%) joints which were tender on physical examination. The relationship between the US and X-ray findings is reported in online supplementary tables 1 and 2.

### The predictive value of the US bone erosions for the development of inflammatory arthritis

A total of 123/400 (30.7%) CCP+ at-risk individuals developed IA (median follow-up: 301 days, IQR 112–721), 95 (77.2%) of whom fulfil the 2010 RA classification criteria. In particular, 25 out of the 41 (61.0%) individuals with US bone erosions, and 98 out of 359 (27.3%) individuals without US bone erosions, developed IA (p<0.01).

The ORs of the US findings for the development of IA are reported in table 3. The results are adjusted for age, sex, smoking exposure, anti-CCP2 titre and RF status, except when the combination of the US and clinical findings was analysed (ie, presence of bone erosions+high titre anti-CCP2 Ab±RF). In this case, the analysis was not adjusted for anti-CCP2 titre and RF status as they were independent variables.

**Table 3** Predictive value of the US findings for the development of inflammatory arthritis (ever, at 1 year at 3 years)

| | Ever | | At 1 year | | At 3 years | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | P value | OR (95% CI) | P value | OR (95% CI) | P value |
| Presence of bone erosion in ≥1 joint (any joint) | **4.0 (1.8 to 8.7)** | **<0.01** | **3.6 (1.7 to 7.5)** | **<0.01** | **3.5 (1.6 to 7.4)** | **<0.01** |
| *in the MCP2 joints* | 2.4 (0.5 to 11.1) | 0.26 | 1.1 (0.2 to 5.8) | 0.94 | 1.7 (0.4 to 7.0) | 0.53 |
| *in the MCP5 joints* | 1.4 (0.1 to 31.0) | 0.85 | 0 | 1 | 0 | 1 |
| *in the MTP5 joints* | **4.8 (2.0 to 11.6)** | **<0.01** | **5.2 (2.3 to 11.8)** | **<0.01** | **5.4 (2.3 to 12.9)** | **<0.01** |
| Presence of bone erosion and synovitis in the same joint (any joint) | **3.9 (1.2 to 12.8)** | **0.02** | **6.0 (2.1 to 17.5)** | **<0.01** | **3.9 (1.3 to 11.8)** | **0.02** |
| Presence of bone erosion and synovitis in the same MTP5 joint | **5.1 (1.4 to 18.9)** | **0.02** | **7.0 (2.3 to 21.7)** | **<0.01** | **4.9 (1.5 to 16.2)** | **<0.01** |
| Presence of bone erosion in >1 joint (any joint) | **10.6 (1.9 to 60.4)** | **<0.01** | **5.7 (1.7 to 19.5)** | **<0.01** | **7.3 (1.7 to 31.7)** | **<0.01** |
| Presence of bone erosion in ≥1 joint (any joint)+high titre anti-CCP2 Ab | **5.3 (2.2 to 12.7)** | **<0.01** | **4.2 (1.9 to 9.3)** | **<0.01** | **4.2 (1.9 to 9.4)** | **<0.01** |
| Presence of bone erosion in ≥1 joint (any joint)+high titre anti-CCP2 Ab and positive RF | **16.9 (2.1 to 132.8)** | **<0.01** | **4.1 (1.4 to 11.5)** | **<0.01** | **7.1 (1.9 to 26.4)** | **<0.01** |

Ab, antibody; Anti-CCP2, second generation anti-cyclic citrullinated peptide; MCP2 and MCP5, second and fifth metacarpophalangeal joints; MTP5, fifth metatarsophalangeal joints; RF, rheumatoid factor.

As shown in table 4, the presence of bone erosion in the MTP5 joints was the most significant factor for the development of IA in the multivariable analysis.

Individuals with bone erosions in ≥1 joint (any joint) show a significantly reduced IA-free survival rate compared with individuals without bone erosion (p<0.01) (figure 1A). At 1 year follow-up, 31.7% of individuals with bone erosions in ≥1 joint (any joint), and 61.5% of individuals with bone erosions in >1 joint (any joint) developed IA, compared with only 14.8% of individuals without bone erosions (p=0.04 and p<0.01, respectively).

The same trend was observed evaluating the US findings at MTP5 joints level (figure 1B). At 1 year of follow-up, 36.6% of individual with bone erosions in ≥1 MTP5 joints, but only 14.6% of subjects without bone erosions, developed IA (p=0.04). At the same time-point, the rate of progression to IA was significantly higher for the subjects showing bone erosion and synovitis in the MTP5 joints (68.8%) than the rate of progression of the individuals with bone erosions only (without synovitis) (p=0.03).

At 1 year follow-up, the rate of progression to IA of individuals with high titre anti-CCP2 Ab (without bone erosion) was 14.8% (figure 1C). Interestingly, this goes up to 40% in presence of bone erosions in ≥1 joint (any joint) (p<0.01), and to 61.1% in case of bone erosions in ≥1 joint (any joint) and positive RF (p<0.01). This last analysis was adjusted for the following confounders: age, sex and smoking exposure.

## DISCUSSION

The results of our study suggest that an efficient, targeted US protocol, evaluating a set of only three joints (bilaterally), provides important information regarding the prevalence, distribution and the predictive role of US bone erosions for the development of IA in CCP+ at-risk individuals. A focused US examination on the classical sites for RA damage (in particular the MTP5 joints) has the potential to improve risk-stratification and inform the management of CCP+ at-risk individuals. We demonstrated that US-detected bone erosions in selected joints are useful to predict progression (and its timing) to IA in CCP+ at-risk individuals, with the risk of progression increasing with the number of joints with bone erosions, and with the presence of bone erosions in the MTP5 joints, especially when in combination with synovitis. Of note, around two-thirds of individuals with bone erosions in more than one joint (any joint), or with bone erosion and synovitis in the same MTP5 joint, progressed to IA within 12 months of observation. Therefore, the detection of such US findings appears particularly useful for the identification of individuals at high risk of imminent arthritis (≤12 months); these individuals should be

**Table 4** Final multivariate logistic regression model for the development of inflammatory arthritis at 1 year (A) and 3 years (B)

| | | | | | | | 95% CI of the OR | |
|---|---|---|---|---|---|---|---|---|
| | B | SE | Wald | df | P value | OR | Lower bound | Upper bound |
| **A** | | | | | | | | |
| Presence of bone erosions in the MTP5 joints | 1.65 | 0.41 | 15.90 | 1 | <0.01 | 5.2 | 2.3 | 11.7 |
| High titre anti-CCP2 Ab | 0.87 | 0.42 | 4.31 | 1 | 0.04 | 2.4 | 1.1 | 5.4 |
| RF positivity | 1.05 | 0.30 | 12.24 | 1 | <0.01 | 2.9 | 1.6 | 5.2 |
| Smoking exposure (current or previous) | 0.70 | 0.34 | 4.20 | 1 | 0.04 | 2.0 | 1.1 | 3.9 |
| Constant | −3.08 | 0.39 | 60.92 | 1 | <0.01 | 0.1 | | |
| Model summary. Nagelkerke $R^2$: 0.21, Cox and Snell $R^2$: 0.13 | | | | | | | | |
| **B** | | | | | | | | |
| Presence of bone erosions in the MTP5 joints | 1.70 | 0.44 | 14.88 | 1 | <0.01 | 5.5 | 2.3 | 12.9 |
| High titre anti-CCP2 Ab | 1.36 | 0.39 | 12.12 | 1 | <0.01 | 3.9 | 1.8 | 8.3 |
| RF positivity | 1.10 | 0.27 | 17.16 | 1 | <0.01 | 3.0 | 1.8 | 5.1 |
| Smoking exposure (current or previous) | 0.71 | 0.35 | 4.18 | 1 | 0.04 | 2.0 | 1.0 | 4.0 |
| Constant | −3.44 | 0.41 | 69.40 | 1 | <0.01 | 0.0 | | |
| Model summary. Nagelkerke $R^2$: 0.30, Cox and Snell $R^2$: 0.21. | | | | | | | | |

Ab, antibody; Anti-CCP2, second generation anti-cyclic citrullinated peptide; MTP5, fifth metatarsophalangeal joints; RF, rheumatoid factor.

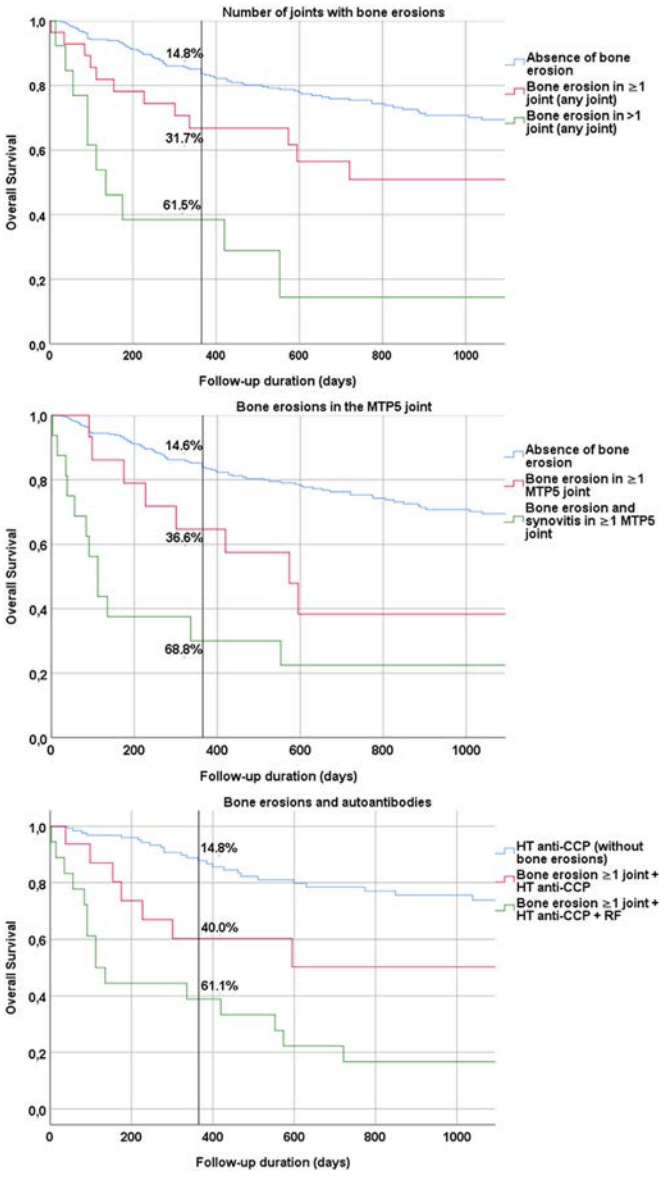


**Figure 1** Kaplan-Meier analysis shows inflammatory arthritis-free survival time in at-risk individuals with positive anti-cyclic citrullinated peptide (CCP) antibodies (Ab). (A) Number of joints with bone erosions (absence of bone erosion, bone erosions in ≥1 joint, bone erosions in >1 joint). (B) Bone erosions in the fifth metatarsophalangeal (MTP5) joints (absence of bone erosion, bone erosions in ≥1 MTP5 joint, bone erosion and synovitis in ≥1 MTP5 joint). (C) High titre (HT) anti-CCP Ab without bone erosions, HT anti-CCP Ab with ≥1 bone erosion (any joint), HT anti-CCP Ab with ≥1 bone erosion (any joint) and positive rheumatoid factor (RF). Percentages refer to the individuals progressing at 12 months follow-up (black lines).

followed closely and potentially considered for preventive intervention (eg, clinical trials), especially if presenting with high titre anti-CCP2 Ab and positive RF.

The prevalence of bone erosions in the MTP5 joints was relatively high (7.4%), and significantly higher than the prevalence of bone erosions in the MCP2 joints (2.7%) and MCP5 joints (0.7%) (p<0.01). Indeed, previous X-rays and US studies have revealed that the foot is one of the earliest sites of joint damage in patients with RA, with the MTP5 joints often representing the first site of bone erosions in those with early disease.[26–28] Moreover, the MTP5 joints appear to be a very specific site for the identification of US bone erosion in patients with RA. In fact, in the above-mentioned study carried out by Zayat *et al*, bone erosions (of any size) in the MTP5 joints were highly specific for RA.[21] Moreover, in a large study carried out on 207 healthy subjects, bone erosions were not detected in any of the MTP5 joints evaluated.[29] The results of our study suggest that a careful examination of the feet is required in CCP+ at-risk individuals given the relatively high prevalence of bone erosions at this level.

To the best of our knowledge, this is the first study evaluating the association between US bone erosions and synovitis (at joint level) in CCP+ at-risk individuals. We found a significant association between bone erosions and synovitis in the MTP5 joints (Cramer's V=0.37, p<0.01). One explanation is that bone erosions may occur as a consequence of persistent, subclinical joint inflammation which, acting alongside site-specific mechanical stress, leads to structural joint damage.[30 31] On the other hand, joint damage could determine the release of bone and cartilage degradation elements. These act as possible triggers for local inflammation, thereby initiating a vicious circle of inflammation and joint damage.[32] However, this appears more likely to occur in patients with already established disease. In the joints with bone erosions but no concomitant synovitis (60%), the presence of structural damage could be interpreted as the result of a previous inflammatory process that was not detected at the time of the US scan. Another very intriguing hypothesis links the development of bone damage to the direct effect of anticitrullinated protein Ab (through the activation of osteoclasts), before the onset of clinical synovitis.[5 33] Interestingly, the OR for the progression to IA increased from 4.8 (95%CI 2.0–11.6) to 5.1 (95%CI 1.4–18.9), when bone erosions in the MTP5 joints were detected in combination with synovitis.

Only a few joints showing bone erosions were tender on physical examination (12.7%), despite the identification of concomitant US synovitis in almost half of these joints. This is an interesting finding for which there might be different explanations. First, we could assume that the presence of low-grade subclinical inflammation might lead to structural damage (in the long term) without significant symptoms. Another explanation could be that the physical examination might be not sensitive (or not enough accurate) at foot level, especially in patients who do not complain of foot pain. Our results highlight the importance of using US for the evaluation of bone erosions (with or without synovitis) in the classic sites for RA damage in CCP+ at-risk individuals, with a particular focus on the MTP5 joints; clinical examination may often be falsely reassuring in these individuals.

Our study has the following limitations. First, the lack of other imaging tools, such as MRI or CT, to confirm the presence of bone erosions, especially when <2 mm. This may have been useful especially in light of the fact that bone erosions have been found in healthy subjects, both on US and MRI.[34–36] However, particular attention in the assessment of cortical bone breaks of small size was paid by the sonographers to avoid misinterpretation of the US findings (ie, anatomical necks or vascular bone channels). Moreover, several studies have already demonstrated the good correlation between US, MRI and CT for the detection of bone erosions,[37–39] thus suggesting that US is reliable and accurate for the assessment of structural damage in patients with RA. The US protocol used in our study did not clearly specify the site of bone erosions at wrist level (radio-carpal joint, ulno-carpal joint, inter-carpal joints or distal ulna) and the distal ulna, which has also been described as a specific site for the detection of US bone erosions in patients with RA,[21] was not included. Finally,

targeting the US evaluation only to the classic sites of RA damage could be considered another limitation of the study, as this might have led to underestimating the prevalence of bone erosions in CCP+ at-risk individuals.

The prevention of RA has the potential to completely transform the clinical approach to this disease, and represents one of the most intriguing challenges in modern rheumatology.[40] In this context, the identification of reliable and clinically available biomarkers of disease progression, which allow identification individuals at high risk of developing clinical disease, becomes extremely important.

## CONCLUSIONS

The MTP5 joints appear to be an early site of erosive damage in individuals at-risk of RA without clinical synovitis. US bone erosions were mainly detected in asymptomatic joints, but frequently in association with subclinical synovitis. In CCP+ at-risk individuals, US bone erosions in >1 joint, and bone erosions in the MTP5 joints in combination with synovitis, are the most predictive for the development of clinical arthritis. Our results suggest that a focused US examination of the classical sites for RA damage, evaluating a set of only three joints (bilaterally), has the potential to improve risk-stratification and therefore inform management of CCP+ at-risk individuals.

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

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CLINICAL SCIENCE

# Ultrasound Doppler and tenosynovial fluid analysis in tenosynovitis

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## ABSTRACT

**Objective** To assess Doppler ultrasound (US) and tenosynovial fluid (TSF) characteristics in tenosynovitis within common rheumatic conditions, as well as their diagnostic utility.

**Methods** Subjects with tenosynovitis underwent Doppler US and US-guided TSF aspiration for white cell count (WCC) and crystal analysis. Tenosynovial Doppler scores (DS) were semiquantitatively graded. TSF WCC and DS were compared using Kruskal-Wallis tests and logistic regression between non-inflammatory conditions (NIC), inflammatory conditions (IC) and crystal-related conditions (CRC). Receiver operating curves, sensitivity and specificity assessed the ability of WCC and DS to discriminate IC from NIC.

**Results** We analysed 100 subjects from 14 sites. The mean age was 62 years, 65% were female, and the mean TSF volume was 1.2 mL. Doppler signal was present in 93.7% of the IC group and was more frequent in IC than in NIC group (OR 6.82, 95% CI 1.41 to 32.97). The TSF median WCC per $10^9$/L was significantly higher in the IC (2.58, p<0.001) and CRC (1.07, p<0.01) groups versus the NIC group (0.38). A TSF cut-off of ≥0.67 WCC per $10^9$/L optimally discriminated IC versus NIC with a sensitivity and specificity each of 81.3%. In the IC group, 20 of 48 (41.7%) subjects had a TSF WCC <2.00 per $10^9$/L.

**Conclusions** A negative DS helps rule out IC in tenosynovitis, but a positive DS is non-specific and merits TSF testing. Unlike synovial fluid, a lower TSF WCC better discriminates IC from NIC. US guidance facilitates aspiration of minute TSF volume, which is critical for diagnosing tenosynovial CRC.

## Key messages

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

- Tenosynovitis is an important manifestation in many rheumatic diseases, but not much is known about the utility of tenosynovial fluid (TSF) analysis and its correlation with tenosynovial Doppler score (DS).

**What does this study add?**

- This is the first study to systematically evaluate tenosynovitis seen in routine rheumatology practices with Doppler ultrasound (US), US-guided TSF aspiration and TSF analysis.
- Doppler scoring of tenosynovitis and TSF white cell count (WCC) provide complementary information to classify patients as having non-inflammatory conditions, inflammatory conditions or crystalline-related conditions.
- Compared with synovial fluid, a lower TSF WCC better discriminates non-inflammatory from inflammatory causes of tenosynovitis.

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

- Tenosynovial DS and TSF aspiration will be helpful in differentiating inflammatory from non-inflammatory conditions and prove useful in identifying crystalline conditions.

## INTRODUCTION

Inflammation of the tendon sheath, termed tenosynovitis, is present in multiple rheumatic conditions. In some circumstances, tenosynovitis may be the initial manifestation of a systemic disease. For example, tenosynovitis may precede the onset of synovitis in rheumatoid arthritis (RA).[1] The Outcome Measures in Rheumatology in Clinical Trials (OMERACT) group has defined tenosynovitis on ultrasound (US) as the presence of abnormal anechoic or hypoechoic tendon sheath widening due to abnormal fluid and/or hypertrophy within the tendon sheath, with or without fluid, with or without Doppler signal, visible in two planes.[2]

Tenosynovial fluid (TSF) aspiration has traditionally been described in infectious flexor tenosynovitis of the hands.[3] Rare case reports exist with US-assisted aspiration in diagnosing tenosynovial gout.[4 5] No study has systemically examined the utility of TSF testing in routine rheumatology clinical practice. There are well-established white cell count (WCC) cut-off criteria for classifying synovial fluid as normal, non-inflammatory, inflammatory and infectious.[6 7] However, no such thresholds exist for categorising TSF WCC.

Doppler modality on US is used to detect active inflammation in the synovium. It also assesses tendon damage in RA.[8] In RA, Doppler signal within the synovium, typically scored semiquantitatively,[9] correlates with histological evidence of hypervascularity that can lead to joint damage.[10 11] The distribution of tenosynovial Doppler scores (DS) across different rheumatic diseases is not well

known. Furthermore, the correlation of DS in tenosynovitis with TSF WCC is relatively unknown.

The aim of this multicentre, cross-sectional pragmatic study was to characterise tenosynovial DS and TSF findings among different rheumatic conditions in clinical rheumatology practices. We also assessed the correlation between tenosynovial DS and TSF WCC and the impact of these findings on diagnosis.

## METHODS

### Study design and patients

Patients for this cross-sectional study were recruited from 14 adult rheumatology private and academic practices in the USA between November 2017 and May 2019. Informed consent was obtained at all sites. Subjects were included if they presented with tenosynovial complaints (eg, pain, swelling and so on) and the clinician felt the need to perform a US examination of the involved area, US demonstrated tenosynovial effusion, and a tenosynovial aspiration was planned due to diagnostic uncertainty and/or therapeutic reasons. Subjects could be patients with established disease or new patients. All evaluations took place during one visit.

The study procedure involved Doppler US assessment of the symptomatic tendon (see the US assessment and scoring section). US-guided TSF aspiration was planned and performed by the treating rheumatologist. Only one TSF sample per subject was obtained. Only subjects with a successful TSF aspiration were included in the final analysis. If the TSF volume was below the minimum required by the local laboratory, the treating rheumatologist used a haemocytometer to perform WCC (but without differential) and polarised microscopy for crystal analysis.

Clinical data collected included patient age, gender, preaspiration diagnosis (if any), tendon location, duration of tendon symptoms and postaspiration diagnosis. TSF characteristics recorded included volume, colour, WCC with differential and crystal (monosodium urate (MSU) or calcium pyrophosphate (CPP)) identification. Microbial testing was only done if an infection was clinically suspected.

### US assessment and scoring

Transverse and longitudinal Doppler images of the involved tendons were recorded. The most symptomatic tendon with an effusion was aspirated. Within that tendon, DS was measured at the level of maximal signal, and aspiration occurred at the level of maximal effusion. All investigators are certified by the American College of Rheumatology in musculoskeletal US and are teachers in the Ultrasound School of North American Rheumatologists. While US equipment varied between clinical sites (see online supplementary table S1), all sites adjusted Doppler settings to match a standardised finger pulp signal showing at least one and not more than two linear signals at room temperature (see online supplementary figure S1), as previously described.[12] Gain was adjusted to just above the noise threshold. These settings were maintained for all study evaluations. Tenosynovitis was defined as per the OMERACT definition.[2] US and Doppler image acquisition (but not grading) preceded TSF aspiration.

Independent and blinded DS grading of still images was performed remotely by three rheumatologists (AC, EYK and FA) on a 0–3 semiquantitative scale.[9] In this method, grade 0 is absent signal, grade 1 is focal peritendinous signal, grade 2 is multifocal Doppler signal, and grade 3 is widespread Doppler signal in the tendon sheath. Additionally, an intratendinous Doppler signal was assigned a score of 1 and added to the final DS only if the sheath DS was 1 or 2. The median DS from the three raters was used for analysis.

### Statistical analysis

Inter-rater agreement for DS was assessed using Fleiss' kappa with quadratic weights.[13] The correlations of the DS among the three raters with TSF WCC were determined by Spearman's correlation coefficients. For analytic purposes, clinical diagnoses were categorised into three groups based on the final clinical impression of the treating physician: non-inflammatory conditions (NIC), inflammatory conditions (IC) and crystal-related conditions (CRC). The final clinical diagnosis, serving as the reference standard, was based on clinical and US evaluation as well as TSF results but independent of the study DS (as this was scored after the evaluation). NIC included metabolic, mechanical and trauma-related causes. IC encompassed RA, spondyloarthritis, polymyalgia rheumatica, systemic lupus erythematosus, vasculitis and so on. The CRC group consisted of subjects with MSU and CPP. If a final clinical diagnosis could not be categorised, it was excluded from intergroup analyses comparing DS and TSF. Intergroup WCC were compared using a Kruskal-Wallis test and Dunn's post-hoc tests with Bonferroni correction for multiple testing. Intergroup DS were compared using ordinal logistic regression. An analysis using subjects whose clinical diagnostic category did not change from preaspiration to postaspiration was performed to determine if the TSF results substantially influenced the diagnostic category assignments. Sensitivity, specificity and receiver operating characteristic (ROC) curve analyses assessed the utility of DS and TSF WCC for discriminating IC from NIC. Optimal cut-points for WCC to discriminate IC from NIC were determined by the Youden index.[14] All analyses were completed using Stata V.14.2.

## RESULTS

### Subject characteristics

Of the 112 subjects recruited for this study, 100 (table 1) were included in the final analysis as 12 aspirations did not produce TSF.

Of the 100 subjects, 90 could be categorised into the three diagnostic groups. When limiting subjects to those whose diagnostic category was unchanged from preaspiration to postaspiration, the total number was 66. Notably, 30% of TSF samples were below the 0.5 mL volume required by many labs to perform an automated cell count and differential. Five tendon locations accounted for 74.0% of the aspirations (table 2).

### Doppler scoring

DS inter-rater agreement was substantial (kappa 0.74, 95% CI 0.65 to 0.83). DS among the three raters was grade 0 in 16%, grade 1 in 21%, grade 2 in 33%, and grade 3 in 30% of subjects. Figure 1 shows representative DS images. Figure 2 shows the distribution of DS across the diagnostic groups. Doppler was absent in only 6.3% of the IC group as opposed to 23.1% of the CRC group and 31.3% of the NIC group. The three diagnostic groups and DS (p=0.09 IC vs NIC, p=0.86 CRC vs NIC) had no statistically significant association. However, the odds of having a Doppler signal were 6.82-fold higher (95% CI 1.41 to 32.97, p=0.02) in the IC group versus the NIC group.

### TSF characteristics

TSF WCC was highly variable within each diagnostic group with median (IQR) counts of 0.38 (IQR 0.15–0.65), 2.58 (IQR 0.77–14.11) and 1.07 (IQR 0.76–6.83) WCC per $10^9$/L in the NIC, IC

**Table 1** Patient demographics and disease characteristics

| Characteristics | Results |
|---|---|
| Mean age, years (±SD) | 61.8 (16.4) |
| Gender, n (%) | |
| Female | 65 (65.0) |
| Male | 35 (35.0) |
| Tendon symptom duration, n (%) | |
| <7 days | 6 (6.0) |
| 7 days–2 months | 34 (34.0) |
| >2 months | 60 (60.0) |
| Presenting with a history of tendon trauma, n (%) | 7 (7.0) |
| TSF volume aspirated, mL | |
| Mean (±SD) | 1.2 (1.7) |
| Range | 0.3–10.0 |
| TSF infection testing if clinically suspected, n=20 (%) | |
| Positive | 1 (5.0) |
| Negative | 19 (95.0) |
| Final postaspiration clinical diagnosis, n (%) | |
| Rheumatoid arthritis | 20 (20.0) |
| CPPD disease* | 18 (18.0) |
| Mechanical cause | 12 (12.0) |
| Spondyloarthropathy | 12 (12.0) |
| Gout† | 8 (8.0) |
| Unknown‡ | 7 (7.0) |
| Inflammatory arthritis | 6 (6.0) |
| Connective tissue disease | 5 (5.0) |
| Polymyalgia rheumatica | 4 (4.0) |
| Trauma | 4 (4.0) |
| Other‡ | 2 (2.0) |
| Infection‡ | 1 (1.0) |
| Vasculitis | 1 (1.0) |
| Diagnostic groups, n (%) | |
| Non-inflammatory conditions | 16 (16.0) |
| Inflammatory conditions | 48 (48.0) |
| Crystal-related conditions | 26 (26.0) |
| Unclassified | 10 (10.0) |

*Final clinical diagnosis of CPPD was made in 18 patients, although only 16 showed presence of CPP crystals on aspiration.
†Final clinical diagnosis of gout was made in eight patients, although only six showed presence of monosodium urate crystals on aspiration.
‡Not classified into one of the analysis diagnostic groups.
CPPD, calcium pyrophosphate deposition; TSF, tenosynovial fluid.

**Table 2** Location of tendons as per underlying condition

| Involved tendon | Overall Total* n=100 (%) | NIC Total n=16 (%) | IC Total n=48 (%) | CPP Total n=16 (%) | MSU Total n=6 (%) |
|---|---|---|---|---|---|
| Wrist extensor compartment 4 | 26 (26.0) | 6 (37.5) | 15 (31.3) | 2 (12.5) | 0 (0.0) |
| Bicipital tendon | 18 (18.0) | 3 (18.8) | 7 (14.6) | 7 (31.8) | 0 (0.0) |
| Posterior tibial tendon | 16 (16.0) | 3 (18.8) | 8 (16.7) | 1 (6.3) | 1 (16.7) |
| Wrist extensor compartment 6 | 7 (7.0) | 0 (0.0) | 3 (6.3) | 3 (18.8) | 0 (0.0) |
| Peroneal tendons | 7 (7.0) | 1 (6.3) | 2 (4.2) | 0 (0.0) | 2 (33.3) |
| Extensor digitorum longus, foot | 6 (6.0) | 0 (0.0) | 2 (4.2) | 1 (6.3) | 3 (50.0) |
| Wrist extensor compartment 1 | 5 (5.0) | 0 (0.0) | 3 (6.3) | 0 (0.0) | 0 (0.0) |
| Wrist flexor tendons | 4 (4.0) | 0 (0.0) | 2 (4.2) | 1 (6.3) | 0 (0.0) |
| Wrist extensor compartment 2 | 2 (2.0) | 0 (0.0) | 1 (2.1) | 1 (6.3) | 0 (0.0) |
| Finger flexor tendon, third digit | 2 (2.0) | 0 (0.0) | 1 (2.1) | 0 (0.0) | 0 (0.0) |
| Anterior tibial tendon | 2 (2.0) | 1 (6.3) | 1 (2.1) | 0 (0.0) | 0 (0.0) |
| Wrist extensor compartment 3 | 1 (1.0) | 0 (0.0) | 1 (2.1) | 0 (0.0) | 0 (0.0) |
| Finger flexor tendon, second digit | 1 (1.0) | 0 (0.0) | 1 (2.1) | 0 (0.0) | 0 (0.0) |
| Wrist extensor compartment 5 | 1 (1.0) | 0 (0.0) | 1 (2.1) | 0 (0.0) | 0 (0.0) |
| Flexor carpi radialis | 1 (1.0) | 1 (6.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Flexor hallucis longus | 1 (1.0) | 1 (6.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) |

*Total of individual categories is not 100 as only 90 patients could be categorised into a diagnostic category and 4 additional patients were diagnosed with CRC, although crystals were not seen at the study visit.
CPP, calcium pyrophosphate; CRC, crystal-related condition; IC, inflammatory conditions; MSU, monosodium urate; NIC, non-inflammatory conditions.

and CRC groups, respectively (see online supplementary table S2). WCC was significantly higher in the IC ($p<0.001$) and CRC ($p<0.01$) groups than in the NIC group (figure 3), and 87.5% of the NIC group patients had TSF WCC <2.00 WCC per $10^9$/L. In the IC group, 41.7% of subjects had a TSF WCC <2.00 WCC per $10^9$/L. TSF WCC and DS correlated weakly (figure 4).

Crystals were observed in 22% of TSF aspirates (CPP 16% and MSU 6%). Coccidioidomycosis, isolated from the peroneal tendon sheath in a subject with disseminated coccidioidomycosis, was the only infection identified (TSF WCC of 2.10 WCC per $10^9$/L and a DS of 1).

## Discrimination of diagnosis groups

A DS of ≥1 had a sensitivity of 93.8% and a specificity of 31.3% for discriminating IC versus NIC groups, while a DS of ≥2 had a sensitivity of 77.1% and a specificity of 43.8%. The synovial fluid-based cut-off of a WCC of ≥2.00 WCC per $10^9$/L had a sensitivity of 58.3% and a specificity of 87.5% for discriminating IC versus NIC groups. Notably, TSF WCC <2.00 WCC per $10^9$/L was observed in 41.7% of the IC group. In reanalysing the data using only subjects whose diagnostic category was unchanged from preaspiration to postaspiration, the

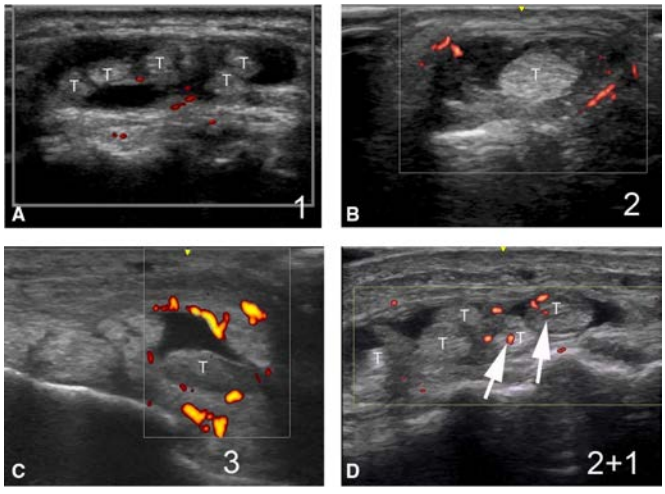


**Figure 1** Representative transverse Doppler scoring images of tenosynovitis based on Doppler signal in the tenosynovial sheath and intratendinous Doppler signal. (A) DS of 1 with Doppler signal in only the focal tendon sheath region, fourth extensor wrist compartment. (B) DS of 2 with multifocal Doppler signal, sixth extensor wrist compartment. (C) DS of 3 with widespread Doppler signal, peroneal tendons. (D) Composite DS of 3 based on DS of 2 from multifocal tendon sheath signal and 1 point from intratendinous signal (arrows), fourth extensor wrist compartment. DS, Doppler score; T, tendon.

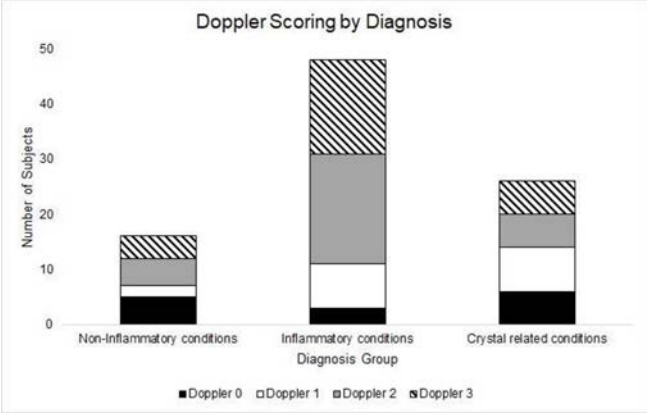


**Figure 2** Distribution of Doppler scores in the diagnosis groups.

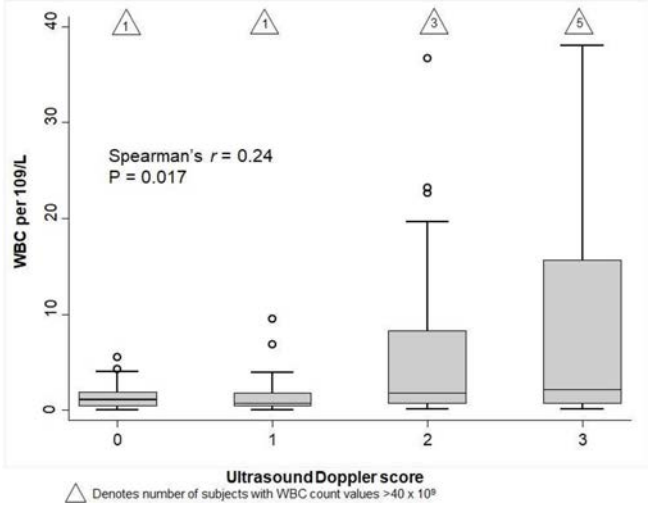


**Figure 4** Correlation of tenosynovial fluid white blood cell count with Doppler score. Total n=100. WBC, white blood cell.

percentage of subjects with IC with WCC <2.00 WCC per $10^9$/L was unchanged (42.9%).

ROC curve analysis (figure 5) identified the optimal TSF WCC cut-off for discriminating IC versus NIC groups at ≥0.67 WCC per $10^9$/L, with a sensitivity and specificity of 81.3% each and a Youden index of 0.625. Since a DS of ≥1 has high sensitivity (93.8%), a higher WCC provides greater specificity. For example WCC of 2.00 and 2.56 WCC per $10^9$/L give a specificity of 87.5% and 93.8%, respectively. Using data from subjects whose diagnostic category was unchanged from preaspiration to postaspiration, the test characteristics were essentially unchanged (sensitivity 81.0%, specificity 75.0%).

To explore the clinical utility of our findings, we created an algorithm using the combination of DS and TSF WCC with a cut-off value of 0.67 WCC per $10^9$/L and determined the agreement between the final clinical diagnosis category, as the reference standard, and the diagnosis category reached through our algorithm. Our algorithm classified subjects into three diagnostic categories as follows: no Doppler (NIC), positive Doppler (any score) and a WCC of ≥0.67 WCC per $10^9$/L (IC), and a positive Doppler with positive crystals (CRC) (figure 6). This algorithm was able to classify subjects into the NIC, IC and CRC-related categories 78% of the time and had substantial agreement (kappa 0.68, 95% CI 0.54 to 0.83) with the final clinical diagnosis when able to categorise subjects. When tested against the 90 clinician-diagnosed and categorised patients, a DS of 0 had a sensitivity of 88% and a specificity of 31% to identify NIC from IC or CRC. Among those with positive Doppler and without crystals, the WCC cut-off of ≥0.67 WCC per $10^9$/L had a sensitivity of 80% and a specificity of 82% to identify IC from NIC.

Because biceps tendon sheath TSF could track from the glenohumeral joint, we analysed the data excluding the biceps tendon. The results were essentially unchanged, with a sensitivity of 80.5% and a specificity of 92.5% for a TSF WCC cut-off of 0.67 WCC per $10^9$/L. However, in the comparison of DS between diagnostic groups, with biceps excluded, DS in those with IC was significantly higher than NIC ($p<0.05$). The tendon location group (biceps vs hand/wrist vs foot/ankle) did not statistically significantly influence DS or WCC.

## DISCUSSION

This is the first multicentre study of paired TSF analysis and Doppler US among patients seen in rheumatology practices. Our results show that a negative DS can help rule out inflammatory causes of tenosynovitis (93.8% sensitivity). Although specificity of positive DS is low (31.8%), TSF analysis can provide complementary information to the DS. TSF WCC was higher in the

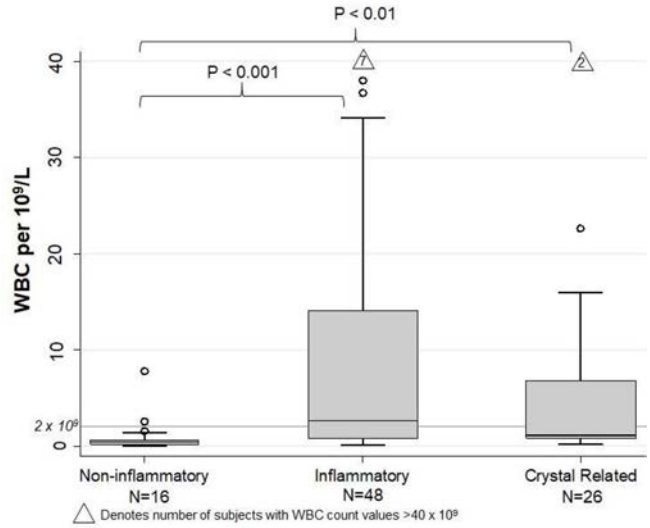


**Figure 3** Comparison of tenosynovial fluid white cell count between diagnosis groups. Total n=90. WBC, white blood cell.

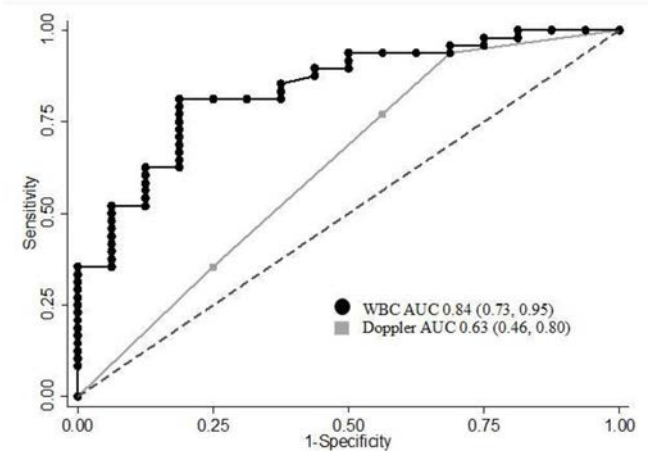


**Figure 5** ROC curve of WBC and Doppler for classifying IC versus NIC. P=0.04 comparing these ROC curves. Total n=90. AUC, area under the curve; ROC, receiver operating characteristic; WBC, white blood cell.

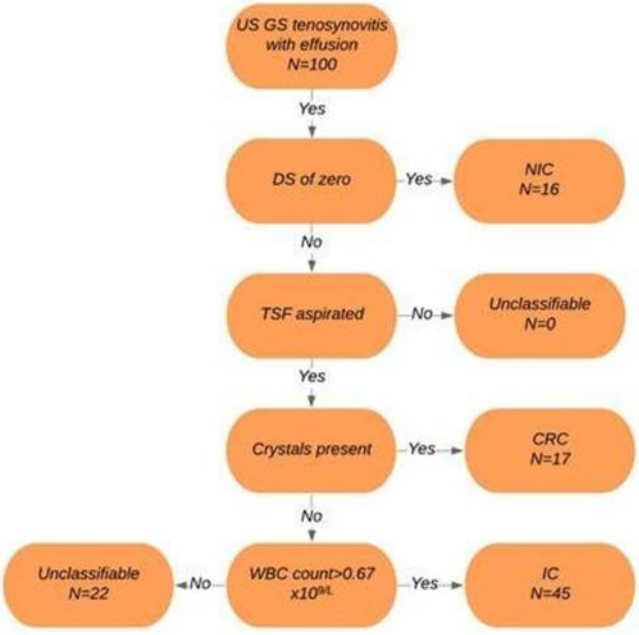


**Figure 6** Flow chart of the diagnostic algorithm for classifying subjects into a diagnostic category. CRC, crystal-related condition; DS, Doppler score; IC, inflammatory conditions; NIC, non-inflammatory conditions; TSF, tenosynovial fluid; US, ultrasound; US GS, ultrasound grey scale; WBC, white blood cell.

IC group than in the NIC group (median TSF WCC per $10^9$/L of 2.58 vs 0.38, respectively), making TSF useful in ruling in inflammatory causes.

We suggest a lower WCC cut-off for discriminating IC versus NIC than that used for synovial fluid. Traditional synovial fluid analysis classifies joint fluid as normal if the WCC is <0.20 per $10^9$/L, non-inflammatory if <2.00 per $10^9$/L, inflammatory if between 2.00 and 50.00 per $10^9$/L, and infectious if >50.00 per $10^9$/L.[7] A cut-off of 2.00 WCC per $10^9$/L is 90% accurate in differentiating inflammatory and non-inflammatory causes.[6] In our study, synovial fluid WCC categories are not applicable to TSF, and a cut-off value of 0.67 WCC per $10^9$/L would better discriminate IC from NIC causing tenosynovitis. The optimal cut-off for TSF WCC requires further validation. The reason for the difference in WCC response is unknown. A study on 18 RA tenosynovectomy specimens[15] showed that the mean WCC was highest in the joint synovium and lowest in invasive tenosynovitis, although the actual numbers and whether WCC was from fluid or tissue were not reported. In contrast, the tenosynovial and synovial histology are reported to be similar.[16] We did not find a correlation between TSF volume and WCC as previously reported for synovial fluid.[17]

Our study establishes the important role of US-guided aspiration to obtain small, yet critical TSF volume to determine tenosynovial pathology. In our study, 30% of the samples were below 0.5 mL. These volumes may be too low for traditional laboratory processing, thus requiring the rheumatologist to perform crystal analysis and WCC. The extra time and skill to evaluate small tenosynovial volumes can be critical in diagnosing CRC.

While infiltrative tendon disease in gout is well known, tenosynovitis is rarely reported.[18] The presence of an articular Doppler signal in asymptomatic hyperuricaemia has been previously reported.[19] Similarly, calcium pyrophosphate deposition (CPPD) is primarily an articular disease, but tendon deposition[20 21] and rare cases of CPPD tenosynovitis[22] exist. Our study identified crystals in 22% of the cases (CPP 16% and MSU 6%). Our findings show that tenosynovitis is common in CPPD or that CPP can reside asymptomatically in the tenosynovium.

In our study, the tenosynovial WCC in the CRC group had a broad range (IQR 0.76–6.83 WCC per $10^9$/L). This may be due to the timing of aspiration (acute vs resolving vs intercritical phase) as we know MSU can be found in previously inflamed but currently asymptomatic joints,[23] or may reflect the different nature of crystalline inflammation in the tenosynovial environment. Synovial joint WCC can also vary substantially in CRC. In one study, about 24% of subjects with CRC had synovial WCC below 2.00 WCC per $10^9$/L.[24] The average WCC in bursal fluid (2.90 WCC per $10^9$/L) was much less than articular WCC (25.50 WCC per $10^9$/L) in acute gouty bursitis.[25] TSF seems similar to bursal fluid in this respect. Similarly, our mean WCC of 0.99 WCC per $10^9$/L (±SD 1.91) in the NIC group is similar to that of 0.88 WCC per $10^9$/L in an idiopathic olecranon bursitis group.[26] These findings support the conclusion that both bursal and TSF produce less WCC response compared with synovial fluid.

Our study is among the first to examine the association of TSF WCC and DS, and showed that correlation was present but weak. A similar weak correlation between DS and synovial fluid (Spearman's r of 0.28) was reported in 194 patients with acute arthritis.[27] Others have explored the relationship between synovial fluid T helper 17 cells and DS but did not report WCC.[28 29] While Doppler and TSF WCC only correlate weakly, Doppler and TSF WCC have important complementary diagnostic roles in tenosynovitis assessment. We show that Doppler signal is sensitive but not specific for inflammatory tenosynovitis, while a high TSF WCC is specific but not sensitive. Our proposed algorithm for tenosynovitis assessment leverages these properties to classify the majority of tenosynovitis with substantial agreement to the recorded final clinical diagnosis. TSF results were a factor in forming the final clinical diagnosis; therefore, we reanalysed the data using subjects whose preaspiration and postaspiration diagnostic category was unchanged and found no substantial change in the results. Moreover, while TSF results were a factor in forming the clinical diagnosis, clinicians had to rely on synovial fluid WCC cut-offs to make a diagnosis, and over 40% of the subjects with WCC below $2.00\times10^9$/L were still categorised as having an IC. Therefore, these data and the exploratory algorithm will facilitate clinical diagnostic decision tool development for future studies.

Our study has limitations. Study findings need validation through an independent cohort. There is selection bias as only subjects with a clinical indication of tenosynovial aspiration were included. Subjects who had tenosynovitis but no effusion were excluded. Reflective of real-world settings, multiple sonographers were involved. Machine heterogeneity and the resulting inherent equipment differences may have affected Doppler comparisons across sites. However, our Doppler standardisation method should have reduced this bias, and this equipment heterogeneity is reflective of routine clinical practices. Prestudy inter-rater reliability assessment was not done. Immunosuppressive medication and disease activity status, which could affect DS and TSF WCC, were not assessed. We did not use disease classification criteria but rather the gold standard measure of clinician diagnosis. This study was not designed to evaluate the impact of US assessment and TSF analysis in diagnosing tenosynovitis. Therefore, we cannot comment on the additional diagnostic information provided by this testing except for finding CRC in previously undiagnosed patients. The biceps tendon sheath accounted for 18% of our tenosynovial sites. Biceps tendon sheath fluid may track from the glenohumeral joint,[30 31] although some literature

states otherwise.[32] However, the presence of Doppler positive tenosynovitis in 15 of 18 (83.3%) biceps tendons in our study supports a primary tenosynovial pathology.[33] Furthermore, TSF WCC results were unchanged and DS comparisons were marginally but positively affected in the sensitivity analysis excluding the biceps tendon.

In conclusion, DS is a sensitive screening tool for inflammatory tenosynovitis, whereas TSF WCC provides specificity. If followed by TSF analysis for cell count and crystals, it shows substantial agreement with clinical diagnosis and can categorise the cause of tenosynovitis into the NIC, IC or CRC groups 78% of the time in our broad rheumatology patient sample. Neither the DS nor the TSF WCC can identify CRC; therefore, US is critical for aspirating minute TSF volumes.

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CLINICAL SCIENCE

# Real-world evidence of TNF inhibition in axial spondyloarthritis: can we generalise the results from clinical trials?

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## ABSTRACT

Management guidelines assume that results from clinical trials can be generalised, although seldom is data available to test this assumption. We aimed to determine the proportion of patients commencing tumour necrosis factor inhibition (TNFi) who would have been eligible for relevant clinical trials, and whether treatment response differs between these groups and the trials themselves. The British Society for Rheumatology Biologics Register for Ankylosing Spondylitis (BSRBR-AS) recruited a real-world cohort of TNFi-naïve spondyloarthritis patients with data collection from clinical records and patient questionnaires. Participant characteristics were extracted from trials identified from a recent Health Technology Assessment of TNFi for ankylosing spondylitis/non-radiographic axial spondyloarthritis. Descriptive statistics were used to determine the differences, including treatment response, between BSRBR-AS participants who would/would not have been eligible for the clinical trials and with trial participants. Among 2420 BSRBR-AS participants, those commencing TNFi (34%) had shorter symptom duration (15 vs 22 years) but more active disease (Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) 6.4 vs 4.0; Bath Ankylosing Spondylitis Disease Functional Index (BASFI) 6.2 vs 3.8). Of those commencing TNFi, 41% met eligibility criteria for ≥1 of fourteen relevant trials; they reported higher disease activity (BASDAI 6.9 vs 6.1) and poorer function (BASFI 6.6 vs 6.0). 61.7% of trial participants reported a positive treatment response, vs 51.3% of BSRBR-AS patients (difference: 10.4%; 95% CI 4.4% to 16.5%). Potential eligibility for trials did not influence treatment response (difference 2.0%; -9.4% to 13.4%). Fewer patients in the real world respond to TNFi than is reported in the trial literature. This has important implications for the generalisability of trial results, and the cost-effectiveness of TNFi agents.

## Key messages

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

► Data on the benefits of tumour necrosis factor inhibition (TNFi) come from clinical trials, although trial populations are seldom representative of the routine clinic population. It is unclear, therefore, how generalisable this data actually is.

**What does this study add?**

► Fewer patients in the real world respond to TNFi than is reported in the trial literature. This has important implications for the generalisability of trial results, and the cost-effectiveness of TNFi agents. Treatment response is unrelated to whether patients would have met eligibility for clinical trials although, overall, it is lower than in trial populations.

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

► The rheumatologist needs to consider that the proportion of patients who achieve a satisfactory treatment response to TNF inhibition will be lower than might be expected from clinical trials. This has implications for cost-effectiveness of therapy and perhaps, therefore, choice of agent.

## INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic arthritis affecting the sacroiliac joints and spine, although peripheral joint involvement is common, as are several extra-articular features. Treatment, historically, has been with non-steroidal anti-inflammatory drugs (NSAIDs), plus physiotherapy/hydrotherapy, although tumour necrosis factor α inhibitors (TNFi) have revolutionised patient management. Targeting inflammation and reducing disease activity,[1] they also have demonstrable effects on outcomes identified as important for patients, such as work productivity,[2] and their use is common: we have shown that, in the Scotland Registry for Ankylosing Spondylitis, around one-third of patients either are, or have been, on TNFi.[3]

In 2009, the Assessment of Spondyloarthritis International Society (ASAS) proposed the patients were classified according to whether they have imaging evidence of sacroiliitis; and if so, whether there are X-ray changes or not.[4] In the UK, early guidelines from the British Society for Rheumatology,[5] and the National Institute of Health and Care Excellence (NICE),[6 7] advocated TNFi only among patients with radiological evidence of sacroiliitis—reflecting the patient population of trials at the time. More recent guidance recommends that in the absence of evidence of radiological sacroiliitis patients may be offered TNFi providing they have a positive MRI and/or elevated acute phase reactants.[8] Similarly, the updated NICE Technology Appraisal approves



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five agents for the treatment of ankylosing spondylitis, three of which may also be used for the treatment of non-radiographic axSpA.[9]

Much of what we know about the benefits of TNFi in axSpA comes from clinical trials, although it is acknowledged that patients recruited to trials may not be representative of the routine clinical population. Trials often have restrictive eligibility criteria and recruit patients from specialist centres. In rheumatoid arthritis, it has been demonstrated that only a small proportion of patients in observational clinical cohorts meet biological agent trial eligibility criteria, raising concern about the extrapolation of the trial results.[10] Others have provided some evidence that disease-modifying anti-rheumatic drugs (DMARD) treatment response may be superior among randomised trial participants than in daily clinical practice, although the data was equivocal.[11] However, the assumption is commonly made that the treatment response observed in trials will be generalisable to the wider patient population—although seldom is data available to test this assumption.

The current study takes advantage of a large nationwide cohort providing real-world evidence on the use of biologics in axSpA and aims to determine the proportion of patients commencing TNFi in a real-world setting who may or may not have been eligible for the clinical trials that led to the licencing and approval of TNFi, and to determine whether there is a difference in treatment response between these groups.

## METHODS

### British Society for Rheumatology Biologics Register for Ankylosing Spondylitis data

Between December 2012 and December 2017, the British Society for Rheumatology Biologics Register for Ankylosing Spondylitis (BSRBR-AS) recruited patients meeting the ASAS classification criteria for axSpA[4] who were naïve to biological therapy. The protocol is published elsewhere,[12] but in brief: patients were recruited from 83 rheumatology departments across Great Britain. Initially, only patients meeting the ASAS imaging criteria were eligible, although from November 2014 patients meeting the ASAS clinical criteria were also included. Patients remaining on conventional therapy were recruited into a non-biologic cohort; whereas those commencing TNFi were recruited into a biological cohort. Eligible biological agents included Adalimumab (Humira) and Etanercept (Enbrel) from the start of study, plus Certolizumab Pegol (Cimzia) from August 2015, and Etanercept (Benepali) from November 2016. All patients were followed up annually, with additional follow-up at 3 months and 6 months for patients commencing TNFi.

Clinical data were collected from medical records, including: spinal mobility (Bath Ankylosing Spondylitis Metrology Index; BASMI[13]), acute phase reactants (C reactive protein (CRP) and erythrocyte sedimentation rate) and extra-articular features (uveitis, psoriasis, inflammatory bowel disease). Information was also collected on the use of other medication, and on various comorbidities (angina, congestive heart failure, stroke, hypertension, diabetes, asthma, bronchitis, peptic ulcer, liver disease, renal disease, tuberculosis, demyelination, depression and malignancy). At each point, participants were sent a postal questionnaires, asking about lifestyle factors, disease activity (Bath Ankylosing Spondylitis Disease Activity Index, BASDAI[14]) and function (Bath Ankylosing Spondylitis Disease Functional Index, BASFI[15]).

### Randomised trial data

Placebo-controlled randomised controlled trials to determine the clinical effectiveness of the TNF inhibitors were identified from the Health Technology Assessment that informed the NICE Technology Appraisal on TNFi for ankylosing spondylitis and non-radiographic axSpA.[16] Key characteristics of trial participants were extracted from the original articles for the TNFi agents included in the BSRBR-AS, including, where available: age and gender, disease duration, BASDAI, BASFI, BASMI, HLA-B27 status, plus an objective measure of inflammation (CRP).

Detailed information on trial inclusion/exclusion criteria was extracted; including the age and disease state of included participants, plus diagnoses which would necessitate exclusion (see online supplementary table S1). For each participant in the BSRBR-AS commencing Humira, a count was made of how many Humira trials that patient would have been eligible for. This was repeated for Cimzia, and Enbrel trials were used for patients starting either Enbrel or Benepali.

### Statistical analysis

Simple descriptive statistics were used to describe the BSRBR-AS cohort and the differences between participants who were/were not commencing TNFi. Characteristics of trial participants were summarised by pooling data across trials using a weighted arithmetic mean. Differences between trial and BSRBR-AS participants were then quantified and presented with 95% CIs. Treatment response was determined using the ASAS-20 response criteria, a four-domain outcome based on patient global assessment, pain, function and inflammation[17] and the most common outcome measure reported in the trials. For BSRBR-AS patients commencing TNFi, treatment response was determined at the first contact with the study in the period 10 weeks to 9 months after commencement of TNFi therapy. This permited measurement of the outcome within the first two follow-up periods of the study (but allowing for early or late clinic visits).

All analyses were conducted in Stata V.14.1 (StataCorp) and used the June 2017 version of the BSRBR-AS dataset.

## RESULTS

The 2419 BSRBR-AS participants had a mean age of 48 years (SD=14); 68% were male; and 67% met the modified New York criteria for ankylosing spondylitis, 29% met the ASAS axSpA imaging criteria but not the modified New York, and 4% met solely the ASAS clinical criteria. Mean age at symptom onset was 29 years (SD=12) and mean symptom duration was 19 years (SD=14) and at recruitment participants had a mean CRP of 43 (SD=216) mg/L. In clinic, 57% had been tested for HLA-B27, of whom 81% were positive. A total of 816 participants (34%) were starting TNFi: 526 (64%) were starting Adalimumab (Humira); 207 (25%) Etanercept (Enbrel); 17 (2%) Etanercept (Benepali) and 66 (8%) Certolizumab Pegol (Cimzia). Median time from treatment decision to commencing therapy was 24 days (IQR: 0–46 days).

Participants commencing TNFi were younger (mean age 44.3 vs 50.1 years; difference 5.8 years (95% CI 4.6 to 7.0 years)). They had shorter symptom duration (mean duration 14.9 vs 21.8 years; difference 6.9 years (5.7 to 8.1 years)) and more severe disease, as determined by the Bath Indices (mean BASDAI 6.4 vs 4.0; difference 2.4 (2.2 to 2.6); mean BASFI 6.2 vs 3.8; difference 2.3 (2.1 to 2.6); and mean BASMI 4.2 vs 3.6; difference 0.6 (0.4 to 0.8)).

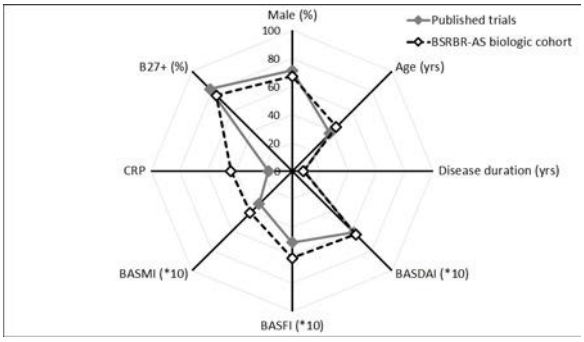


**Figure 1** Differences between BSRBR-AS biological cohort and randomised trial participants. BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; BSRBR-AS, British Society for Rheumatology Biologics Register for Ankylosing Spondylitis; CRP, C reactive protein.

### Randomised trial data

Fourteen randomised trials were identified, comprising 2437 participants, including six for Humira (n=1018), one for Cimzia (n=325) and seven for Enbrel (n=1094). Trial inclusion criteria were broadly similar, requiring active disease (commonly defined as a BASDAI ≥4 out of 10) plus a combination of back pain, morning stiffness and a failure to tolerate NSAIDs. Whereas, exclusion criteria mainly related to prior/current therapy or persons with a relevant history in relation to safety issues under investigation (see online supplementary table S1).

Trial participants had a mean age of 38 years; 71% were male. Participants had a mean disease duration of 8.5 years and mean BASDAI, BASFI and BASMI of 6.2, 5.1 and 3.3, respectively. Eighty-two per cent were HLA-B27 positive and participants had a mean CRP of 17 mg/L.

There were several differences between the randomised trial participants and the BSRBR-AS biological cohort (see figure 1 and table 1). Although the differences were not large, the BSRBR-AS had a significantly smaller proportion of male participants (67% vs 71%) and a lower proportion of participants who were HLA-B27 positive (76% vs 82%). BSRBR-AS participants were approximately 6 years older than trial participants although no real difference in disease duration. They reported similar disease activity (BASDAI: 6.4 vs 6.2; difference 0.2; 95% CI −0.3 to 0.7), although poorer function (BASFI: 6.2 vs 5.1; difference 1.1; 95% CI 0.5 to 1.8) and poorer spinal mobility (BASMI: 4.2 vs 3.3; difference 1.0; 95% CI 0.8 to 1.1).

### Treatment response

Of the 816 BSRBR-AS participants commencing TNFi, only 333 (41%) would have been eligible for any of the relevant trials (see table 2). There were differences between agents: adalimumab (30%), certolizumab pegol (50%) and etanercept (64%).

There were no large differences between BSRBR-AS biological cohort participants who did/did not meet any clinical trial eligibility criteria (see table 3). However, a slightly higher disease activity was reported among participants who would have been eligible for at least one trial, vs those eligible for none (BASDAI: 6.9 vs 6.1; difference 0.8; 95% CI 0.5 to 1.1). Similarly, participants eligible for at least one trial reported poorer function (BASFI: 6.6 vs 6.0; difference 0.6; 95% CI 0.2 to 1.0).

Ten of the 14 trials reported ASAS20 response criteria, and 864/1401 participants reported a positive treatment response (61.7%). In the BSRBR-AS biological cohort, follow-up data were available for 318 (39%), in whom 163 (51.3%) achieved an ASAS20 treatment response (difference: 10.4%; 95% CI 4.4% to 16.5%). Exactly 50% of participants who would have been eligible for at least one clinical trial achieved a positive treatment response, compared with 52% of those who did not meet any trial eligibility criteria (difference 2.0%; 95% CI −9.4% to 13.4%).

## DISCUSSION

This is the first paper of which we are aware to examine the generalisability of results from trials to real world prescribing in axSpA. We have shown that there are a number of differences in patients commencing TNFi, versus those in the trials that led to the licensing of these drugs. In the clinical trials, participants are more likely to be male, younger and HLA-B27 positive. Also, despite similar disease activity, they are likely to report better function prior to treatment. Reassuringly, treatment response in BSRBR-AS biological patients was unrelated to whether they would have met eligibility for the trials although, overall, treatment response was significantly lower than that reported in clinical trials.

It is important to consider what might explain these findings—especially because the likelihood of meeting response criteria was not related to factors determining eligibility for trials. BSRBR-AS participants were approximately 6 years older and we have previously demonstrated that for every additional year of age, there is a 1% reduction in the odds of achieving

**Table 1** Key characteristics of BSRBR-AS biological cohort, versus randomised trial participants

| | BSRBR-AS* | | Randomised trials* | | Difference† (95% CI) | |
|---|---|---|---|---|---|---|
| Sex (male) | 67.2% | (n=816) | 71.3% | (n=2437) | −4.1% | (−7.8% to −0.4%) |
| Mean age (years) | 44.3 | (n=816) | 37.9 | (n=2114) | 6.4 | (5.4 to 7.3) |
| Disease duration (years) | 8.1 | (n=816) | 8.5 | (n=1534) | −0.4 | (−1.1 to 0.4) |
| Bath indices | | | | | | |
| Mean BASDAI | 6.4 | (n=699) | 6.2 | (n=2036) | 0.2 | (−0.3 to 0.7) |
| Mean BASFI | 6.2 | (n=707) | 5.1 | (n=2076) | 1.1 | (0.5 to 1.8) |
| Mean BASMI | 4.2 | (n=604) | 3.3 | (n=1512) | 1.0 | (0.8 to 1.1) |
| CRP (mg/L) | 43.3 | (n=699) | 16.7 | (n=1644) | 26.6 | (16.1 to 37.1) |
| HLA-B27 positive | 75.7% | (n=543) | 82.3% | (n=1939) | −6.6% | (−10.6% to −2.6%) |

*Numbers vary for BSRBR-AS, due to missing data. Numbers vary for randomised trials because not each trial reported each characteristic.
†BSRBR-AS minus trials. Therefore, a positive result indicates a higher value in the BSRBR-AS biologic cohort.
BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; BSRBR-AS, British Society for Rheumatology Biologics Register for Ankylosing Spondylitis; CRP, C reactive protein.

**Table 2** Count of trials for which BSRBR-AS participants met eligibility criteria

| N | Adalimumab (six trials) | | | | Etanercept (seven trials) | | | | Certolizumab pegol (one trial) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (Trials) | N | % | Trials | Cumv %* | N | % | Trials | Cumv %* | N | % | Trials | Cumv %* |
| 0 | 370 | 70.3 | – | – | 80 | 35.7 | – | – | 33 | 50.0 | – | – |
| 1 | 42 | 8.0 | ≥1 | 30 | 39 | 17.4 | ≥1 | 64 | 33 | 50.0 | 1† | 50.0 |
| 2 | 78 | 14.8 | ≥2 | 22 | 23 | 10.3 | ≥2 | 47 | | | | |
| 3 | 7 | 1.3 | ≥3 | 7 | 14 | 6.3 | ≥3 | 37 | | | | |
| 4 | 29 | 5.5 | ≥4 | 6 | 33 | 14.7 | ≥4 | 30 | | | | |
| 5 | 0 | 0 | ≥5 | 0 | 35 | 15.6 | ≥5 | 16 | | | | |
| 6 | 0 | 0 | 6† | 0 | 0 | 0 | ≥6 | 0 | | | | |
| 7 | | | | | 0 | 0 | 7† | 0 | | | | |

*Cumulative percent—that is, proportion of patients who meet eligibility criteria for at least this number of trials.
†Maximum number of trials available, for this agent.
BSRBR-AS, British Society for Rheumatology Biologics Register for Ankylosing Spondylitis.

ASAS20 treatment response. However, this difference is not statistically significant (OR 0.99, 95% CI 0.97 to 1.004)[18] and cannot explain the difference observed in the current study. Across several different response criteria this previous work has also shown that persons who do not respond to TNFi are characterised by not being in full-time employment, and by leaving formal education earlier; they report worse scores on questionnaires of mood and mental health, and experience fewer comorbidities.[18] With the exception of various specific comorbidities, none of these were trial exclusion criteria. It is possible that there is a selection of patients into trials favouring those who are better educated, with higher socioeconomic status, better mental and overall health. This would explain why treatment response in the BSRBR-AS was lower than that achieved in the trials, even though basic clinical characteristics between studies were broadly similar. Alternatively, and equally plausible, it may be that non-specific effects are stronger in randomised trials, with the overt experimental testing of a novel agent, and with intensive follow-up. This is speculative, although underlines the importance of harnessing these effects in the real world.

There is an argument that, due to classification criteria being misused for diagnostic purposes, a proportion of patients, in reality, do not have axSpA. It is possible, therefore, that if this occurs with greater frequency in the BSRBR-AS than in the trial populations, then this might contribute to the difference in treatment response between groups. However, 96% of the BSRBR-AS participants had objective evidence of sacroiliitis—the defining feature of axSpA—and thus any effect of this in the current study is likely to be small.

Could the findings be explained by selective attrition? Although fewer BSRBR-AS participants provided follow-up data than in the clinical trials, to account for the observed difference BSRBR-AS participants lost to follow-up would have to be one-third more likely to achieve ASAS20 response than those who provided follow-up data. We believe this is unlikely.

ASAS-20 is one of many available outcome measures. Indeed, recently, other trials have adopted a higher bar, such as ASAS-40. However, ASAS-20 was chosen for the pragmatic reason that, among the relevant trials it was the most common outcome measure (10 of 14 trials) and was therefore the most appropriate measure for comparison. Ideally, we could have examined a composite measure encapsulating patient-reported and objectively measured aspects, such as change in the Ankylosing Spondylitis Disease Activity Score (ASDAS). Pooling observational data from 12 registries across Europe, others have shown that nearly twice as many patients report ASAS-20 response at 6 months, compared with the proportion who achieve ASDAS inactive disease: 64% vs 33%.[19] There were also differences in the timing of outcome measurement between the clinical trials and the BSRBR-AS. All trials measured treatment outcome at 12 weeks; whereas, in the BSRBR-AS, treatment response was assessed at the first study contact between 10 weeks and 9 months. This is a pragmatic reflection of clinical practice, where data collection is not mandated by study protocol. Could this explain the current findings? In the longer term, it may be that patients who achieve satisfactory treatment response are less likely to attend clinic (although infrequent

**Table 3** Comparison of BSRBR-AS biological cohort participants, those who did/did not meet any clinical trial eligibility criteria

| | Eligible for any clinical trials | Not eligible for any clinical trials | Difference* (95% CI) | |
|---|---|---|---|---|
| Sex (male) | 68.8% | 66.0% | –2.7% | (–3.8% to 9.3%) |
| Mean age (years) | 43.0 | 45.2 | –2.2 | (–4.0 to –0.3) |
| Disease duration (years) | 7.1 | 8.5 | –1.7 | (–3.2 to –0.3) |
| Bath indices | | | | |
| Mean BASDAI | 6.9 | 6.1 | 0.8 | (0.5 to 1.1) |
| Mean BASFI | 6.6 | 6.0 | 0.6 | (0.2 to 1.0) |
| Mean BASMI | 4.2 | 4.3 | –0.03 | (–0.3 to 0.3) |
| CRP (mg/L) | 34.8 | 49.2 | –1.4 | (–4.7 to 1.8) |
| HLA-B27 positive | 72.9% | 77.5% | –4.6 | (–12.1% to 2.9%) |
| ASAS20 treatment response | 50.0% | 52.0% | 2.0% | (–9.4% to 13.4%) |

*Eligible minus non-eligible. Therefore, a positive result indicates a higher value in the 'eligible' subcohort.
ASAS, Assessment of Spondyloarthritis International Society; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; BSRBR-AS, British Society for Rheumatology Biologics Register for Ankylosing Spondylitis; CRP, C reactive protein.

attendance may result in cessation of treatment). However, it is harder to argue that this is likely for the first follow-up, when the initial treatment response (and maintenance of therapy) is to be determined.

The context of a randomised trial and routine clinical practice differ markedly. In trials, treatment is likely to commence soon after randomisation, and the identification of early treatment failure is important because of the ethical imperative to get participants randomised to placebo switched to the treatment that is believed to be superior. Whereas, in the real world, after the clinical decision has been made for a patient to start TNFi, it may take several weeks for a patient to receive the medication. The counterargument is that, in the real-world, a physician may wish to start certain patients on active treatment immediately, rather than 'risk' randomisation to placebo. These patients, unlikely to be in the trials, may be less likely to achieve a good treatment response.

In the BSRBR-AS treatment response was determined at the first follow-up data point at least 10 weeks, but no more than 9 months after commencing TNF inhibition. The median (IQR) follow-up was 14 weeks (12–7 weeks) and all participants, by definition, had been on therapy for at least 2.5 months. Although the timing of outcome in the clinical trials was more consistent, at 12 weeks, it is unlikely that the superior treatment response in the trials is due to large differences in follow-up.

Finally, one must also consider the generalisability of findings to other TNFi agents. Patients in trials probably get better overall care: the follow-up response rate is certainly greater, and this emphasises the importance of regular patient follow-up, perhaps even with treat-to-target strategies. Indeed, one may argue that this may be more important than which specific agent is administered. Although there are other anti-TNF agents (and indeed non-TNFi biologics), we only included clinical trials reporting adalimumab (Humira), certolizumab pegol (Cimzia) and etanercept (Enbrel and Benepali). This omission was important to preserve comparability between the trial data and the BSRBR-AS in which patients commencing other agents were not eligible for recruitment. It would be interesting to replicate this analysis with other agents, including recent biosimilars, although it is hard to think of why the similarities and differences between real-world and trial data would be different to the results that are reported here.

In summary, using a large nationally representative sample of patients with axSpA, we have shown several differences between patients commencing TNFi and the trial populations that led to the treatment guidelines for—and, ultimately, access to—these agents. Participants in clinical trials tend to have better function and spinal mobility, and lower CRP prior to the commencement of therapy. However, we found no difference in disease activity, the key feature indicating commencement of TNFi.

While the rheumatologist will already exercise caution when generalising trial results to the patients in clinic—and we provide evidence in support of this—the development of clinical guidelines is based on data from randomised trials. We have shown that in the real world, the proportion of patients who achieve a satisfactory treatment response is lower than is observed in the trials themselves. The inferior treatment response in patients outside the trial setting has important implications for the cost-effectiveness of these agents, particularly with incremental cost effectiveness ratios approaching the upper limit of what is considered acceptable.[16] In the era of biosimilars the cost-effectiveness of originator products is already being challenged.

Patients with an increasing number of comorbidities are likely to be excluded from clinical trials. We have shown elsewhere that this is also a predictor of response to TNFi in a real-world spondyloarthritis population.[18] In addition, we found that higher socioeconomic status, longer education and better mental health were independent predictors of response. We hypothesise that there is a selection bias into randomised clinical trials favouring the more educated and more affluent, and those with better mental health, and that this results in trial participants having a superior chance of positive outcome, compared with the real-world patients that they ostensibly represent.

**Correction notice** This article has been corrected since it published Online First. A minus sign has been added to difference 2.0%; -9.4% to 13.4% in the abstract.

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**Contributors** GTJ and GJM designed the study and oversaw data collection (primary data). LED extracted the data (literature review). GTJ conducted the analysis and all authors were involved in interpretation of data. GTJ drafted the manuscript to which all authors provided critical contribution. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. All authors have given final approval of the version to be submitted for publication and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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**Competing interests** None declared.

**Patient and public involvement** The British Society for Rheumatology's Registers Committee, which oversees the running of the BSRBR-AS, has patient representation from several arthritis charities, including the (UK) National Axial Spondyloarthritis Society. Although no patients or public were involved in the analysis for this manuscript, the research question was identified at a priority setting exercise involving scientists, rheumatology consultants, and patient representatives.

**Patient consent for publication** Not required.

**Ethics approval** The study was approved by the National Research Ethics Service (NRES) Committee North East – County Durham and Tees Valley (Research Ethics Committee (REC) reference 11/NE/0374).

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**Data availability statement** Data from the British Society for Rheumatology Biologics Register for Ankylosing Spondylitis are available to external investigators, on reasonable request. For information on how to access data, see: http://www.rheumatology.org.uk/.

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CLINICAL SCIENCE

# Maintenance of clinical remission in early axial spondyloarthritis following certolizumab pegol dose reduction

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## ABSTRACT

**Background** The best strategy for maintaining clinical remission in patients with axial spondyloarthritis (axSpA) has not been defined. C-OPTIMISE compared dose continuation, reduction and withdrawal of the tumour necrosis factor inhibitor certolizumab pegol (CZP) following achievement of sustained remission in patients with early axSpA.

**Methods** C-OPTIMISE was a two-part, multicentre phase 3b study in adults with early active axSpA (radiographic or non-radiographic). During the 48-week open-label induction period, patients received CZP 200 mg every 2 weeks (Q2W). At Week 48, patients in sustained remission (Ankylosing Spondylitis Disease Activity Score (ASDAS) <1.3 at Weeks 32/36 and 48) were randomised to double-blind CZP 200 mg Q2W (full maintenance dose), CZP 200 mg every 4 weeks (Q4W; reduced maintenance dose) or placebo (withdrawal) for a further 48 weeks. The primary endpoint was remaining flare-free (flare: ASDAS ≥2.1 at two consecutive visits or ASDAS >3.5 at any time point) during the double-blind period.

**Results** At Week 48, 43.9% (323/736) patients achieved sustained remission, of whom 313 were randomised to CZP full maintenance dose, CZP reduced maintenance dose or placebo. During Weeks 48 to 96, 83.7% (87/104), 79.0% (83/105) and 20.2% (21/104) of patients receiving the full maintenance dose, reduced maintenance dose or placebo, respectively, were flare-free ($p<0.001$ vs placebo in both CZP groups). Responses in radiographic and non-radiographic axSpA patients were comparable.

**Conclusions** Patients with early axSpA who achieve sustained remission at 48 weeks can reduce their CZP maintenance dose; however, treatment should not be completely discontinued due to the high risk of flare following CZP withdrawal.

**Trial registration number** NCT02505542, ClinicalTrials.gov.

## Key messages

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

- Tumour necrosis factor inhibitors (TNFi) are effective for the management of axial spondyloarthritis (axSpA), including radiographic and non-radiographic axSpA, with many patients able to achieve a state of low disease activity and remission.
- Previous studies exploring remission induction-and-maintenance strategies have shown that discontinuing TNFi after achieving remission can lead to flares in the majority of patients. However, few studies have assessed remission maintenance in a broad axSpA population, and none have formally tested a dose reduction strategy in axSpA.

**What does this study add?**

- C-OPTIMISE is the first randomised controlled trial to compare both TNFi dose continuation and dose reduction with the effects of treatment withdrawal in patients with axSpA who achieved sustained clinical remission after 48 weeks' open-label certolizumab pegol (CZP) treatment.
- During the randomised period of the study, significantly higher proportions of patients who continued on either a full or reduced CZP maintenance dose remained flare-free (83.7% and 79.0%, respectively) than patients who had CZP treatment withdrawn (20.2%).

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

- CZP maintenance dose reduction is a feasible option for the long-term management of patients with axSpA in remission, preserving the clinical benefits of remaining on TNFi treatment, reducing costs and limiting patients' long-term exposure to immunosuppressive therapy.

## INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory rheumatic disease that affects the spine and sacroiliac joints, causing pain, stiffness and fatigue.[1–3] It usually manifests in early adulthood,[4] and encompasses patients with radiographic sacroiliitis (radiographic axSpA) and those without (non-radiographic axSpA). Symptoms cause considerable impairment to patients' physical function, work productivity and quality of life.[5 6]

Achievement of a state of low disease activity or remission is key to optimising health-related

quality of life in patients with axSpA, and in many patients this can be reached through treatment with tumour necrosis factor inhibitors (TNFi). The high costs of TNFi[7] and the possible consequences of long-term immunosuppression have raised the question of how remission, once achieved, should best be maintained. Trials in different systemic autoimmune diseases have explored remission induction-and-maintenance strategies.[8–10] Such strategies have not been formally tested in patients with axSpA, although previous studies have suggested that complete treatment withdrawal often leads to relapse.[11 12] Therefore, a key question remaining for clinicians is whether to maintain or reduce TNFi treatment in patients in whom sustained remission has been induced.

The PEGylated, Fc-free TNFi certolizumab pegol (CZP) is an effective and well tolerated treatment across the axSpA spectrum.[13 14] C-OPTIMISE is the first phase 3b randomised treatment strategy trial that evaluated TNFi dose reduction in patients with early axSpA in whom sustained remission had been induced. The study included a 48-week open-label induction period, followed by a 48-week randomised, double-blind maintenance period evaluating maintenance of remission following CZP dose continuation, CZP dose reduction or complete withdrawal.

## METHODS

### Study design

C-OPTIMISE was a two-part, phase 3b multicentre study evaluating maintenance of remission in adult patients with early active axSpA. Patients were enrolled into the study from 108 study sites between 31 July 2015 and 24 March 2017.

During the induction period (baseline to Week 48) patients received open-label CZP 200 mg every 2 weeks (Q2W; after a loading dose of CZP 400 mg at Weeks 0, 2 and 4) for 48 weeks. Patients who achieved sustained remission in this period were eligible to enter the second part of the trial (maintenance period; Weeks 48 to 96). Sustained remission was defined as Ankylosing Spondylitis Disease Activity Score[15 16] (ASDAS) inactive disease (ASDAS-ID: ASDAS <1.3) at Week 32 or 36, and at Week 48 (with ASDAS <2.1 for Weeks 32 and 36).

The maintenance period (Weeks 48 to 96) was a randomised, parallel-group, double-blind, placebo-controlled 48-week study period, which evaluated the efficacy and safety of CZP in patients with sustained remission who received CZP 200 mg Q2W (full maintenance dose), CZP 200 mg every 4 weeks (Q4W; reduced maintenance dose) or placebo. Randomisation (1:1:1) was stratified by geographical region and presence or absence of radiographic sacroiliitis. The primary outcome was remaining flare-free during the maintenance period. Flare was defined as: ASDAS ≥2.1 (high disease activity) at two consecutive visits, or ASDAS >3.5 (very high disease activity) at any visit.

The maintenance period included an early escape arm for those patients who experienced a flare. Patients who escaped received open-label CZP 200 mg Q2W for a minimum of 12 weeks to assess possible return to clinical remission. Those escaping from the placebo arm received a loading dose of CZP 400 mg at 0, 2 and 4 weeks into the escape arm.

### Patient and public involvement

Patients were not involved in the study design or conduct. A lay summary reporting study outcomes will be made available on the study sponsor website approximately 1 year after the last patient assessment.

### Patients

Patients eligible for inclusion were 18 to 45 years of age, had a documented diagnosis of axSpA (starting at age 18 or older) meeting the Assessment of SpondyloArthritis international Society (ASAS) classification criteria,[17] symptom duration ≥3 months and <5 years and active disease (defined as ASDAS ≥2.1, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) ≥4 and spinal pain ≥4 on a 0 to 10 numerical rating scale (BASDAI item 2)).

Patients were subclassified as having either radiographic axSpA (fulfilling the imaging criterion of the modified New York classification criteria[18] (radiographic sacroiliitis was confirmed by two central readers, plus an adjudicator if necessary)) or non-radiographic axSpA (fulfilling the ASAS but not the modified New York criteria imaging criterion). In addition, patients with non-radiographic axSpA had to have either a C-reactive protein level above the upper limit of normal or evidence of active sacroiliitis on MRI (using ASAS/Outcome Measures in Rheumatology (OMERACT) definition of a positive MRI, confirmed by two central readers and an adjudicator if necessary). All patients must have had inadequate response, contraindication or intolerance to ≥2 non-steroidal anti-inflammatory drugs. Permitted concomitant medications included stable doses of certain analgesics, non-steroidal anti-inflammatory drugs and disease-modifying anti-rheumatic drugs (online supplementary table S1).

Full patient selection criteria are provided in the online supplementary appendix. All patients provided informed consent to participate.

### Outcome measures

The primary outcome of the C-OPTIMISE study was the percentage of patients remaining flare-free during the maintenance period; the main secondary outcome was time to flare. Additional key secondary outcomes included the percentage of patients achieving sustained remission at Week 48, and assessment of disease activity at Week 96. Disease activity measures included assessment of ASDAS status (ASDAS-ID, low disease activity, high disease activity and very high disease activity),[16] ASDAS major improvement (MI; ASDAS reduction from baseline of ≥2.0) and clinically important improvement (CII; ASDAS reduction from baseline of ≥1.1),[16] ASAS response (ASAS20, ASAS40, ASAS5/6), ASAS partial remission,[19 20] BASDAI50 response and change from maintenance period baseline (Week 48) in ASDAS, BASDAI,[21] Bath Ankylosing Spondylitis Functional Index (BASFI),[22] Bath Ankylosing Spondylitis Metrology Index (BASMI; linear definition)[23 24] and MRI outcomes, including sacroiliac joint Spondyloarthritis Research Consortium of Canada (SIJ SPARCC) score[25] and the Berlin modification of the Ankylosing Spondylitis spine MRI score for activity (ASspiMRI-a).[26]

Additional outcomes included assessment at Week 96 of Maastricht Ankylosing Spondylitis Enthesitis Score[27] and tender and swollen joint counts (44 joints evaluation). For patients who experienced a flare during the maintenance period, outcomes at 12 weeks after escape to open-label CZP 200 mg Q2W are reported.

All treatment-emergent adverse events (TEAEs) were reported for the Safety Set (patients who received ≥1 dose CZP) up to 70 days after the last dose of study medication. TEAEs were coded according to Medical Dictionary for Regulatory Activities (MedDRA) V.19.0.

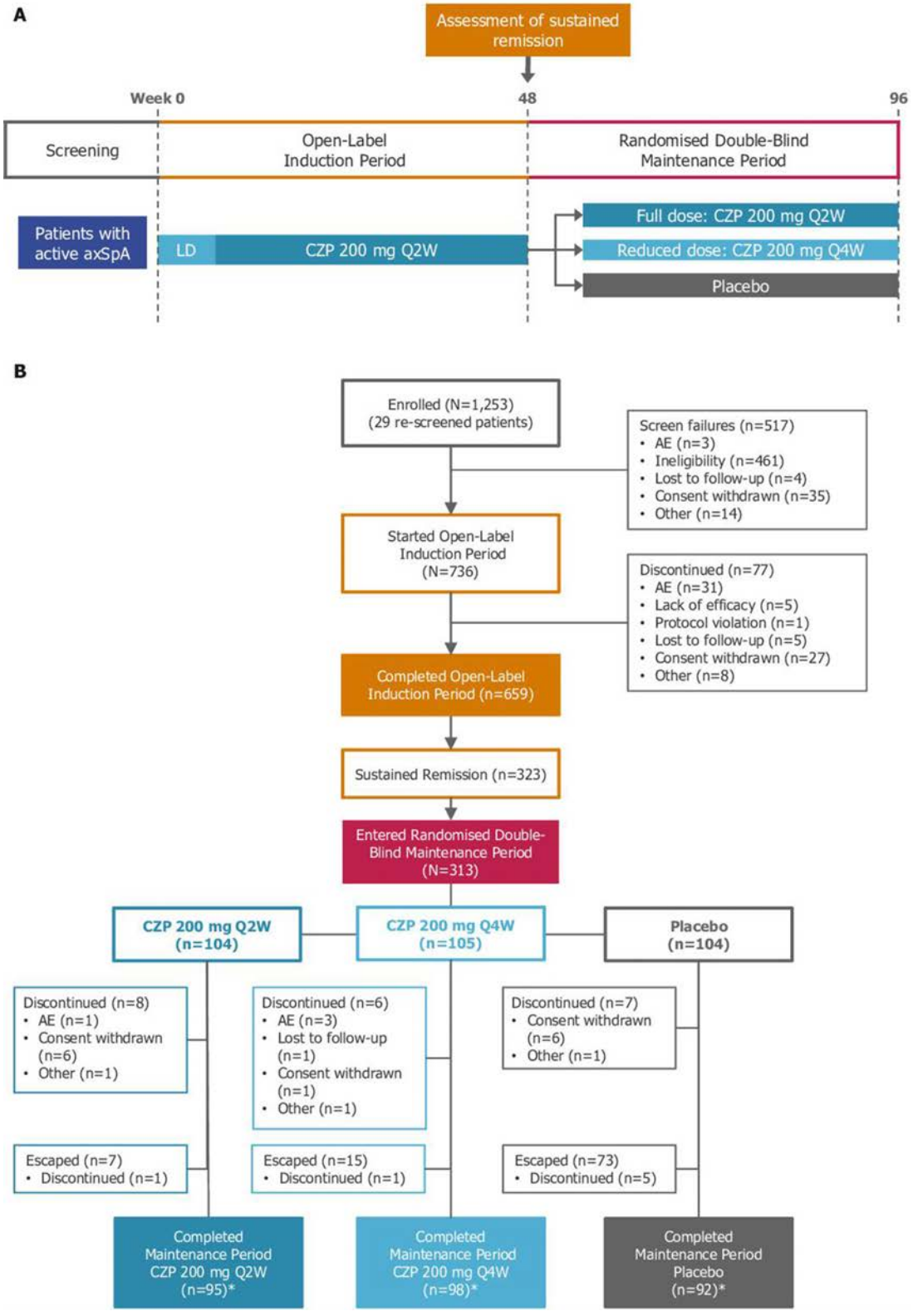


**Figure 1** C-OPTIMISE study design (panel A) and patient disposition (panel B). AE, adverse event; axSpA, axial spondyloarthritis; CZP, certolizumab pegol; LD, loading dose; Q2W, every 2 weeks; Q4W, every 4 weeks. *Includes patients in the escape arm who completed week 96.

### Study procedures and evaluations

Outcomes were assessed during study visits, scheduled for Weeks 2, 4, 12, 24, 32, 36, 48, 52, 60, 72, 84 and 96. During the maintenance period, ASDAS (used to define disease flare) was evaluated at Weeks 48, 50, 52, then every 4 weeks up to Week 96. For patients escaping to open-label treatment after experiencing a flare in the maintenance period, ASDAS components were assessed at 0, 2 and 4 weeks into the escape arm, then every 4 weeks up to Week 96.

### Statistical analysis

Assuming that 80%, 75% and 45% of CZP 200 mg Q2W, CZP 200 mg Q4W and placebo patients, respectively, would remain

**Table 1** Demographics and disease characteristics for patients enrolled in C-OPTIMISE

| | Induction period | Patients randomised into maintenance period (n=313) | | |
|---|---|---|---|---|
| | All axSpA (n=736) | CZP 200 mg Q2W (n=104) | CZP 200 mg Q4W (n=105) | Placebo (n=104) |
| **Baseline demographics** | | | | |
| Age, years | | | | |
| Mean (SD) | 32.9 (7.0) | 32.6 (7.2) | 32.4 (6.9) | 31.2 (6.6) |
| Range | 18–45 | 18–45 | 18–45 | 18–45 |
| Male, n (%) | 514 (69.8) | 79 (76.0) | 83 (79.0) | 85 (81.7) |
| BMI, kg/m$^2$, mean (SD) | 25.7 (4.9) | 25.1 (4.2) | 25.9 (4.6) | 24.7 (3.6) |
| Race, n (%) | | | | |
| Caucasian | 681 (92.5) | 97 (93.3) | 97 (92.4) | 98 (94.2) |
| Asian | 38 (5.2) | 5 (4.8) | 6 (5.7) | 5 (4.8) |
| Other/mixed/missing | 17 (2.3) | 2 (1.9) | 2 (1.9) | 1 (1.0) |
| Geographical region, n (%) | | | | |
| North America | 33 (4.5) | 3 (2.9) | 3 (2.9) | 4 (3.8) |
| Western Europe | 91 (12.4) | 10 (9.6) | 9 (8.6) | 8 (7.7) |
| Eastern Europe | 537 (73.0) | 82 (78.8) | 83 (79.0) | 82 (78.8) |
| Asia | 75 (10.2) | 9 (8.7) | 10 (9.5) | 10 (9.6) |
| mNY positive, n (%) | 407 (55.3) | 56 (53.8) | 56 (53.3) | 56 (53.8) |
| Symptom duration, years | | | | |
| Mean (SD) | 3.3 (2.2) | 3.8 (2.8) | 3.4 (1.8) | 3.1 (1.6) |
| Median | 3.5 | 3.9 | 3.5 | 3.3 |
| Time since diagnosis, years | | | | |
| Mean (SD) | 2.2 (1.7) | 2.5 (1.7) | 2.0 (1.7) | 2.1 (1.7) |
| Median | 1.6 | 2.7 | 1.3 | 1.3 |
| HLA-B27 positive, n (%) | 617 (83.8) | 91 (87.5) | 97 (92.4) | 94 (90.4) |
| CRP >ULN, n (%) | 344 (46.7) | 55 (52.9) | 51 (48.6) | 44 (42.3) |
| Prior TNFi therapy, n (%) | 32 (4.3) | 4 (3.8) | 6 (5.7) | 7 (6.7) |
| History of enthesitis (heel), n (%) | 184 (25.0) | 30 (28.8) | 35 (33.3) | 24 (23.1) |
| History of EAMs, n (%) | | | | |
| Uveitis | 111 (15.1) | 16 (15.4) | 20 (19.0) | 17 (16.3) |
| Inflammatory bowel disease | 17 (2.3) | 2 (1.9) | 3 (2.9) | 1 (1.0) |
| Psoriasis | 45 (6.1) | 8 (7.7) | 2 (1.9) | 7 (6.7) |
| Concomitant medication,* n (%) | | | | |
| NSAIDs | 618 (84.0) | 85 (81.7) | 92 (87.6) | 85 (82.5) |
| DMARDs | 166 (22.6) | 21 (20.2) | 24 (22.9) | 24 (23.3) |
| **Disease characteristics at Week 0, mean (SD)** | | | | |
| ASDAS | 3.7 (0.8) | 3.7 (0.7) | 3.7 (0.8) | 3.5 (0.8) |
| BASDAI | 6.7 (1.4) | 6.5 (1.4) | 6.7 (1.5) | 6.3 (1.3) |
| BASFI | 5.3 (2.1) | 5.2 (1.8) | 5.3 (2.1) | 4.8 (1.9) |
| BASMI | 3.1 (1.5) | 3.0 (1.3) | 2.8 (1.4) | 2.8 (1.6) |
| Tender joint count | 2.6 (5.0) | 1.6 (2.9) | 2.5 (4.1) | 1.9 (3.6) |
| Swollen joint count† | 0.7 (2.1) | 0.4 (1.3) | 0.8 (1.7) | 0.7 (1.6) |
| MASES | 2.5 (3.0) | 2.1 (2.8) | 2.5 (3.1) | 1.7 (2.5) |
| Imaging (MRI) | | | | |
| SIJ SPARCC | 8.0 (11.4) | 8.4 (11.6) | 10.9 (12.5) | 9.4 (14.3) |
| ASspiMRI-a | 3.1 (5.2) | 3.5 (6.0) | 2.9 (5.0) | 3.2 (5.4) |
| **Disease characteristics at Week 48, mean (SD)** | | | | |
| ASDAS | — | 0.8 (0.2) | 0.8 (0.2) | 0.8 (0.2) |
| BASDAI | — | 0.4 (0.5) | 0.4 (0.5) | 0.5 (0.6) |
| BASFI | — | 0.4 (0.5) | 0.3 (0.5) | 0.5 (0.7) |
| BASMI | — | 2.1 (1.2) | 1.9 (1.2) | 2.2 (1.5) |
| Tender joint count | — | 0.2 (0.6) | 0.1 (0.5) | 0.2 (0.6) |
| Swollen joint count† | — | 0.0 (0.1) | 0.0 (0.2) | 0.0 (0.1) |
| MASES | — | 0.1 (0.5) | 0.1 (0.4) | 0.3 (1.3) |
| Imaging (MRI) | — | | | |
| SIJ SPARCC | — | 1.0 (2.4) | 1.1 (2.9) | 0.7 (1.6) |
| ASspiMRI-a | — | 0.7 (1.5) | 0.7 (1.6) | 0.5 (1.3) |

*Any intake during induction period (Weeks 0 to 48) or maintenance period (Weeks 48 to 96).
†44 joints.
ASDAS, Ankylosing Spondylitis Disease Activity Score; ASspiMRI-a, Ankylosing Spondylitis spine MRI score for activity; axSpA, axial spondyloarthritis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; BMI, body mass index; CRP, C-reactive protein; CZP, certolizumab pegol; DMARD, disease-modifying anti-rheumatic drug; EAMs, extra-articular manifestations; HLA-B27, human leucocyte antigen-B27; MASES, Maastricht Ankylosing Spondylitis Enthesitis Score; mNY, modified New York; NSAID, non-steroidal anti-inflammatory drug; Q2W, every 2 weeks; Q4W, every 4 weeks; SIJ SPARCC, sacroiliac joint Spondyloarthritis Research Consortium of Canada; TNFi, tumour necrosis factor inhibitor; ULN, upper limit of normal.

flare-free during the 48-week maintenance period, a sample size of 210 patients was deemed sufficient to provide 98% power to detect a difference between CZP 200 mg Q2W versus placebo, and 94% power for CZP 200 mg Q4W versus placebo, using a two-sided significance level of 0.05. Based on the assumption that ~28% of patients would achieve sustained remission at the end of the induction period, 750 patients were planned for study enrolment.

The primary analysis was based on a logistic regression model which included treatment group, region and presence or absence of radiographic sacroiliitis as factors. ORs for each CZP dose versus placebo (with 95% two-sided CIs) were derived from the model, based on the percentage of patients who did not experience a flare. A fixed sequence testing procedure was used to account for testing of multiple doses: hypothesis testing at the 0.05 level was first conducted for CZP 200 mg Q2W versus placebo, followed by CZP 200 mg Q4W versus placebo. The second test was only interpreted as statistically significant if the first test was significant at the 0.05 level. No statistical testing was planned for CZP 200 mg Q2W versus CZP 200 mg Q4W.

Non-responder imputation (NRI) was used to account for missing data for analysis of the primary outcome; if patients withdrew or had two consecutive missing ASDAS values these were designated as flares. The time to flare was analysed using the log-rank test and Kaplan-Meier methods. The percentage of patients achieving sustained remission is summarised using descriptive statistics (counts and percentages). Continuous data are summarised using mean and SD. In the maintenance period,

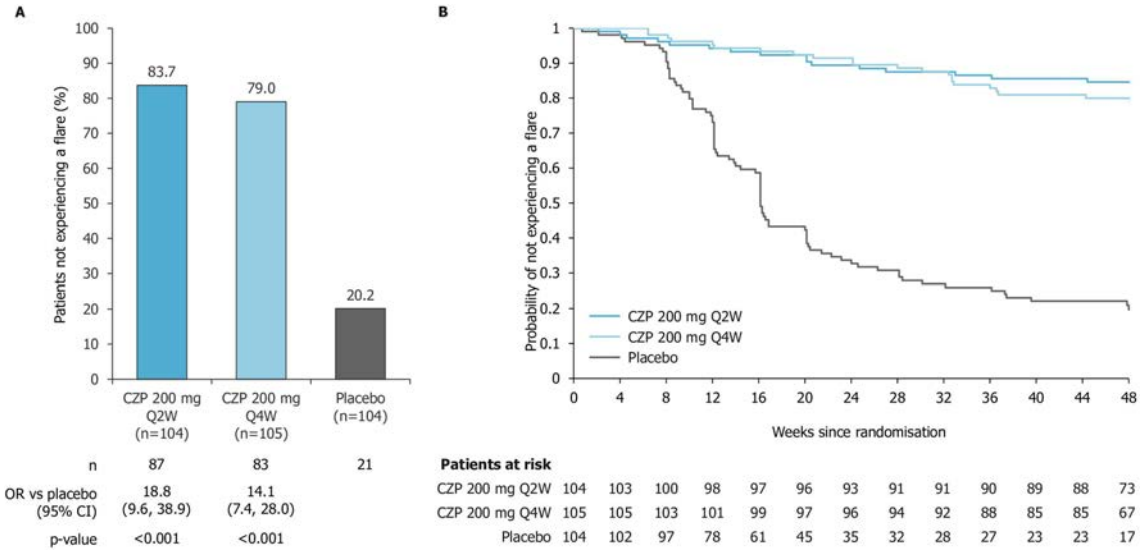


**Figure 2** Patients free of flares during the maintenance period of C-OPTIMISE. Panel A shows the proportions of patients who did not experience flares following randomisation to CZP full maintenance dose (200 mg Q2W), CZP reduced maintenance dose (200 mg Q4W) or placebo. Panel B shows a Kaplan-Meier plot of time to flare. Missing values were imputed using non-responder imputation. Flare was defined as ASDAS ≥2.1 at two consecutive visits, or ASDAS >3.5 at any visit. CZP, certolizumab pegol; Q2W, every 2 weeks; Q4W, every 4 weeks.

binary variables were analysed using logistic regression (using NRI to impute missing values), and continuous data were analysed using a mixed model for repeated measures. For patients entering the escape arm, ASDAS status and ASDAS clinical responses (calculated from the start of escape medication) are reported using descriptive statistics.

Post-hoc analysis of predictors of flare was performed using a stepwise logistic regression model (details in online supplementary material).

Statistical analyses were performed using SAS Version 9.3.

## RESULTS

### Patient disposition and baseline characteristics

Of 1253 patients screened, 736 were enrolled into the induction part of C-OPTIMISE, including 407 patients with radiographic axSpA and 329 with non-radiographic axSpA (figure 1). The mean age at study entry was 32.9 years, with an average symptom duration of 3.3 years (table 1). Baseline characteristics were comparable between radiographic and non-radiographic axSpA, but the former included a higher percentage of patients who were male, humanleucocyte antigen-B27 (HLA-B27)

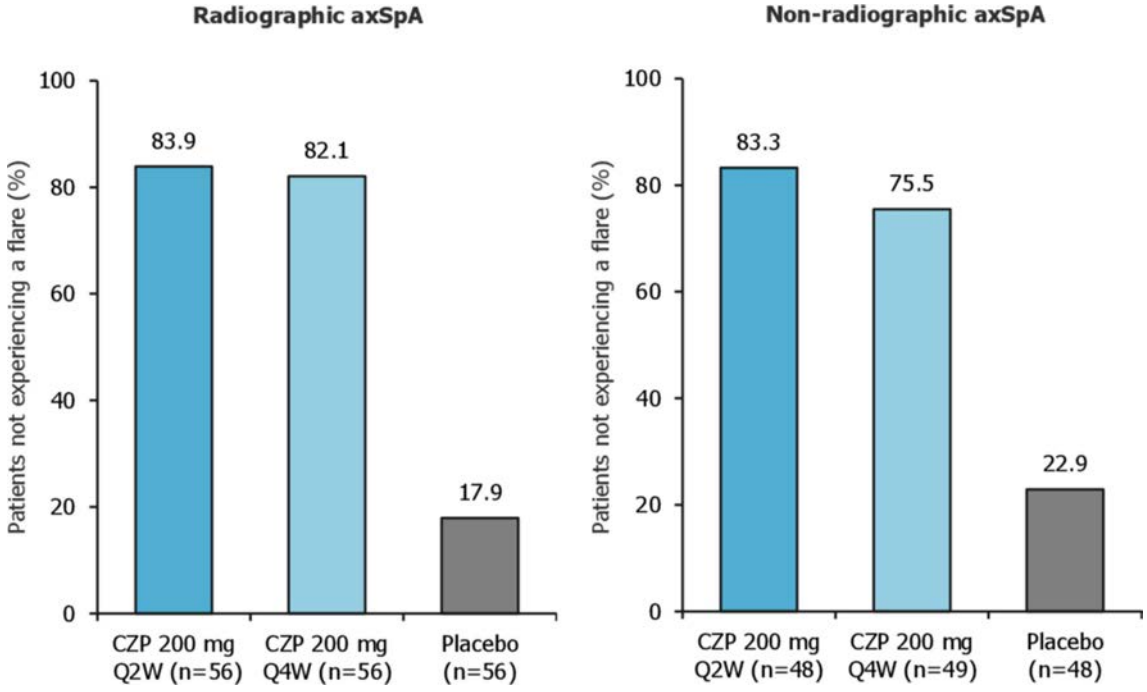


**Figure 3** Patients with radiographic and non-radiographic axial spondyloarthritis not experiencing flares during the maintenance period of C-OPTIMISE. axSpA, axial spondyloarthritis; CZP, certolizumab pegol; Q2W, every 2 weeks; Q4W, every 4 weeks.

**Table 2** Efficacy outcomes at the end of the maintenance period (Week 96) of C-OPTIMISE (n=313)

| | Imputation | CZP 200 mg Q2W (n=104) | P vs placebo | CZP 200 mg Q4W (n=105) | P vs placebo | Placebo (n=104) |
|---|---|---|---|---|---|---|
| ASDAS disease activity state, n (%) | | | | | | |
| ID (<1.3) | OC | 75/87 (86.2) | – | 58/83 (69.9) | – | 14/24 (58.3) |
| LD (≥1.3 and <2.1) | OC | 12/87 (13.8) | – | 19/83 (22.9) | – | 6/24 (25.0) |
| HD (≥2.1 and ≤3.5) | OC | 0/87 (0) | – | 6/83 (7.2) | – | 4/24 (16.7) |
| vHD (>3.5) | OC | 0/87 (0) | – | 0/83 (0) | – | 0/24 (0) |
| **Disease activity responses from Week 0, n (%)** | | | | | | |
| ASDAS clinical improvement* | | | | | | |
| CII | NRI | 86 (82.7) | <0.001 | 79 (75.2) | <0.001 | 22 (21.2) |
| MI | NRI | 70 (67.3) | <0.001 | 61 (58.1) | <0.001 | 11 (10.6) |
| ASAS responder rates* | | | | | | |
| 20 | NRI | 89 (85.6) | <0.001 | 82 (78.1) | <0.001 | 24 (23.1) |
| 40 | NRI | 88 (84.6) | <0.001 | 77 (73.3) | <0.001 | 22 (21.2) |
| 5/6 | NRI | 73 (70.2) | <0.001 | 66 (62.9) | <0.001 | 13 (12.5) |
| Partial remission | NRI | 81 (77.9) | <0.001 | 74 (70.5) | <0.001 | 18 (17.3) |
| BASDAI50[a] | NRI | 87 (83.7) | <0.001 | 81 (77.1) | <0.001 | 23 (22.1) |
| **Change from Week 48** | | | | | | |
| Efficacy outcomes, LS mean±SE | | | | | | |
| ASDAS | MMRM | 0.2±0.1 | <0.001 | 0.5±0.1 | <0.001 | 1.7±0.1 |
| BASDAI | MMRM | 0.6±0.2 | <0.001 | 0.8±0.2 | <0.001 | 3.0±0.2 |
| BASFI | MMRM | 0.3±0.2 | <0.001 | 0.5±0.2 | <0.001 | 1.9±0.2 |
| BASMI | MMRM | 0.0±0.1 | 0.074 | −0.0±0.1 | 0.036 | 0.2±0.1 |
| MRI outcomes, mean (SD; n) | | | | | | |
| SIJ SPARCC score | OC | 0.2 (2.4; 79) | 0.195 | 0.6 (3.8; 77) | 0.432 | 1.1 (3.6; 24) |
| ASspiMRI-a | OC | 0.0 (0.8; 79) | 0.040 | 0.0 (0.8; 78) | 0.074 | 0.4 (0.9; 24) |
| Additional outcomes, mean (SD; n) | | | | | | |
| MASES | OC | 0.1 (0.6; 90) | – | 0.1 (0.6; 84) | – | −0.1 (0.9; 24) |
| Tender joint count | OC | −0.1 (0.6; 90) | – | 0.1 (0.9; 84) | – | 0.0 (1.0; 24) |
| Swollen joint count | OC | 0.0 (0.2; 90) | – | 0.0 (0.2; 84) | – | 0.0 (0.0; 24) |

P values were obtained using a logistic regression model or, for MRI outcomes, an ANCOVA model, with factors for treatment group, geographical region and mNY classification (Week 48 baseline was included as a covariate in the ANCOVA model).
*Calculated from Week 0 baseline.
ANCOVA, analysis of covariance; ASAS, Assessment of Spondyloarthritis international Society; ASDAS, Ankylosing Spondylitis Disease Activity Score; ASDAS-ID/LD/HD/vHD, ASDAS-inactive disease/low disease/high disease/very high disease; ASspiMRI-a, Ankylosing Spondylitis spine MRI score for activity; BASDAI50, Bath Ankylosing Spondylitis Disease Activity Index 50% improvement; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; CII, clinically important improvement; CZP, certolizumab pegol; LS, least squares; MASES, Maastricht Ankylosing Spondylitis Enthesitis Score; MI, major improvement; MMRM, mixed effect model for repeated measures; mNY, modified New York; NRI, non-responder imputation; OC, observed case; Q2W, every 2 weeks; Q4W, every 4 weeks; SIJ SPARCC, sacroiliac joint Spondyloarthritis Research Consortium of Canada.

positive and with elevated C-reactive protein (CRP) levels (online supplementary table S2).

By Week 48, 323 patients had achieved sustained remission. Of these, 313 underwent 1:1:1 randomisation: 104 were randomised to CZP full maintenance dose, 105 to CZP reduced maintenance dose and 104 to placebo. A further 10 patients with sustained remission did not enter the maintenance period due to subject withdrawal or ineligibility. Compared with patients who entered the induction period, those entering the maintenance period were more likely to be male and HLA-B27 positive. Week 48 disease characteristics were similar among patients entering the maintenance period (table 1).

## Efficacy

### Induction period

During the 48-week induction period in which patients received open-label CZP 200 mg Q2W treatment, 43.9% of patients (323/736) achieved sustained remission according to the study definition. Results were similar among patients with radiographic and non-radiographic axSpA: 42.8% (174/407) and 45.3% (149/329) achieved sustained remission, respectively.

### Maintenance period

During the maintenance period, 83.7% (87/104) and 79.0% of patients (83/105) who were randomised to the CZP full maintenance dose or CZP reduced maintenance dose, respectively, remained flare-free. Only 20.2% of patients (21/104) randomised to placebo remained flare-free (p<0.001 vs placebo for both CZP maintenance doses; figure 2A). The time to flare was significantly different for each CZP dose versus placebo (p<0.001 vs placebo for both CZP treatment groups, log-rank test). In the placebo arm, the median time to flare following randomisation was 113 days (95% CI: 101 to 141), with the majority of flares occurring between 8 and 20 weeks post-randomisation (figure 2B). For CZP patients, no median time to flare could be determined within the 48-week timeframe. Among patients with radiographic or non-radiographic axSpA, similar percentages of patients did not experience flares (figure 3).

Post-hoc logistic regression analysis of predictors of flares in CZP (full and reduced maintenance dose groups combined) and placebo patients identified HLA-B27 negativity as a potential predictor of flares in patients randomised to CZP, but not in those randomised to placebo (online supplementary table

S3). The model did not identify any other variables as possible predictors of flare.

### Escape arm

During the maintenance period, 95 patients who experienced flares (7 randomised to CZP full maintenance dose, 15 to CZP reduced maintenance dose and 73 to placebo) entered an open-label escape arm. The mean (SD) ASDAS at the time of flare for patients in the CZP full and reduced maintenance dose groups was 2.5 (1.1) and 2.3 (0.6), respectively, while in the placebo group ASDAS was 3.4 (1.0). Twelve weeks after treatment re-initiation with open-label CZP 200 mg Q2W following flare, clinical remission (ASDAS-ID) was regained in 63.4% (45/71), 60.0% (9/15) and 16.7% (1/6) of patients escaping from the placebo, CZP reduced maintenance dose and CZP full maintenance dose arms, respectively. An ASDAS <2.1 (ASDAS low disease activity) was reached in 90.1% (64/71), 80.0% (12/15) and 66.7% (4/6) of patients, respectively. For other efficacy measures, including BASDAI, BASFI and SIJ SPARCC, disease activity was highest in the placebo group at the time of flare, but showed improvements after 12 weeks of escape treatment (online supplementary table S4).

## Other efficacy outcomes

At Week 96, a significantly higher percentage of patients randomised to the CZP full or reduced maintenance dose achieved an ASDAS clinical improvement (CII or MI), ASAS20/40 or BASDAI50 response compared with placebo (table 2), with responses calculated from Week 0.

Between Weeks 48 and 96, in patients randomised to the CZP full or reduced maintenance dose, disease activity (ASDAS and BASDAI), function (BASFI) and mobility (BASMI) remained stable (table 2). In patients randomised to placebo, disease activity (ASDAS and BASDAI) and function (BASFI) worsened between Weeks 48 and 96 (table 2).

In all three treatment groups, there were minimal changes in MRI outcomes (SIJ SPARCC and ASspiMRI-a) in patients who remained on randomised treatment (table 2).

## Safety

During the maintenance period, TEAEs were reported in 57.7%, 61.0% and 54.4% of patients randomised to CZP 200 mg Q2W, CZP 200 mg Q4W or placebo, respectively (table 3). Serious TEAEs were reported in five patients randomised to CZP 200 mg Q2W: these included one case each of acute pancreatitis, Crohn's disease and anal abscess, which the study investigators did not consider to be treatment-related, and one case each of intestinal obstruction and latent tuberculosis, which were considered by the study investigators to be treatment-related. No serious TEAEs led to patient withdrawal from the study, and complete recovery was reported for all five cases. There were no malignancies, serious cardiovascular events or deaths during the study.

**Table 3** Treatment-emergent adverse events during the C-OPTIMISE maintenance period (Weeks 48 to 96)

| N (%), unless otherwise specified | CZP 200 mg Q2W (n=104) | CZP 200 mg Q4W (n=105) | Placebo (n=104) |
|---|---|---|---|
| CZP exposure duration (days) | | | |
| Mean (SD) | 306.9 (78.9) | 300.5 (77.7) | 171 (104.7) |
| Median (range) | 336.0 (14 to 346) | 336.0 (44 to 350) | 126.0 (14 to 345) |
| Total patient-years at risk | 101.0 | 96.4 | 52.7 |
| Any TEAE | 60 (57.7) | 64 (61.0) | 56 (54.4) |
| Event rate per 100 PY | 177.2 | 140.0 | 237.1 |
| Serious TEAEs | 5 (4.8) | 0 | 0 |
| Discontinuation due to TEAEs | 1 (1.0) | 3 (2.9) | 0 |
| Drug-related TEAEs | 14 (13.5) | 20 (19.0) | 14 (13.6) |
| Severe TEAEs | 1 (1.0) | 0 | 2 (1.9) |
| TEAEs of Interest | | | |
| Opportunistic infections | 1 (1.0) | 3 (2.9) | 2 (1.9) |
| Oral candidiasis | 0 | 1 (1.0) | 0 |
| Malignant or unspecified tumours* | 0 | 0 | 0 |
| Serious cardiovascular events† | 0 | 0 | 0 |
| Serious haematopoietic cytopenia | 0 | 0 | 0 |
| Serious bleeding events‡ | 0 | 0 | 0 |
| Hepatic events§ | 3 (2.9) | 5 (4.8) | 3 (2.9) |
| Liver function analyses¶ | 3 (2.9) | 4 (3.8) | 2 (1.9) |
| Hypersensitivity and anaphylactic reactions** | 0 | 0 | 0 |
| Demyelinating disorders | 0 | 0 | 0 |
| Deaths | 0 | 0 | 0 |

*Identified using SMQs 'malignant or unspecified tumours' and 'malignant tumours'; also include incidence of 'any malignancy'.
†Identified using study sponsor-defined search criteria based on a two-step process using identification via a predefined list of preferred terms in addition to manual review by the study physician.
‡Identified using SMQ 'haemorrhage terms (excluding laboratory terms)' in the subset of serious TEAEs.
§Identified using SMQs 'cholestasis and jaundice of hepatic origin', 'hepatic failure, fibrosis and cirrhosis and other liver damage-related conditions', 'hepatitis, non-infectious', 'liver-related investigations, signs and symptoms' and 'liver-related coagulation and bleeding disturbances'.
¶Includes increased levels of alanine aminotransferase, aspartate aminotransferase, hepatic enzyme, blood bilirubin or transaminases.
**Includes incidence of 'any hypersensitivity and anaphylactic reactions', 'any hypersensitivity reactions' and 'any anaphylactic reactions'. Safety events are reported for the Safety Set (n=736) according to MedDRA Version 19.0.
CZP, certolizumab pegol; MedDRA, Medical Dictionary for Regulatory Activities; PY, patient-years; Q2W, every 2 weeks; Q4W, every 4 weeks; SD, standard deviation; SMQ, standard MedDRA query; TEAE, treatment-emergent adverse event.

## DISCUSSION

C-OPTIMISE demonstrates that patients with early axSpA who achieve sustained remission after 48 weeks' full dose CZP (200 mg Q2W) treatment can reduce their dose without further increasing their risk of flares in disease activity, but that they should not completely stop treatment. This is an important finding for clinicians who face decisions on how best to manage axSpA patients in sustained remission. These results also have implications for patients, who are typically in their late 20s or early 30s at symptom onset and who fear long-term continuation of immunosuppressive therapy. Furthermore, the option to reduce the maintenance dose can ease the economic burden of TNFi treatment.

While previous studies have explored TNFi tapering or withdrawal in patients with axSpA following remission

induction,[11 12 28] C-OPTIMISE is the first randomised controlled trial to compare both dose continuation and reduction with the effects of TNFi withdrawal. In keeping with the results from C-OPTIMISE, ABILITY-3 showed that adalimumab withdrawal led to significantly more flares than continuation; however, there was no comparison with a reduced dose arm in ABILITY-3, which also included only patients with non-radiographic axSpA.[11] C-OPTIMISE recruited patients with both subforms of axSpA (radiographic and non-radiographic), and patients were on average younger (32.9 vs 37.3 years) and had shorter symptom duration (3.3 vs 7.7 years) compared with ABILITY-3. The induction period of C-OPTIMISE was also longer (48 vs 28 weeks), although in both studies patients had to be in remission (ASDAS-ID) for at least 12 weeks to be eligible for randomisation. In C-OPTIMISE, more patients randomised to CZP (full or reduced dose) maintained remission (~80%) compared with ABILITY-3 (70%); however, in the withdrawal arm more patients in ABILITY-3 maintained remission (47%) than in C-OPTIMISE (20%). This difference may be attributable to differences in patient populations. The inclusion of both radiographic and non-radiographic axSpA patients in C-OPTIMISE demonstrated the benefits of dose reduction across the entire axSpA spectrum.

The use of ASDAS-ID (<1.3) as the definition of remission in C-OPTIMISE aligns with recommendations in current treat-to-target guidelines for axSpA.[2] Additionally, the recently published ASAS-flare definition is based on ASDAS, but was not yet available at the time of the design of the current study.[29] The advantages of ASDAS are the combination of patient-reported outcomes and an objective measure of inflammation (ie, CRP) as well as validated cut-offs for various levels of disease activity and improvement.

Approximately two-thirds of patients who experienced flares following complete withdrawal of CZP were able to regain their status of remission following 12 weeks of rescue treatment with open-label CZP 200 mg Q2W; similar results were seen for patients escaping from the reduced maintenance dose arm, although patient numbers in this group were smaller. A high proportion of escapers from the placebo arm achieved ASDAS <2.1 (~90%) after 12 weeks of rescue treatment, so it is possible that with continued treatment beyond 12 weeks more patients would regain their initial response (ASDAS-ID).

Identification of predictors of flare could help to optimise chances of successfully maintaining remission following either dose reduction or withdrawal. Post-hoc analyses in C-OPTIMISE identified HLA-B27 negativity as a possible predictor of flare in patients who continued on CZP treatment, but did not identify any predictors in placebo-randomised patients. Given the small number of flares in the CZP treatment groups, further investigation into this result is required.

No new safety concerns were identified throughout the entire study period. Five serious TEAEs were reported, all of which occurred in patients continuing on the full CZP maintenance dose. A full recovery was made for all five events, including the two serious TEAEs considered by the study investigator to be treatment-related. Nevertheless, this may add to the relevance of TNFi dose reduction in patients when clinically possible.

A potential limitation of the C-OPTIMISE study is the fact that enrolment was limited to patients with <5 years' symptom duration, so it is unclear whether the results may be generalised to patients with more established disease.

In summary, in early axSpA patients in sustained remission after 1 year of open-label treatment with CZP, reducing the maintenance dose of CZP enabled patients to maintain their state of remission, while completely stopping treatment resulted in flares in the majority of patients. CZP maintenance dose reduction is therefore a feasible option for the long-term management of this chronic rheumatic disease, which has the advantage of preserving the clinical benefits of remaining on TNFi treatment, reducing costs and limiting patients' long-term exposure to immunosuppressive therapy.

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

**Patient consent for publication** Not required.

**Ethics approval** The study was approved by institutional review boards and independent ethics committees at participating sites and was conducted in accordance with local regulations and the International Conference on Harmonisation Good Clinical Practice requirements, based on the Declaration of Helsinki.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Underlying data from this manuscript may be requested by qualified researchers six months after product approval in the US and/or Europe, or global development is discontinued, and 18 months after trial

completion. Investigators may request access to anonymized individual patient-level data and redacted trial documents which may include: analysis-ready datasets, study protocol, annotated case report form, statistical analysis plan, dataset specifications, and clinical study report. Prior to use of the data, proposals need to be approved by an independent review panel at www.Vivli.organd a signed data sharing agreement will need to be executed. All documents are available in English only, for a pre-specified time, typically 12 months, on a password protected portal.



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CLINICAL SCIENCE

# High prevalence of spondyloarthritis-like MRI lesions in postpartum women: a prospective analysis in relation to maternal, child and birth characteristics

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## ABSTRACT

**Objectives** Bone marrow oedema (BMO) on MRI of sacroiliac joints (SIJs) represents a hallmark of axial spondyloarthritis (SpA), yet such lesions may also occur under augmented mechanical stress in healthy subjects. We therefore sought to delineate the relationship between pregnancy/delivery and pelvic stress through a prospective study with repeated MRI. Results were matched with maternal, child and birth characteristics.

**Methods** Thirty-five women underwent a baseline MRI-SIJ within the first 10 days after giving birth. MRI was repeated after 6 months and, if positive for sacroiliitis according to the Assessment of SpondyloArthritis International Society (ASAS) definition, after 12 months. BMO and structural lesions were scored by three trained readers using the Spondyloarthritis Research Consortium of Canada (SPARCC) method.

**Results** Seventy-seven per cent of the subjects (27/35) displayed sacroiliac BMO immediately postpartum, 60% fulfilled the ASAS definition of a positive MRI. After 6 months, 46% of the subjects (15/33) still showed BMO, representing 15% (5/33) with a positive MRI. After 12 months, MRI was still positive in 12% of the subjects (4/33). Few structural lesions were detected. Intriguingly, in this study, the presence of BMO was related to a shorter duration of labour and lack of epidural anaesthesia.

**Conclusion** A surprisingly high prevalence of sacroiliac BMO occurs in women immediately postpartum. Our data reveal a need for a waiting period of at least 6 months to perform an MRI-SIJ in postpartum women with back pain. This study also underscores the importance of interpreting MRI-SIJ findings in the appropriate clinical context.

## Key messages

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

- Bone marrow oedema (BMO) on MRI of the sacroiliac joints (SIJs) lacks specificity for spondyloarthritis and can also occur under circumstances of augmented biomechanical stress.

**What does this study add?**

- A strikingly high number of postpartum women display sacroiliac BMO on MRI.
- The occurrence of sacroiliac BMO on MRI in postpartum women is associated with a shorter duration of labour and the lack of epidural anaesthesia.
- Sacroiliac BMO on MRI in postpartum women decreases significantly over time, but persists mainly in subjects older than 30 years.

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

- Our data indicate the need for a waiting period of at least 6 months to perform an MRI of the SIJs in postpartum women with back pain.

## INTRODUCTION

Axial spondyloarthritis (axSpA) is an inflammatory rheumatic condition, characterised by involvement of the spine and/or sacroiliac joints (SIJs). Bone marrow oedema (BMO) on MRI of the SIJs plays a central role in the Assessment of SpondyloArthritis International Society (ASAS) classification criteria for axSpA, with a sensitivity of the imaging arm of 66%.[1 2] SIJ BMO on MRI is present in up to 84% of patients with non-radiographic axSpA.[3] However, it is frequently seen in a non-inflammatory setting. Recently, a high prevalence of BMO meeting the ASAS definition of a positive MRI for sacroiliitis was seen even in young, active individuals, such as military recruits (36%) and professional ice hockey players (41%).[4 5] A significant number of healthy volunteers (23%) and patients with mechanical chronic back pain (6%–8%) also fulfil the ASAS definition of a positive MRI for active sacroiliitis.[6 7] Seventeen per cent of the mechanical chronic back pain patients show structural SIJ lesions on MRI; however, the different combinations of structural lesions are more seen in patients with axSpA.[7] Although structural lesions of the SIJ are also important characteristics of axSpA, they are not included in the ASAS MRI definition.[8] Importantly, structural lesions may differentiate patients with axSpA from patients with a non-SpA back pain.

In contrast to ankylosing spondylitis, non-radiographic axSpA has a more equal sex distribution.[9–11] Hence, a broad differential diagnosis has to be considered in young women with back pain. Peripartum low back pain is common. In approximately

4% of women, pain persists for more than 6 months postpartum and is occasionally inflammatory in nature.[12–17] Up until now, little is known regarding the presence of sacroiliac MRI lesions in postpartum women, which complicates the distinction with axSpA. Sacroiliac BMO during pregnancy and after childbirth has been reported in previous studies,[6 12 14] yet the extent and frequency of MRI lesions is inadequately described and no prospective follow-up was performed. Thus, it can be challenging to discriminate patients with axSpA from postpartum women with persistent back pain. To date, there are no data regarding the evolution of sacroiliac BMO over time in postpartum women, nor links with maternal, child or birth characteristics.

Therefore, our goal was to explore the association between pregnancy and giving birth, and the occurrence of sacroiliac MRI lesions. Furthermore, this study also aimed to detect the time frame in which these lesions disappear. In addition, MRI findings were correlated with maternal, child and birth characteristics.

## METHODS

### Subjects

Thirty-five subjects were recruited from the Department of Obstetrics of the Ghent University Hospital. All subjects provided written informed consent. All included women were between 18 and 45 years old, after an uncomplicated, vaginal childbirth. Exclusion criteria were a known diagnosis of SpA and/or inflammatory bowel disease, severe scoliosis, treatment with anti-tumour necrosis factor-α agents, any kind of contraindication for MRI, childbirth through caesarean section and multiple pregnancy (pregnancy with more than one fetus). Baseline demographic and clinical data (SpA criteria, visual analogue scale (VAS) back pain at night and VAS back pain day and night, duration of labour, gravida/para/abortus status, weight gain during pregnancy, epidural anaesthesia, and sex, weight, length and head circumference of the newborn) were collected. HLA-B27 status was determined.

### MRI assessment

Within the first 10 days after giving birth, an MRI-SIJ was performed, which was repeated after 6 months, and, if the second MRI fulfilled the ASAS definition of a positive MRI for sacroiliitis, another MRI-SIJ was performed 12 months after giving birth. Identical settings as in routine clinical practice were adopted. Images were obtained on a 1.5 T MRI unit (Aero/Avanto, Siemens Medical, Erlangen, Germany). A body flexed array coil was used to scan the SIJs. The sequence protocol included the following: semicoronal (along long axis of the sacral bone) T1-weighted turbo spin echo (tse) (slice thickness (ST): 3 mm; repetition time/echo time (TR/TE): 679/20 ms); semicoronal short tau inversion recovery (STIR) (ST: 3 mm; TR/TE/TI: 5030/70/150 ms); and axial STIR (ST: 5 mm; TR/TE/TI: 7540/70/150 ms). All images were scored for BMO, capsulitis, enthesitis, high signal intensity in joint space, erosions, sclerosis, fat metaplasia and (partial) ankylosis, as defined by the ASAS MRI working group, by three experienced and calibrated readers (MdH, LJ, NH).[1] Scored lesions were regarded by the readers as characteristic for axSpA. Readers were blinded for time sequence and demographic/clinical data. BMO was scored using the Spondyloarthritis Research Consortium of Canada (SPARCC) method, with a maximum score of 72.[18] BMO was evaluated for depth (deep lesions=extending >1 cm from the articular surface) and intensity (intense lesions=high signal intensity as bright or brighter as vascular structures or intervertebral discs). Additionally, fulfilment of the ASAS definition of a positive MRI for sacroiliitis (≥2 BMO lesions on one slice or ≥1 lesion on two consecutive slices and lesions highly suggestive of SpA) was assessed.[8] Structural lesions (erosions, fatty lesions, sclerosis and ankylosis) were scored using an adjusted SPARCC method. In addition, the proposed cut-off values for erosions and fatty lesions of de Hooge *et al* were applied for each subject for each time point.[19] Individual reader scores were combined and for further analyses the median scores were reported. Regarding dichotomous outputs, the consensus of two out of three readers was reported. In case the month 6 MRI fulfilled the ASAS definition of sacroiliitis, the third MRI was provided to the readers for an independent evaluation. A summary of the inter-reader agreement and the measurement error is shown in the online supplementary text.

### Statistical analyses and data management

Statistical analyses were performed using R (V.3.5.2; R Core Team (2018), Vienna, Austria; http://www.R-project.org/) and RStudio (RStudio Team (2018), Boston, Massachusetts, USA; http://www.rstudio.com/). Mean and median values and confidence intervals were determined using descriptive statistics. The significance of SPARCC score differences between time points was calculated by the Wilcoxon signed-rank test. Difference in proportion of subjects having a positive MRI-SIJ was calculated using the McNemar test. Fisher's exact test was used to compare proportions between two independent groups. Correlation with clinical data was assessed using Spearman's rank correlation coefficient. P values≤0.05 were considered as statistically significant. Non-significant p values were labelled in the main text as NS. Study data were collected and managed using REDCap electronic data capture tools.[20 21]

## RESULTS

### Subjects

Thirty-five subjects were included and underwent the baseline MRI, which was acquired, on average, 5 days postpartum. Thirty-three subjects underwent the month 6 MRI, two subjects were lost to follow-up. Demographics and clinical data are displayed in table 1. Eleven subjects (31%) had back pain at the time of the first MRI. In 8 out of 11 subjects (73%) back pain was chronic (≥3 months) and in four subjects (36%) back pain was inflammatory according to the ASAS criteria. Two subjects had a positive family history for SpA. No extra-articular SpA manifestations were present, except for three subjects (9%) with a history of skin psoriasis.

### Sacroiliac MRI lesions

A summary of the detected MRI lesions is presented in table 2. At baseline, the majority of subjects (77%) displayed BMO on MRI-SIJ, with a median SPARCC score of 5. BMO was numerically, but not significantly, more prevalent at the iliac compared with the sacral side of the joint (14.5% vs 11.3% of the quadrants), and significantly more prevalent at the upper SIJ compared with the lower (15.4% vs 10.5%, p≤0.01), and at the anterior part compared with the posterior (19.6% vs 6.2%, p≤0.001). BMO was equally present at the right SIJ compared with the left (13.7% vs 12.1%). Three subjects (9%) had deep BMO lesions at baseline, whereas seven subjects (20%) had intense BMO lesions. Twenty-one subjects (60%) had a positive MRI according to the ASAS definition. High signal intensity in the SIJ space was seen in 13 subjects (37%); however, median score was low (0). Capsulitis and enthesitis were rarely seen.

**Table 1** Demographic and clinical data of subjects

| | |
|---|---|
| **Demographics at baseline (n=35)** | |
| Age, years (mean, SD) | 29.7 (2.62) |
| Age>30 years, n (%) | 12 (34) |
| Smoking status, n (%) | |
| Never | 29 (83) |
| Cessation>3 years | 2 (6) |
| Cessation<3 years | 3 (9) |
| Current smoker | 1 (3) |
| Profession, n (%) | |
| Physical labour | 6 (17) |
| Non-physical labour | 27 (77) |
| Unemployed/student | 2 (6) |
| **Clinical characteristics at baseline (n=35)** | |
| Weight, kg (mean, SD) | 68 (10.9) |
| BMI, kg/m² (mean, SD) | 25 (3.7) |
| Weight gain during pregnancy, kg (median, 95% CI) | 10 (9 to 10) |
| HLA-B27 positivity, n (%) | 1 (3) |
| Back pain symptoms, n (%) | 11 (31) |
| Duration back pain, weeks (mean, SD)* | 13.8 (7.65) |
| ≥3 months of back pain (=chronic), n (%)* | 8 (73) |
| VAS back pain at night (median, 95% CI)* | 0.5 (0 to 2) |
| VAS back pain day and night (median, 95% CI)* | 1 (1 to 3) |
| Insidious onset of back pain, n (%)* | 9 (82) |
| Back pain improvement with exercise, n (%)* | 9 (82) |
| No improvement of back pain with rest, n (%)* | 3 (27) |
| Nocturnal back pain, n (%)* | 5 (46) |
| Alternating buttock pain, n (%)* | 3 (27) |
| Morning stiffness, n (%)* | 1 (9) |
| Inflammatory back pain (ASAS criteria), n (%)* | 4 (36) |
| Family history of SpA, n (%) | 2 (6) |
| Arthritis (history or current), n | 0 |
| Enthesitis (history or current), n | 0 |
| Dactylitis (history or current), n | 0 |
| Uveitis (history or current), n | 0 |
| Psoriasis (history or current), n (%) | 3 (9) |
| Inflammatory bowel disease (history or current), n | 0 |
| Current pregnancy and delivery (n=35) | |
| First pregnancy (G=1), n (%) | 18 (51) |
| First delivery (P=1), n (%) | 22 (63) |
| Duration of labour, hours (median, 95% CI) | 8 (6 to 12) |
| Epidural anaesthesia, n (%) | 22 (63) |
| Male newborn, n (%) | 17 (49) |
| Weight newborn, g (mean, SD) | 3341 (502) |
| Length newborn, cm (mean, SD) | 50 (2.4) |
| Head circumference newborn, cm (mean, SD) | 34 (1.5) |

*Only those patients with back pain were retained.
ASAS, Assessment of SpondyloArthritis international Society; G, gravida; P, para; SpA, spondyloarthritis; VAS, visual analogue scale.

A significant decrease in SPARCC score was seen after 6 months ($p \leq 0.001$) (figure 1). Five subjects (15%) still had a positive MRI ($p \leq 0.001$), persisting in four subjects (12%) after 12 months. A significant drop in high signal intensity in the joint space was seen after 6 months ($p \leq 0.01$), while no residual capsulitis or enthesitis was reported. There were no deep or intense BMO lesions detected at follow-up.

Almost no structural MRI lesions were seen, neither at baseline nor at follow-up (table 2). No subjects displayed erosions on ≥3 quadrants at baseline, while only one subject showed erosions on three quadrants at month 12. Two subjects had ≥3 quadrants showing fatty lesions at month 6 and one subject had five quadrants showing fatty lesions at month 12. In three subjects (7.4% of the quadrants) BMO transformed to fatty lesions during follow-up. An example of a subject with postpartum sacroiliac MRI lesions is shown in figure 2.

### Correlation between MRI lesions and clinical data

The correlation of MRI findings with relevant clinical data is shown in table 3. No significant association was found between baseline MRI findings and the presence of back pain. Both subjects developing erosions or fatty lesions in ≥3 quadrants after 12 months had back pain. One subject was HLA-B27 positive; she did not have back pain and had a baseline SPARCC score of 8 with one intense BMO lesion. Unfortunately, she was lost to follow-up. Four subjects would have fulfilled the ASAS classification criteria if there was a suspicion of axSpA: three fulfilled the ASAS definition of a positive MRI for sacroiliitis and had inflammatory back pain, one had chronic back pain, a positive MRI and skin psoriasis. Baseline SPARCC scores and a positive MRI-SIJ were not significantly associated with the subject's age, although all five subjects with persistent BMO up to 12 months were older than 30 years. No significant association was found between baseline MRI lesions and the subject's gravidity and parity. A shorter duration of labour was associated with higher baseline SPARCC scores and consequently also with a higher percentage of women fulfilling the ASAS definition of a positive MRI for sacroiliitis at baseline. When epidural anaesthesia was performed, significantly lower baseline SPARCC scores were found. Baseline MRI findings were not associated with the sex and biometry of the newborn.

### Other aberrant MRI findings

In 21 subjects (60%), aberrant MRI findings, other than sacroiliac inflammatory or structural lesions, were seen at baseline. Fifteen subjects (43%) had both sacroiliac lesions and other aberrant MRI findings. The most frequent unforeseen MRI finding was symphysis pubis BMO, which was present in 18 subjects (51%) at baseline and persisted in 7 (39%) women after 6 months. Fourteen out of 18 subjects (82%) with symphysis pubis BMO at baseline also had sacroiliac BMO. Degenerative disc disease was seen in one subject (3%) at baseline. Two subjects (6%) had a sacral fracture on baseline MRI (figure 3). Both fractures were asymptomatic and healed spontaneously after 6 months.

## DISCUSSION

This is the first prospective study investigating the evolution of sacroiliac MRI lesions in postpartum women. In addition, the correlation of MRI findings with clinical data of mother and child was assessed, which has never been done before. A high prevalence of BMO was seen on MRI-SIJ performed immediately after giving birth, even in subjects without back pain. Notably, a significant portion had a positive MRI for sacroiliitis according to the ASAS definition. Four subjects even fulfilled the ASAS classification criteria for axSpA. A significant decrease in BMO was seen over time, but persisted mainly in subjects older than 30 years. Interestingly, the presence of BMO was related to a shorter labour and the lack of epidural anaesthesia.

Since pregnancy-related low back pain in women is common[22] and they occasionally develop an inflammatory pain pattern, our findings affirm concern about the risk of overdiagnosis of axSpA solely based on MRI findings. Although the BMO lesions do not necessarily occur in SIJ locations most specific for SpA,[4 23] most

**Table 2** Inflammatory and structural sacroiliac MRI lesions at baseline, after 6 months and after 12 months

| | Baseline (n=35) | | | Month 6 (n=33) | | | Month 12 (n=5) | | |
|---|---|---|---|---|---|---|---|---|---|
| Inflammatory lesions | Subjects with ≥1 lesion (n, %) | Range (min–max) | Median (95% CI) | Subjects with ≥1 lesion (n, %) | Range (min–max) | Median (95% CI) | Subjects with ≥1 lesion (n, %) | Range (min–max) | Median (95% CI) |
| Sacroiliitis (ASAS definition) | 21 (60) | – | – | 5 (15) | – | – | 4 (80) | – | – |
| SPARCC score | 27 (77) | 0–30 | 5 (1 to 8) | 15 (46) | 0–16 | 0 (0 to 1) | 4 (80) | 0–14 | 4 (0 to 4) |
| Capsulitis | 4 (11) | 0–12 | 0 (0 to 0) | 0 | 0–0 | 0 (0 to 0) | 0 | 0–0 | 0 (0 to 0) |
| Enthesitis | 1 (3) | 0–2 | 0 (0 to 0) | 0 | 0–0 | 0 (0 to 0) | 0 | 0–0 | 0 (0 to 0) |
| High signal intensity joint space | 13 (37) | 0–12 | 0 (0 to 1) | 4 (12) | 0–10 | 0 (0 to 0) | 0 | 0–0 | 0 (0 to 0) |
| Structural lesions | | | | | | | | | |
| Sclerosis | 4 (11) | 0–13 | 0 (0 to 0) | 4 (12) | 0–10 | 0 (0 to 0) | 3 (60) | 0–13 | 1 (0 to 6) |
| Erosions | 1 (3) | 0–1 | 0 (0 to 0) | 2 (6) | 0–2 | 0 (0 to 0) | 1 (20) | 0–3 | 0 (0 to 3) |
| Fatty lesions | 0 | 0–1 | 0 (0 to 0) | 5 (15) | 0–10 | 0 (0 to 0) | 1 (20) | 0–5 | 0 (0 to 5) |
| (Partial) ankylosis | 0 | 0–0 | 0 (0 to 0) | 0 | 0–0 | 0 (0 to 0) | 0 | 0–0 | 0 (0 to 0) |

ASAS, Assessment of SpondyloArthritis International Society; SPARCC, Spondyloarthritis Research Consortium of Canada.

rheumatologists and radiologists would score these lesions as suggestive for sacroiliitis. This assumption is supported by the findings of Agten *et al*, showing that BMO on MRI-SIJ of postpartum women is indistinguishable from SpA-related sacroiliitis regarding the extent and distribution of the lesions.[12] In addition, the median SPARCC score in our study is relatively high considering the mean SPARCC score of 4.9 in the total study population of the ABILITY-1 trial and a median score of 10.2 in a study by Varkas *et al* in newly diagnosed patients with axSpA warranting treatment.[24 25] Recently, several studies highlighted that BMO lacks specificity for axSpA. Hence, fulfilment of the ASAS definition of a positive MRI for sacroiliitis can also be seen in a non-SpA context, such as in recreational runners, professional ice hockey players, military recruits, chronic back pain patients and healthy controls.[4–6] In a study by Seven *et al*, sacroiliitis on MRI was seen in 41.3% and 21.4% of the postpartum women with and without back pain, respectively.[26] Other recent studies also demonstrated a relatively high presence of sacroiliac BMO in pregnant and postpartum women.[6 12 14]

The question about the need for a higher threshold for sacroiliitis on MRI arises. Particularly the incorporation of structural lesions in the MRI definitions could augment the specificity. Active lesions remain the hallmark for assessment of inflammation in sacroiliitis, but structural lesions increasingly play a role in SpA diagnosis.[27] In the present study, few postpartum women demonstrated structural lesions on MRI-SIJ, which endorses this assumption. The lack of development of fat metaplasia could indicate towards a more mechanical, compared with inflammatory origin of the BMO lesions.[14] However, the follow-up period may not be long enough to detect this transformation. The lack of structural lesions in our study population is in concordance with the existing literature. Intermediate to high levels of erosions appear to offer a high level of specificity for axSpA.[26] Weber *et al* suggested that incorporating erosions in the ASAS MRI definitions would enhance sensitivity from 67% to 81% while maintaining specificity.[28] De Winter *et al* concluded that deep BMO lesions are almost exclusively found in patients with axSpA.[6] A recent retrospective, cross-sectional study of pelvic MRI in a large population of individuals without a rheumatologic condition found that erosions were uncommon and had no age-dependent increase.[29] In another study, no structural changes on MRI were found in pregnant or postpartum women.[14] In the aforementioned study by Seven *et al*, erosions were only present in patients with axSpA and women with postpartum pain, however, with significantly higher prevalence and severity

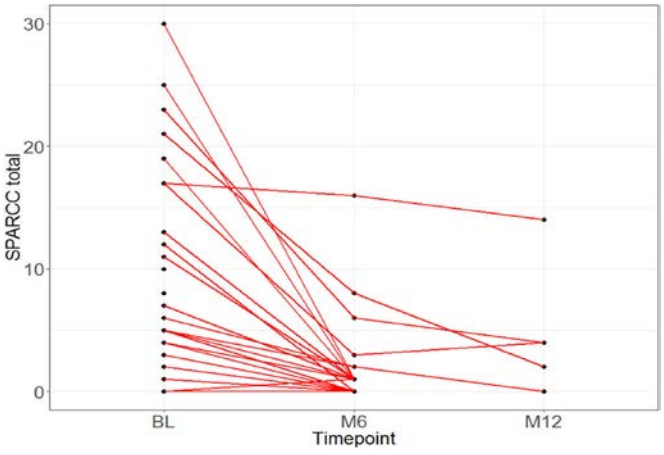


**Figure 1** Evolution of the Spondyloarthritis Research Consortium of Canada (SPARCC) scores over time. Each dot represents an MRI of the sacroiliac joints. MRI examinations of the same subject are connected by a straight line. BL, baseline MRI; M6, month 6 MRI; M12, month 12 MRI.

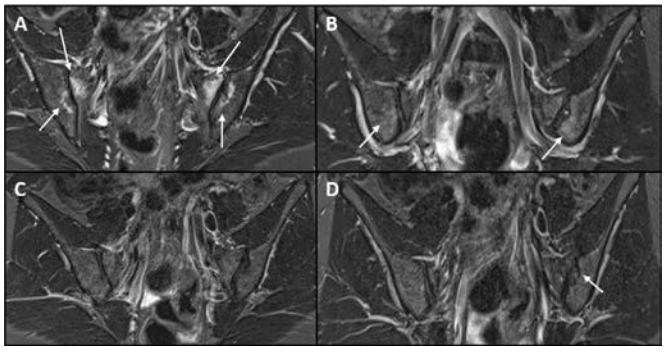


**Figure 2** Sacroiliac joint MRI examinations of a 31-year-old postpartum woman. (A) Extensive sacroiliac bone marrow oedema (BMO) on shorttau inversion recovery images at baseline; (B) decrease of the BMO after 6 months; (C) vanishing of the BMO after 12 months; (D) T1 sequences of the month 12 MRI showing sacroiliac erosions.

**Table 3** Correlation of baseline SPARCC scores and the presence of sacroiliitis on baseline MRI with clinical data

| | Baseline SPARCC | | Sacroiliitis on baseline MRI | |
|---|---|---|---|---|
| | Mean | P value | Yes (n, %) | P value |
| Back pain | | 0.36 | | 0.72 |
| Yes | 11.6 | | 6/11 (55) | |
| No | 5.6 | | 15/24 (63) | |
| First pregnancy | | 0.56 | | 1.00 |
| Yes | 8.3 | | 11/18 (61) | |
| No | 6.7 | | 10/17 (59) | |
| Primipara | | 0.69 | | 0.69 |
| Yes | 7.2 | | 12/22 (55) | |
| No | 8.2 | | 9/13 (69) | |
| Epidural anaesthesia | | **0.050** | | 0.22 |
| Yes | 5.2 | | 11/22 (50) | |
| No | 11.5 | | 10/13 (77) | |
| Newborn's sex | | 0.12 | | 0.24 |
| Male | 5.8 | | 8/17 (47) | |
| Female | 9.2 | | 13/18 (72) | |

| | Rho | P value | Yes | No | P value |
|---|---|---|---|---|---|
| Subject's age (years) | 0.16 | 0.41 | 29.9* | 29.3* | 0.61 |
| Duration of labour (hours) | −0.46 | **0.005** | 8.4* | 12.6* | **0.02** |
| Newborn's weight (g) | −0.02 | 0.91 | 3391* | 3266* | 0.69 |
| Length (cm) | 0.12 | 0.50 | 50.2* | 49.9* | 0.54 |
| Head circumference (cm) | −0.05 | 0.80 | 34.3* | 34.0* | 0.94 |

Significant correlations are shown in bold.
*Mean values.
SPARCC, Spondyloarthritis Research Consortium of Canada.

in the first. Ankylosis and backfill were only seen in patients with axSpA, making these features highly specific.[26]

As back pain is common in postpartum women,[17 30 31] the differential diagnosis with axSpA is a factual issue in clinical practice. In contrast to the pre-MRI era, in which underdiagnosis of SpA was common, nowadays the risk of overdiagnosis is apparent. This holds several pitfalls. Back pain patients with a false diagnosis of axSpA will likely have less therapeutic effect of non-steroidal anti-inflammatory drugs and are subsequently more likely to receive ineffective biological therapy, which has significant potential side effects and encompasses high socioeconomic costs. Unnecessarily, those patients suffer from the psychological consequences of dealing with a chronic, incurable condition. Considering the significant drop in BMO over time in our study, it seems advisable to wait at least 6 months to perform an MRI-SIJ in postpartum women presenting with back pain. When the MRI is considered as suggestive of SpA, it should be repeated more than 1 year after giving birth.

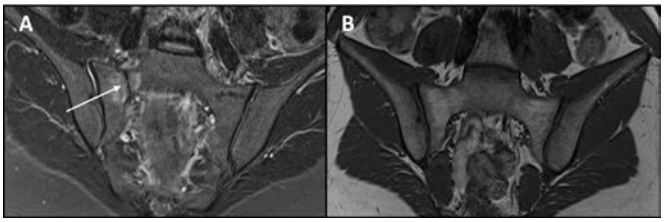


**Figure 3** A postpartum sacral fracture on sacroiliac joint MRI of a 28-year-old woman. (A) Shorttau inversion recovery (STIR) sequences of the baseline MRI show a clear fracture of the sacral bone. (B) STIR sequences show a healed sacral fracture after 6 months.

Interestingly, the presence of sacroiliac BMO was associated with a shorter duration of labour. At first sight, this could appear counterintuitive. However, a shorter labour likely reflects an association with more biomechanical stress in a shorter time period. SPARCC scores were significantly lower in subjects undergoing epidural anaesthesia. A more painful labour may be associated with higher levels of biomechanical stress due to an inefficient labour. Both correlations indicate towards a more important role of giving birth compared with the pregnancy itself in the occurrence of BMO on MRI-SIJ. Nevertheless, Eshed *et al* showed a high frequency of sacroiliac BMO, both prepartum and postpartum.[14] In a study by Agten *et al*, no differences in BMO between women with and without caesarean section were found.[12] The dual relationship between biomechanical stress-induced MRI lesions mimicking sacroiliitis and the role of biomechanical stress in the pathophysiology of SpA complicates the interpretation of MRI-SIJ in postpartum women with back pain even further.[32 33]

In 60% of the subjects, other aberrant MRI findings were reported. Symphysis pubis BMO was seen in a significant portion of postpartum women. Although this is generally not regarded as an SpA lesion, a study by Jans *et al* found a high specificity of symphysis pubis BMO on MRI in patients with axial SpA at time of diagnosis.[34] Sacral fracture is considered to be a rare complication of giving birth.[35] Nonetheless, in this rather small study population, MRI detected two sacral fractures. The fractures were asymptomatic and giving birth was atraumatic and without complications, making these findings accidental. Thus, presumably, the prevalence of postpartum sacral fractures is higher than previously thought.

Major strengths of the present study are the prospective acquisition of postpartum women who would not have been symptomatic enough to warrant further investigation, the correlation with clinical data from mother and child, and the repeated MRI examinations, allowing evaluation of the evolution of the lesions. Other strengths include the blinded reads by three independent, experienced and calibrated readers. Limitations are the small sample size and the exclusion of postpartum women who gave birth through caesarean section to better investigate the role of pregnancy versus childbirth in the occurrence of sacroiliac MRI lesions. Considering the small study population, a multivariate analysis is not reliable and therefore not added to this manuscript.

In conclusion, women immediately postpartum show a markedly high prevalence of sacroiliac BMO on MRI. A significant proportion of the women even fulfilled the ASAS definition of a positive MRI for sacroiliitis, which questions the threshold of this definition. These MRI findings decrease over time, even though a fraction retains BMO over 1 year. When suspecting axSpA, our data indicate the need to wait at least 6 months to perform an MRI-SIJ in postpartum women, and, if positive, repeat the MRI after 12 months. Our data also underscore that interpretation of MRI in the appropriate clinical context is extremely important.

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CLINICAL SCIENCE

# Central reader evaluation of MRI scans of the sacroiliac joints from the ASAS classification cohort: discrepancies with local readers and impact on the performance of the ASAS criteria

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## ABSTRACT

**Objectives** The Assessment of SpondyloArthritis international Society (ASAS) MRI working group conducted a multireader exercise on MRI scans from the ASAS classification cohort to assess the spectrum and evolution of lesions in the sacroiliac joint and impact of discrepancies with local readers on numbers of patients classified as axial spondyloarthritis (axSpA).

**Methods** Seven readers assessed baseline scans from 278 cases and 8 readers assessed baseline and follow-up scans from 107 cases. Agreement for detection of MRI lesions between central and local readers was assessed descriptively and by the kappa statistic. We calculated the number of patients classified as axSpA by the ASAS criteria after replacing local detection of active lesions by central readers and replacing local reader radiographic sacroiliitis by central reader structural lesions on MRI.

**Results** Structural lesions, especially erosions, were as frequent as active lesions (≈40%), the majority of patients having both types of lesions. The ASAS definitions for active MRI lesion typical of axSpA and erosion were comparatively discriminatory between axSpA and non-axSpA. Local reader overcall for active MRI lesions was about 30% but this had a minor impact on the number of patients (6.4%) classified as axSpA. Substitution of radiography with MRI structural lesions also had little impact on classification status (1.4%).

**Conclusion** Despite substantial discrepancy between central and local readers in interpretation of both types of MRI lesion, this had a minor impact on the numbers of patients classified as axSpA supporting the robustness of the ASAS criteria for differences in assessment of imaging.

## Key messages

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

- MRI of the sacroiliac joints is a crucially important evaluation tool for patients presenting with undiagnosed back pain and suspicion of axial spondyloarthritis (axSPA) although there is limited expertise in image interpretation which may compromise accurate diagnosis and classification of this disease.

**What does this study add?**

- The Assessments in SpondyloArthritis international Society MRI working group reports an expert reader assessment of MRI scans from patients presenting to rheumatologists with undiagnosed back pain and characterises MRI lesions that are highly specific for a diagnosis of axSpA.
- This central reader assessment demonstrates substantial differences in imaging interpretation with local readers. However, this does not affect the number of patients classified as having this disease because the clinical arm of the criteria compensates for differences in disease assignment by the imaging arm.

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

- This report demonstrates the importance of both active and structural MRI lesions in diagnostic decision making and the importance of educational initiatives aimed at enhancing interpretation of these lesions. These data also provide reassurance that the Assessment of SpondyloArthritis international Society classification criteria have performance characteristics that may circumvent the limitations posed by the widespread lack of reader expertise in the interpretation of MRI scans.

## INTRODUCTION

The Assessment of SpondyloArthritis international Society classification cohort study (ASAS-CC) recruited patients referred to a rheumatologist with undiagnosed back pain. It led to the ASAS classification criteria in which patients diagnosed with axial spondyloarthritis (axSpA) could be classified as having axSpA by either an imaging or clinical arm.[1] Imaging criteria for sacroiliitis could be either radiographic or the presence of bone marrow oedema (BME) as elaborated in the ASAS consensus definition.[2 3] The sensitivity and specificity of the



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criteria were 83% and 84%, respectively, and follow-up after 4.4 years indicated a high positive predictive value for a rheumatologist's diagnosis of axSpA.[4]

The assessment of MRI scans from the ASAS-CC by local readers was limited to determination whether the baseline scan demonstrated active and/or structural lesions typical of axSpA.[1] In the decade since this study our understanding of MRI lesions in the sacroiliac joint (SIJ) has increased substantially[5] but longitudinal data have been obtained from cohorts of patients with symptoms restricted to 2–3 years and not the typical patient referred to a rheumatologist where symptom duration averages 8–9 years.[6 7] Moreover, it has been recognised that BME can be observed in the SIJ in other disorders and even in 20%–40% of healthy individuals.[8–10] This has led to concerns focused on the accuracy of local reader interpretations of imaging findings on MRI in the ASAS-CC and whether discrepancies found between local and central readers might alter which patients are classified as having axSpA according to the ASAS criteria. Moreover, diagnosis of axSpA was changed by the local rheumatologist in only 11.2% of patients who were available at follow-up after 4.4 years in the ASAS-CC which has also raised concerns regarding diagnostic ascertainment bias.[4] Evaluation of follow-up MRI scans from this cohort to determine whether evolution of MRI findings supports these diagnostic conclusions has not been reported.

These considerations led to the decision by ASAS to convene the ASAS-MRI working group to conduct a multireader exercise to examine both the baseline and follow-up MRI scans from the ASAS-CC. We aimed to address the following questions: (A) What was the relative frequency of MRI lesions in the SIJ at baseline and follow-up according to the recently updated ASAS definitions[11] and expert rheumatologist diagnosis of axSpA? (B) What was the discrepancy between local and central readers in the detection of active and structural MRI lesions in the SIJ and how did this impact which patients were classified as having axSpA? (C) Did replacement of local reader assignment of radiographic sacroiliitis by central reader assignment of MRI structural lesions impact which patients were classified as having axSpA? (D) What was the evolution of MRI features of axSpA from baseline to follow-up and to what degree did this reflect diagnostic assignment by the local rheumatologist?

## METHODS

The study cohort, local rheumatologist assessments, imaging assessments and follow-up of the ASAS-CC have been reported previously.[1 4 11 12]

### ASAS eCRF for evaluation of MRI lesions in the SIJ

The online-available[12] electronic case report form (eCRF) comprised two sections: (A) A global scoring page where readers recorded the presence/absence of each type of MRI lesion according to published ASAS definitions.[11] Central readers provided a yes/no response to two primary MRI questions that local readers also addressed in the original baseline ASAS-CC CRF[1]: MRI Q1. 'Are there typical acute/active inflammatory lesions compatible with axial SpA present in SI joints or at entheseal sites outside the SI joint?' MRI Q2. 'Are typical chronic inflammatory (structural) lesions present in or around SI joints?' (B) A granular scoring web-based interface where inflammatory and structural lesions were recorded according to established rules.[12–14]

### ASAS-CC MRI resource

Baseline and follow-up MRI scans of the SIJ were available from 278 and 170 cases, respectively. Granular assessment for MRI lesions was conducted only in cases where a Digital Imaging and Communications in Medicine (DICOM) series was available in semicoronal orientation.

### Reading exercises

Two multireader exercises were conducted. Validated calibration modules aimed at standardisation of slice selection and defining SIJ quadrants were provided online for review prior to the readings.[15 16] In the first (exercise A), seven central readers assessed baseline MRI scans from 275 cases. In the second exercise (exercise B), eight central readers assessed MRI scans blinded to time point from 108 cases who had MRI performed at baseline and at 4.4 years follow-up. The eCRF for this exercise included an additional question that asked the reader to indicate whether the MRI scan was indicative of the presence of axSpA (yes/no).

### Statistics

Frequencies of each MRI lesion were assessed descriptively according to individual and majority reader data (≥4/7 and ≥5/8 readers for exercises A and B, respectively). Comparison of lesion frequencies according to the local rheumatologist final diagnostic ascertainment of axSpA was analysed using the unpaired t-test and $X^2$ test for continuous and categorical variables, respectively. Agreement for detection of MRI lesions between central and local readers was assessed descriptively and using the kappa statistic. We calculated the number of patients who were classified differently after central reader detection of active lesions on MRI replaced local readers and after central reader detection of structural lesions on MRI replaced local reader detection of radiographic sacroiliitis for overall fulfilment of the ASAS criteria and for the imaging arm of the criteria.

## RESULTS

### Spectrum of MRI lesions at baseline and follow up in the ASAS-CC

In exercise A, 199/275 (72.3%) were diagnosed as having axSpA and 131/170 (77.1%) were diagnosed with axSpA at follow-up. For MRI Q1, active lesions typical of axSpA were observed by a majority of readers in 43.2% and 44.3% of cases diagnosed with axSpA at baseline and follow-up, respectively, as compared with 3.9% and 5.1% diagnosed without axSpA (table 1). The most frequent lesion was subchondral inflammation, which was observed in 51.3% and 13.2% of cases diagnosed with and without axSpA, respectively. Inflammation at the site of erosion, enthesitis and joint space fluid were each observed in 5%–10% of cases diagnosed as axSpA. The first two lesions were also 100% specific for axSpA. For MRI Q2, structural lesions typical of axSpA were observed in 39.4% and 44.6% of cases diagnosed with axSpA at baseline and follow-up, respectively, as compared with 9.7% and 6.5% without axSpA (table 1). The most frequent lesion was erosion followed by fat lesion. The frequencies of MRI lesions were similar when individual reader observations were analysed (online supplementary table 1). Most patients with lesions typical of axSpA had a combination of acute and structural lesions with only 4.6% of cases having only acute lesions and 4.6% having only structural lesions typical of axSpA (online supplementary table 2). There were 13% of cases who had active or structural lesions typical of axSpA by the majority of

**Table 1** Frequencies of active and structural lesions in the SIJ of baseline MRI scans at the level of the majority of readers (≥4/7 reader agreement for the same case) according to local rheumatologist diagnosis of AxSpA (present yes/no) at baseline and follow-up

| | Local rheumatologist diagnosis | | | | | |
|---|---|---|---|---|---|---|
| | Baseline | | | Follow-up | | |
| Baseline variables | Axial SpA=Yes (n=199) | Axial SpA=No (n=76) | P value | Axial SpA=Yes (n=131) | Axial SpA=No (n=39) | P value |
| Mean age | 30.3 (9.4) | 33.6 (10.2) | 0.016 | 30.1 (9.8) | 35.6 (8.4) | 0.001 |
| Mean symptom duration | 5.0 (5.8) | 6.1 (7.4) | 0.25 | 5.3 (6.1) | 6.6 (7.0) | 0.34 |
| Males, % | 109 (54.8) | 30 (39.5) | 0.024 | 77 (58.8) | 13 (33.3) | 0.005 |
| Mean no of SpA features | 2.8 (1.3) | 1.3 (1.1) | <0.0001 | 2.9 (1.4) | 1.2 (0.9) | <0.0001 |
| B27 positive, % | 126 (63.3) | 18 (23.7) | <0.0001 | 93 (71.0) | 6 (15.4) | <0.0001 |
| Elevated CRP, % | 80 (40.2) | 10 (13.2) | <0.0001 | 51 (38.9) | 4 (10.3) | 0.0008 |
| Definite radiographic sacroiliitis, % | 36 (18.4) | 1 (1.4) | 0.0003 | 22 (17.3) | 1 (2.6) | 0.02 |
| **Active MRI lesion variable, no (%) of cases** | | | | | | |
| Active lesions typical of axSpA (MRI Q1) | 86 (43.2) | 3 (3.9) | <0.001 | 58 (44.3) | 2 (5.1) | <0.001 |
| Active lesions typical of axSpA and meets ASAS definition for positive MRI | 79 (39.7) | 2 (2.6) | <0.001 | 52 (39.7) | 2 (5.1) | <0.001 |
| Subchondral inflammation (any) | 102 (51.3) | 10 (13.2) | <0.001 | 65 (49.6) | 7 (17.9) | <0.001 |
| Inflammation at the site of erosion | 20 (7.2) | 0 (0) | <0.001 | 12 (9.2) | 0 (0) | 0.07 |
| Capsulitis | 8 (2.9) | 0 (0) | 0.11 | 5 (3.8) | 0 (0) | 0.59 |
| Joint space fluid | 16 (8.0) | 2 (2.6) | 0.17 | 10 (7.6) | 0 (0) | 0.12 |
| Enthesitis | 14 (5.0) | 0 (0) | 0.013 | 9 (6.9) | 0 (0) | 0.12 |
| BME score, mean (SD)* | 6.3 (12.0) | 0.4 (0.6) | <0.001 | 6.0 (12.5) | 0.5 (0.8) | <0.001 |
| **MRI structural lesion variable, no (%) of cases** | | | | | | |
| | Axial SpA=yes (n=175) | Axial SpA=no (n=62) | P value | Axial SpA=yes (n=112) | Axial SpA=no (n=31) | P value |
| Structural lesions typical of axSpA (MRI Q2) | 69 (39.4) | 6 (9.7) | <0.001 | 50 (44.6) | 2 (6.5) | <0.001 |
| Subchondral sclerosis | 32 (18.3) | 8 (12.9) | 0.43 | 20 (17.9) | 5 (16.1) | 1.000 |
| Erosion | 64 (36.6) | 3 (4.8) | <0.001 | 45 (40.2) | 2 (6.5) | <0.001 |
| Fat lesion | 44 (25.1) | 3 (4.8) | <0.001 | 28 (25) | 3 (9.9) | 0.085 |
| Bone bud | 1 (0.6) | 0 (0) | 1.00 | 1 (0.9) | 0 (0) | 1.00 |
| Fat metaplasia in an erosion cavity | 16 (9.1) | 2 (3.2) | 0.17 | 14 (12.5) | 1 (3.3) | 0.19 |
| Ankylosis | 6 (3.4) | 0 (0) | 0.34 | 5 (4.5) | 0 (0) | 0.59 |
| Erosion score, mean (SD)† | 3.1 (5.0) | 0.8 (2.5) | <0.001 | 3.6 (5.6) | 0.6 (1.7) | <0.001 |
| Fat lesion score, mean (SD)† | 3.4 (6.4) | 0.7 (4.0) | 0.003 | 4.2 (7.6) | 0.2 (0.6) | <0.001 |
| Sclerosis score, mean (SD)† | 2.0 (4.3) | 1.9 (6.2) | 0.95 | 1.9 (4.2) | 3.3 (9.9) | 0.61 |
| Fat metaplasia in an erosion cavity† | 0.7 (4.1) | 0.0 (0.1) | 0.11 | 1.0 (5.2) | 0.0 (0.0) | 0.12 |
| Ankylosis score† | 0.1 (0.2) | 0.05 (0.2) | 0.55 | 0.1 (0.2) | 0.0 (0.0) | 0.002 |

*Cases with detailed scoring per SIJ quadrant/halve (mean (SD)) available: axSpA at baseline yes, n=109 No, n=49; axSpA at follow-up yes, n=69 no, n=17.
†Cases with detailed scoring per SIJ quadrant/halve (mean (SD)) available: axSpA at baseline yes, n=102 no, n=44; axSpA at follow-up yes, n=63 no, n=16.
ASAS, Assessment of SpondyloArthritis international Society; axSPA, axial spondyloarthritis; BME, bone marrow oedema; CRP, C reactive protein; SIJ, sacroiliac joint.

readers but were diagnosed as not having axSpA at baseline and follow-up.

In exercise B, assessment of MRI scans blinded to baseline and follow-up time points demonstrated that central reader detection of active lesions typical of axSpA was 100% and 95.2% specific for rheumatologist diagnosis of axSpA, respectively (table 2). Sensitivity for diagnosis of axSpA was 41% at baseline and 28% at follow-up. There was a decrease of 9.3% in the proportion of cases from the entire cohort with active inflammatory lesions typical of axSpA (MRI Q1) from baseline to follow-up (p=0.05). Subchondral inflammation was observed in 49% of cases diagnosed as axSpA at baseline and 36% at follow-up but also in 4.2% and 14.3% of baseline and follow-up scans from cases without axSpA. There were 19 (17.8%) cases that were started on tumour necrosis factor inhibitor (TNF) therapy during the course of follow-up. Of these cases, 57.9% had a reduction in inflammatory lesions compared with 5.7% of cases not receiving anti-TNF therapy (p<0.001).

Structural lesions typical of axSpA (MRI Q2) were observed in 38.2% and 51.2% of baseline and follow-up scans of cases diagnosed with axSpA, respectively. For the entire cohort, there was a significant increase of 9.4% (p=0.02) in cases with structural lesions from baseline to follow-up, and this was composed of an increased proportion with a fat lesion and ankylosis (table 2). Erosion was the structural lesion observed most frequently in axSpA, was more highly discriminatory than any active lesion per follow-up diagnostic assessment and was highly specific, being present in only a single case diagnosed at baseline as non-axSpA, and in no cases diagnosed as non-axSpA at follow-up.

**Table 2** Frequencies of active and structural lesions in the SIJ of baseline and follow-up MRI scans at the level of the majority of readers (≥5/8 reader agreement for the same case) according to local rheumatologist diagnosis of axSpA (present yes/no) at baseline and follow-up

| | Local rheumatologist diagnosis | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | | | | Follow-up | | | |
| | All cases (n=108) | Axial SpA=Yes (n=86) | Axial SpA=No (n=22) | P value | All cases (n=108) | Axial SpA=Yes (n=87) | Axial SpA=No (n=21) | P value |
| MRI indicative of axSpA according to central readers, (%) | 44 (40.7) | 43 (50.0) | 1 (4.5) | <0.001 | 47 (43.9) | 46 (52.9) | 1 (4.8) | <0.001 |
| **Active MRI lesion variable, no (%) of cases** | | | | | | | | |
| **Cases with global assessment of active lesions** | **All Cases (n=107)** | **Axial SpA=Yes (n=85)** | **Axial SpA=No (n=22)** | **P-value** | **All Cases (n=107)** | **Axial SpA=Yes (n=86)** | **Axial SpA=No (n=21)** | **P-value** |
| Active lesions typical of axSpA | 35 (32.7) | 35 (41.2) | 0 (0) | <0.001 | 25 (23.4) | 24 (27.9) | 1 (4.8) | 0.023 |
| Active lesions typical of axSpA and meets ASAS definition for positive MRI | 35 (32.7) | 35 (41.2) | 0 (0) | <0.001 | 24 (22.4) | 23 (26.7) | 1 (4.8) | 0.039 |
| Subchondral inflammation | 43 (40.2) | 42 (49.4) | 1 (4.5) | <0.001 | 34 (31.8) | 31 (36.0) | 3 (14.3) | 0.056 |
| Inflammation at the site of erosion | 3 (2.8) | 3 (3.5) | 0 (0) | 1.00 | 2 (1.9) | 2 (2.3) | 0 (0) | 1.00 |
| Capsulitis | 3 (2.8) | 3 (3.5) | 0 (0) | 1.00 | 0 (0) | 0 (0) | 0 (0) | 1.00 |
| Joint space fluid | 12 (11.2) | 12 (14.1) | 0 (0) | 0.121 | 4 (3.7) | 4 (4.7) | 0 (0) | 0.58 |
| Enthesitis | 2 (1.9) | 2 (2.4) | 0 (0) | 1.00 | 2 (1.9) | 2 (2.3) | 0 (0) | 1.00 |
| **Cases with Detailed Scoring of Active Lesions** | **All cases (n=80)** | **Axial SpA=yes (n=64)** | **Axial SpA=no (n=16)** | **P value** | **All cases (n=66)** | **Axial SpA=yes (n=66)** | **Axial SpA=no (n=14)** | **P value** |
| BME score, mean (SD) | 4.6 (8.8) | 5.8 (9.5) | 0.4 (0.5) | <0.001 | 3.4 (7.5) | 4.0 (8.1) | 0.8 (2.1) | 0.007 |
| **Structural MRI lesion variable, no (%) of cases** | | | | | | | | |
| **Cases with global assessment of structural lesions** | **All n=85** | **Axial SpA=yes (n=68)** | **Axial SpA=no (n=17)** | **P value** | **All n=85** | **Axial SpA=yes (n=70)** | **Axial SpA=no (n=15)** | **P value** |
| Structural lesions typical of axSpA | 28 (32.9) | 26 (38.2) | 2 (11.8) | 0.039 | 36 (42.3) | 36 (51.4) | 0 (0) | <0.001 |
| Subchondral sclerosis | 8 (9.4) | 6 (8.8) | 2 (11.8) | 0.66 | 5 (5.9) | 5 (7.1) | 0 (0) | 0.58 |
| Erosion | 24 (28.2) | 23 (33.8) | 1 (5.9) | 0.032 | 24 (28.2) | 24 (34.3) | 0 (0) | 0.005 |
| Fat lesion | 21 (24.7) | 18 (26.5) | 3 (17.6) | 0.55 | 23 (27.1) | 22 (31.4) | 1 (6.7) | 0.059 |
| Bone bud | 1 (1.2) | 1 (1.5) | 0 (0) | 1.00 | 0 (0) | 0 (0) | 0 (0) | 1.00 |
| Fat metaplasia in an erosion cavity (FM-EC) | 5 (5.9) | 4 (5.9) | 1 (5.9) | 1.00 | 5 (5.9) | 5 (7.1) | 0 (0) | 0.58 |
| Ankylosis | 3 (3.5) | 3 (4.4) | 0 (0) | 1.00 | 5 (5.9) | 5 (7.1) | 0 (0) | 0.58 |
| **Cases with Detailed Scoring of Structural Lesions** | **All cases (n=49)** | **Axial SpA=yes (n=39)** | **Axial SpA=no (n=10)** | **P value** | **All cases (n=49)** | **Axial SpA=yes (n=41)** | **Axial SpA=no (n=8)** | **P value** |
| Erosion score, mean (SD) | 2.3 (4.2) | 2.6 (4.3) | 1.2 (3.8) | 0.37 | 2.3 (5.3) | 2.8 (5.8) | 0.1 (0.2) | 0.004 |
| Fat lesion score, mean (SD) | 4.0 (7.7) | 4.3 (7.5) | 2.7 (8.6) | 0.57 | 4.5 (7.8) | 5.4 (8.3) | 0.2 (0.3) | <0.001 |
| Sclerosis score, mean (SD) | 1.0 (2.9) | 1.1 (3.2) | 0.6 (1.3) | 0.43 | 0.9 (2.8) | 1.1 (3.0) | 0.0 (0.0) | 0.032 |
| FM-EC | 0.3 (0.8) | 0.3 (0.9) | 0.3 (0.8) | 0.78 | 0.6 (1.5) | 0.7 (1.6) | 0.0 (0.0) | 0.008 |
| Ankylosis score | 0.7 (4.5) | 0.9 (5.1) | 0.0 (0.1) | 0.30 | 0.9 (4.5) | 1.05 (4.89) | 0.0 (0.0) | 0.18 |

ASAS, Assessment of SpondyloArthritis international Society; axSpA, axial spondyloarthritis; BME, bone marrow oedema; SIJ, sacroiliac joint.

In exercise B, MRI was considered indicative of axSpA in 44/108 (40.7%) of cases at baseline and in 43/86 (50.0%) diagnosed as axSpA by the rheumatologist. Change in MRI diagnosis from baseline to follow-up assessments was recorded in only 10/108 (9.3%) cases (four from axSpA to not axSpA and six from not axSpA to axSpA) according to agreement by ≥2 readers (table 3). Change in MRI diagnosis was recorded in only three cases according to a majority of readers (≥5/8). Change in rheumatologist diagnosis was recorded in 9/108 (8.3%) cases, two of which had a change in MRI diagnosis.

### Local versus central reader detection of MRI lesions in the SIJ

The frequency of active lesions reported by local readers (61%) in cases diagnosed with axSpA was greater than for central readers (43.2% and 49.7% for majority (≥4/7) and ≥2 reader data, respectively) (table 4). This difference was similar for scans limited to cases that attended for follow-up evaluation and cases where only data from DICOM scans was analysed (online supplementary table 3).

Structural lesions typical of axSpA were reported by local readers in 44.4% of cases who were diagnosed with axSpA. This compares with 39.5% and 54.9% of cases when assessed by a majority and ≥2 central readers, respectively.

Discordance between central and local readers for detection of active lesions (MRI Q1) was recorded in 46 (17.8%) and 47 (18.2%) of cases according to ≥2 and majority (≥4/7) central reader data, respectively (kappa (95% CI) of 0.64 (0.54 to 0.73) and 0.62 (0.53 to 0.72)) (table 5). With central reading as external standard the false-positive rate for active lesions was 27.4% and 33.3% ('local overcall') for ≥2 and majority reader data, respectively. Reliability between the seven central readers was higher with a median kappa value of 0.74 and range of 0.63–0.83 for all possible reader pairs (online supplementary table 4). Discordance between central and local readers for detection of structural lesions (MRI Q2) was noted in 66 (30.0%) and 67 (30.5%) of cases according to ≥2 and majority (≥4/7) central reader data, respectively (kappa (95% CI) of 0.44 (0.32 to 0.55) and 0.38 (0.25 to 0.50)). Local versus central reader discrepancies were less evident when only data from DICOM scans was assessed (table 5).

**Table 3** MRI considered indicative of axSpA at baseline and follow-up at the level of any two central readers or the majority of central readers (≥5/8 reader agreement for the same case) according to local rheumatologist diagnosis of axSpA (present yes/no) at baseline and follow-up

| Rheumatologist's diagnosis | MRI indicative of axSpA (any two readers) | | | |
|---|---|---|---|---|
| | Yes at baseline and yes at follow-up (n=48), (%) | Yes at baseline and no at follow-up (n=4), (%) | No at baseline and yes at follow-up (n=6), (%) | no at baseline and No at follow-up (n=50), (%) |
| SpA yes at baseline and follow-up (n=82) | 46 (56.1) | 2 (2.4) | 4 (4.9) | 30 (36.6) |
| SpA no at baseline and yes at follow-up (n=5) | 1 (20) | 0 (0) | 1 (20) | 3 (60) |
| SpA yes at baseline and no at follow-up (n=4) | 1 (25) | 1 (25) | 0 (0) | 2 (50) |
| SpA no at baseline and no at follow-up (n=17) | 0 (0) | 1 (5.9) | 1 (5.9) | 15 (88.2) |
| **Rheumatologist's diagnosis** | **MRI indicative of axSpA (majority (≥5) of readers)** | | | |
| | Yes at baseline and yes at follow-up (n=43) | Yes at baseline and no at follow-up (n=1) | No at baseline and yes at follow-up (n=4) | No at baseline and no at follow-up (n=60) |
| SpA yes at baseline and follow-up (n=82) | 42 (51.2) | 1 (1.2) | 2 (2.4) | 37 (61.7) |
| SpA no at baseline and yes at follow-up (n=5) | 1 (20) | 0 (0) | 1 (20) | 3 (60) |
| SpA yes at baseline and no at follow-up (n=4) | 0 (0) | 0 (0) | 0 (0) | 4 (100) |
| SpA no at baseline and no at follow-up (n=17) | 0 (0) | 0 (0) | 1 (5.9) | 16 (94.1) |

axSpA, axial spondyloarthritis.

### Impact of central versus local reader discrepancies in detection of active lesions typical of axSpA (MRI Q1) on classification of axial SpA

There were 159 (63.1%) patients who fulfilled the ASAS axSpA criteria based on local-reading, and 148 (58.7%) and 143 (56.7%) patients based on ≥2 and majority central-reading, respectively (table 6). A total of 19 (7.5%) and 20 (7.9%) patients who were classified as axSpA after local reading were reclassified as not having axSpA after ≥2 and majority reader central evaluation. Conversely, eight (3.2%) and four (1.6%) cases who were classified as having axSpA after ≥2 and majority reader central evaluation, respectively, would have been reclassified as not having axSpA after local assessment. The numbers were similar when fulfilment of the imaging arm was the primary consideration (irrespective of the clinical arm).

### Impact of replacing local reader detection of radiographic sacroiliitis by central reader detection of MRI structural lesions (MRI Q2) on classification of axSpA

In total, 120 (55.3%) cases fulfilled the axSpA criteria based on local reading of radiographic sacroiliitis and central reading of active inflammation on MRI. This changed to 125 (57.6%) and 117 (53.9%) of cases after replacement of radiographic sacroiliitis by ≥2 and majority central reader MRI structural lesions, respectively (table 6). A total of nine (4.1%) and four (1.8%) cases who were classified as not having axSpA were reclassified as having axSpA after replacing radiographic sacroiliitis with ≥2 and majority reader MRI structural lesions, respectively. Conversely, seven (3.2%) and eight (3.7%) cases were reclassified as not having axSpA after substitution by ≥2 and majority reader MRI structural lesions, respectively. The numbers were similar when fulfilment of the imaging arm was the primary consideration (irrespective of the clinical arm).

## DISCUSSION

This first central reader evaluation of MRI scans from the ASAS-CC study applying consensus definitions for MRI lesions recently reported by ASAS[11] demonstrates several observations of major importance to the interpretation of MRI scans relevant to both diagnosis and classification of axSpA. First, structural lesions occur almost as frequently as active lesions in patients presenting with undiagnosed back pain to a rheumatologist. Second, subchondral bone marrow inflammation may occur in 10%–15% of cases diagnosed as non-axSpA while other active lesions such as inflammation in an erosion cavity, capsulitis, and enthesitis are highly specific for axSpA but each occur in only 5%–10% of cases. Third, central reader detection of active MRI lesions considered typical of axSpA and erosions was comparatively discriminatory between axSpA and non-axSpA. Fourth, there was relatively little change in the frequencies of active and structural lesions over a mean follow-up period of 4.4 years in this cohort of patients who received mainly conservative therapy. Fifth, although clear discrepancy between local and central readers in detection of MRI lesions was evident this had a minor impact on the total number of patients classified as axSpA using the ASAS criteria. Even substitution of radiography with structural lesions detected on T1W MRI by central readers did not materially impact the number of patients classified as having axSpA.

This is the first report that describes the frequencies of the broad spectrum of active and structural MRI lesions according to recently published ASAS definitions in patients presenting to the rheumatologist with undiagnosed back pain. Active or structural lesions typical of axSpA were observed by a majority of central readers in 55% of patients diagnosed by local rheumatologists with axSpA but also in 12.9% of non-axSpA cases suggesting that axSpA may have been under-recognised by local rheumatologists. Subchondral BME was observed in about 50% of cases diagnosed with axSpA although the definition of an ASAS positive MRI was met in only 40%. The corresponding frequencies in non-axSpA cases were 13.2% for subchondral BME and 2.6% for an ASAS positive MRI. This is much lower than the 20%–40% frequency often cited for an ASAS positive MRI in controls, both healthy and those diagnosed with non-specific back pain, in other cohorts.[8–10] This could be explained by central reader expertise in distinguishing BME lesions suggestive of axSpA versus non-specific findings and also the concomitant presence of structural lesions. It reinforces the importance of contextual interpretation of T1W and fat-suppressed scans for diagnostic interpretation of MRI scans previously emphasised in an ASAS consensus exercise.[3]

The revised ASAS definition of erosion was highly discriminatory and was detected in fewer than 10% of non-axSpA cases in both reading exercises although sensitivity of 30%–40% was lower than the 50%–60% reported in some previous studies of

**Table 4** Central and local MRI reader assessment of active and structural MRI lesions in the SIJ according to diagnostic ascertainment by the local physician at baseline and follow-up in the ASAS classification study

| Reader | MRI lesion type | Local rheumatologist diagnosis at baseline | | P value | Local rheumatologist diagnosis at follow-up | | P value |
|---|---|---|---|---|---|---|---|
| | | AxSpA (n=187) | Not AxSpA (n=70) | | AxSpA (n=122) | Not AxSpA (n=35) | |
| **Active lesions** | | | | | | | |
| Local | Active lesions typical of axSpA | 114 (61.0%) | 3 (4.3%) | <0.001 | 75 (61.5%) | 5 (14.3%) | <0.001 |
| Central (≥4/7 reader agreement) | Active lesions typical of axSpA | 83 (43.2%) | 3 (4.3%) | <0.001 | 56 (45.9%) | 2 (5.7%) | <0.001 |
| Central (≥4/7 reader agreement) | ASAS MRI positive | 76 (40.6%) | 2 (2.9%) | <0.001 | 50 (41%) | 2 (5.7%) | <0.001 |
| Central (any 2 readers) | Active lesions typical of axSpA | 93 (49.7%) | 6 (8.6%) | <0.001 | 60 (49.2%) | 5 (14.3%) | <0.001 |
| Central (any 2 readers) | ASAS MRI positive | 89 (47.6%) | 5 (7.1%) | <0.001 | 57 (46.7%) | 4 (11.4%) | <0.001 |
| **Structural lesions** | | | | | | | |
| | | AxSpA (n=162) | Not AxSpA (n=58) | | AxSpA (n=103) | Not AxSpA (n=28) | |
| Local | Structural lesions typical of axSpA | 72 (44.4%) | 3 (5.2%) | <0.001 | 44 (42.7%) | 4 (14.3%) | 0.007 |
| Central (any 2 readers) | Structural lesions typical of axSpA | 89 (54.9%) | 10 (17.2%) | <0.001 | 56 (54.4%) | 6 (21.4%) | 0.003 |
| Central (≥4/7 reader agreement) | Structural lesions typical of axSpA | 64 (39.5%) | 6 (10.3%) | <0.001 | 46 (44.7%) | 2 (7.1%) | <0.001 |

ASAS, Assessment of SpondyloArthritis international Society; AxSpA, axial spondyloarthritis; SIJ, sacroiliac joint.

MRI in axSpA.[17 18] This may reflect differences in the definition of erosion. The first ASAS publication on MRI definitions in the SIJ cited only the requirement for a bony defect at the joint margin without specifying alteration in the signal from adjacent bone marrow.[2] The revised ASAS definition stipulates both a bony defect as well as loss of the adjacent bright marrow signal observed on a T1W sequence.[11] Fat lesion with the distinct features of axSpA, namely a sharp border and homogeneous increased T1W signal, was also discriminatory but sensitivity was less than for erosion at 25%–30% while specificity was 90%–95%, which was comparable to findings in other cohorts of early SpA that applied a similar definition.[18–20]

We observed local reader overcall in the range of 25%–35% when using the central reader assessment as external standard raising the possibility of diagnostic overcall. However, this had little impact on the number of patients classified with axSpA since patients could still be classified as axSpA by the clinical arm. Conversely, local readers detected fewer structural lesions than central readers. This could reflect the requirement for good quality T1W images so that the more complex structural lesions can be adequately visualised as the discrepancy was less evident when DICOM images were assessed. Nevertheless, substitution of radiographic sacroiliitis by structural lesions on MRI detected by central readers had a minor impact on the number of patients classified as axSpA. This may not be surprising as most patients with structural lesions also had active lesions typical of axSpA. Similar observations have been reported in two early axSpA cohorts.[21 22]

There are some limitations of our data. It has been over a decade since the local MRI reads were conducted and it is possible that discrepancy might be less evident if the study was a contemporary comparison. However, recent clinical trials of non-radiographic axSpA[23 24] have reported similar symptom duration prior to diagnosis as noted for the ASAS-CC suggesting that diagnostic delay has not changed a great deal over the past decade and that imaging findings may therefore not be different. Interpretation of local reader data is compromised by lack of data recorded in the ASAS-CC CRF as to which types of MRI lesion were observed. The assessment of structural lesions, especially erosion, is increasingly being performed using MRI sequences that can enhance the contrast between the joint space and bone.[25]

**Table 5** Agreement between central and local readers for active (MRI Q1) and structural (MRI Q2) lesions typical for axSpA observed on all available MRI scans from patients in the ASAS classification cohort

| Local reader | | Central readers (all MRI scans)* | | | | Central readers (DICOM MRI scans)† | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Active lesion (≥2 readers) | | Active lesion (≥4 readers) | | Active lesion (≥2 readers) | | Active lesion (≥4 readers) | |
| | | Yes | No | Yes | No | Yes | No | Yes | No |
| **Active lesion** | Yes | 85 | 32 | 78 | 39 | 42 | 17 | 37 | 22 |
| | No | 14 | 127 | 8 | 133 | 11 | 90 | 7 | 94 |
| **Kappa (95% CI)** | | 0.64 (0.54 to 0.73) | | 0.62 (0.53 to 0.72) | | 0.62 (0.49 to 0.74) | | 0.59 (0.46 to 0.72) | |
| | | Structural lesion (≥2 readers) | | Structural lesion (≥4 readers) | | Structural lesion (≥2 readers) | | Structural lesion (≥4 readers) | |
| **Structural lesion** | Yes | 58 | 25 | 43 | 40 | 29 | 9 | 21 | 17 |
| | No | 41 | 130 | 27 | 144 | 25 | 75 | 14 | 86 |
| **Kappa (95% CI)** | | 0.44 (0.32 to 0.55) | | 0.38 (0.25 to 0.50) | | 0.62 (0.49 to 0.74) | | 0.59 (0.46 to 0.72) | |

*Total with MRI data for assessment of active lesions=258, total with MRI data for assessment of structural lesions=220.
†Total with MRI data for assessment of active lesions=160, total with MRI data for assessment of structural lesions=138.
ASAS, Assessment of SpondyloArthritis international Society; axSpA, axial spondyloarthritis.

**Table 6** Impact of reader discrepancy (central vs local) for detection of active SIJ lesions on MRI and replacement of radiographs by MRI structural lesions on classification of axial SpA in the ASAS classification cohort

| MRI assessment used | Overall SpA Classification=yes after MRI assessment N (%) | Overall SpA Classification=no after MRI assessment N (%) | Imaging Arm SpA Classification=yes after MRI assessment N (%) | Imaging Arm SpA Classification=no after MRI assessment N (%) |
|---|---|---|---|---|
| **Impact of central versus local reader SIJ MRI inflammation assessment on SpA classification in cases with all clinical, radiographic, and central and local MRI inflammation data available (n=252)** | | | | |
| **Local reader SIJ MRI Inflammation positive** | 159 (63.1) | 93 (36.9) | 126 (50) | 126 (50) |
| **≥2 central reader SIJ MRI inflammation assessment positive** | 148 (58.7) | 104 (41.3) | 111 (44.0) | 141 (56.0) |
| **Majority central reader (≥4/7) SIJ MRI inflammation assessment positive** | 143 (56.7) | 109 (43.2) | 102 (40.5) | 150 (59.5) |
| **Impact of replacement of radiographic sacroiliitis by MRI structural lesions on SpA classification in cases with all clinical, radiographic, and central and local MRI inflammation data available (n=217)** | | | | |
| **Central reader MRI Inflammation Positive*** | 120 (55.3) | 97 (44.7) | 83 (38.2) | 134 (61.8) |
| **Replace radiographic sacroiliitis with central reader (≥2) MRI structural positive†** | 125 (57.6) | 92 (42.4) | 100 (46.1) | 117 (53.9) |
| **Replace radiographic sacroiliitis with central reader (≥4/7) MRI structural positive†** | 117 (53.9) | 100 (46.1) | 85 (39.2) | 132 (60.8) |

*Positive imaging for classification is defined by either local reader positive for radiographic sacroiliitis or majority of central readers positive for MRI inflammation.
†Positive imaging for classification is defined by either central readers positive for MRI structural lesions or majority of central readers positive for MRI inflammation.
ASAS, Assessment of SpondyloArthritis international Society; axSpA, axial spondyloarthritis; SIJ, sacroiliac joint.

In conclusion, our analysis of MRI scans from patients referred to rheumatologists with undiagnosed back pain demonstrates the importance of both active and structural lesions in diagnostic decision making and the importance of educational initiatives aimed at enhancing interpretation of these lesions. These data also provide reassurance that the ASAS classification criteria have performance characteristics that may circumvent the limitations posed by the widespread lack of reader expertise in the interpretation of MRI scans. However, our study design was retrospective in nature and could not assess the impact of reader discrepancy on diagnostic ascertainment. Consequently, the performance of the ASAS criteria will require further testing in a study design where the impact of differences in interpretation of imaging on diagnostic ascertainment can be addressed.

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CLINICAL SCIENCE

# Remission in systemic lupus erythematosus: testing different definitions in a large multicentre cohort

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## ABSTRACT

**Objectives** Remission in systemic lupus erythematosus (SLE) is defined through a combination of 'clinical SLE Disease Activity Index (cSLEDAI)=0', 'physician's global assessment (PGA) <0.5' and 'prednisone (PDN) ≤5 mg/day'. We investigated the performance of these items, alone or in combination, in defining remission and in predicting SLICC/ACR Damage Index.

**Methods** We tested seven potential definitions of remission in SLE patients followed-up for ≥5 years: PDN ≤5 mg/day; PGA <0.5; cSLEDAI=0; PGA <0.5 plus PDN ≤5 mg/day; cSLEDAI=0 plus PGA <0.5; cSLEDAI=0 plus PDN ≤5 mg/day; cSLEDAI=0 plus PDN ≤5 mg/day plus PGA <0.5. The effect of these definitions on damage was evaluated by Poisson regression analysis; the best performance was identified as the lowest Akaike and Bayesian information criterion (AIC and BIC). Positive and negative predictive values in identifying no damage increase were calculated.

**Results** We included 646 patients (mean±SD disease duration 9.2±6.9 years). At multivariate analysis, ≥2 consecutive year remission according to all definitions protected against damage (OR, 95% CI: PGA <0.5 0.631, 0.444 to 0.896; cSLEDAI=0 0.531, 0.371 to 0.759; PGA <0.5 plus PDN ≤5 mg/day 0.554, 0.381 to 0.805; cSLEDAI=0 plus PGA <0.5 0.574, 0.400 to 0.826; cSLEDAI=0 plus PDN ≤5 mg/day 0.543, 0.376 to 0.785; cSLEDAI=0 plus PDN ≤5 mg/day plus PGA <0.5 0.532, 0.363 to 0.781, p<0.01 for all), except PDN ≤5 mg/day, which required four consecutive years (OR 0.534, 95% CI 0.325 to 0.877, p=0.013). Positive and negative predictive values were similar; however, cSLEDAI=0 showed the best performance (AIC 1082.90, BIC 1109.72, p<0.0001). Adding PGA <0.5 and/or PDN ≤5 mg/day to cSLEDAI=0 decreased remission duration (−1.8 and −1.5 year/patient, respectively) without increasing cSLEDAI=0 performance in predicting damage accrual.

**Conclusions** cSLEDAI=0 is the most attainable definition of remission, while displaying the best performance in predicting damage progression in the short-to-mid-term follow-up.

### Key messages

**What is already known about this subject?**
- Remission is the most desirable target in systemic lupus erythematosus (SLE); however, a universally accepted definition is still missing.

**What does this study add?**
- A simple definition of remission based on clinical SLE Disease Activity Index (SLEDAI)=0 is easy-to-achieve and protective against damage.
- Adding Physician Global Assessment (<0.5) to clinical SLEDAI=0 decreases the proportion of remitted patients without increasing the performance of clinical SLEDAI in predicting damage accrual.

**How might this impact on clinical practice or future developments?**
- Clinical SLEDAI=0 could serve as a manageable and meaningful outcome in SLE trials and treat-to-target strategy.

## INTRODUCTION

Remission is the most desirable target in the management of systemic lupus erythematosus (SLE), as it leads to a significant improvement across several disease outcome measures (death, organ damage, disease flare-up and health-related quality of life (HRQoL))[1–9]; however, a universally accepted definition of remission combining evidence-based medicine and expert opinion is still missing.[3 10 11] The Definitions Of Remission In SLE (DORIS) international task force defined remission as a sustained state without any symptoms and signs of SLE, assessing disease activity according to the clinical SLE Disease Activity Index 2000 (cSLEDAI),[10] which does not take into account serology,[12] and evaluating the global patient status by Physician Global Assessment (PGA). Addition of PGA was meant to reflect the overall clinician-based evaluation, thereby filling the gaps of cSLEDAI and being also suggested to reflect the patient's perspective.[10]

Since moderate-to-high glucocorticoid dosages contribute to damage accrual in the long term, a prednisone (PDN) dose (or equivalent) ≤5 mg/day was included in the validated definitions of remission.[3 10 13 14]

It should be noted that no clear agreement was reached so far in defining durability in time of any definition of remission, which remains a research priority.

To our knowledge, no head-to-head comparisons were made on the impact of each item or combination

of items included in the definitions of remission proposed by the DORIS task force (ie, cSLEDAI=0, PGA <0.5, PDN ≤5 mg/day) on disease outcomes in a clinical practice setting. Our aim was to compare the prevalence and duration of different potential definitions of remissions and to evaluate their effect on organ damage in a large, closely monitored cohort of patients with SLE.

## PATIENTS AND METHODS

### Data source

We carried out a multicentre study enrolling patients with SLE recruited in seven referral lupus centres in Italy: University of Padova, Campus Bio-Medico University (Rome), Sapienza University (Rome), University of Brescia, University Hospital S. Anna (Ferrara), Fondazione Ca' Granda IRCCS Ospedale Maggiore Policlinico (Milano) and University of Pisa. All patients provided an informed consent before the inclusion in the study.

Patients with SLE were enrolled in the study according to the fulfilment of all the following criteria: (1) ≥4 revised American College of Rheumatology (ACR) Classification Criteria for SLE; (2) SLE diagnosis between 1990 and 2013; (3) active disease, that is, at least one clinical manifestation scored in the SLEDAI-2K, or remission lasting no more than 12 months at study entry; (4) at least five consecutive years of follow-up between January 2009 and September 2018; (5) at least three visits per year, no more than 5 months apart. We analysed a period of 5-year for all patients. Data regarding disease manifestations, autoantibody profile, medical history and organ damage were recorded since SLE diagnosis. The cumulative PDN dose taken before baseline was calculated. Clinical and laboratory findings were collected at each visit.

### Study variables

At each visit SLE activity was measured by SLEDAI-2K and PGA (scale 0–3). Flares were defined according to SELENA-SLEDAI flare index.[15] Organ damage was assessed by Systemic Lupus International Collaborating Clinics/ACR Damage Index (SDI), which was calculated at baseline and annually thereafter. SDI increase was defined as the difference between SDI at the end and at the beginning of the 5-year follow-up. Damage was categorised as related to or independent from glucocorticoids, as defined by Gladman *et al*[16]

We considered the three major items included in the DORIS definition of 'remission on therapy': (1) PGA <0.5; (2) cSLEDAI=0; (3) PDN ≤5 mg/day (or equivalent) daily intake. We also tested their combinations: (4) PGA <0.5 plus PDN ≤5 mg/day; (5) cSLEDAI=0 plus PGA <0.5; (6) cSLEDAI=0 plus PDN ≤5 mg/day; (7) cSLEDAI=0 plus PDN ≤5 mg/day plus PGA <0.5.[10] All seven potential definitions are presented in table 1. For composite definitions, remission was considered as achieved when the two or three items were concomitantly met. One consecutive year was considered the shortest duration of remission for a clinically meaningful definition. In patients with a relapsing-remitting disease, only the longest period of remission was considered in the analysis.

For all definitions, concomitant lupus medications (antimalarials and/or immunosuppressive drugs and/or biologics) were allowed if on a stable dose. Haemolytic anaemia, myelitis and gastrointestinal lupus involvement prevented the fulfilment of all remission definitions.

We also performed a separate analysis, where the cumulative time spent in remission was evaluated and expressed as proportion of the follow-up in remission, that is, <25%, 25%–49%, 50%–74%, 75%–99% and 100%.

### Statistical analysis

T-test was used to compare continuous data with a parametric distribution. Comparison of categorical variables were performed using $\chi^2$ test (Fisher's exact test if necessary). Remission definitions were considered as six-levels categorical variables according to duration (0, 1, 2, 3, 4 and 5 consecutive years). Cohen's kappa coefficient (κ) and 95% CI were used to assess agreement among remission definitions. The patient's demographic and clinical characteristics and remission definitions were tested as predictors of damage accrual by univariate Poisson regression analysis. Those variables with p<0.2 were considered in a multivariate Poisson regression analysis. ORs were estimated with 95% CI. The goodness-of-fit was assessed using Akaike information criterion (AIC) and Bayesian information criterion (BIC): the best performance in predicting damage accrual was identified by the lowest AIC and BIC. Analyses were performed by SAS V.9.4 (SAS Institute) and SPSS (V.25 for Windows), and p<0.05 was considered statistically significant.

This research was done without patient involvement and patients were not invited to comment on the study design or to develop patient outcomes.

## RESULTS

Six hundred and forty-six consecutive lupus patients fulfilled inclusion criteria: 621 (96.1%) Caucasian, 585 (90.6%) female, mean±SD age at baseline 40.6±12.1 years, disease duration 9.2±6.9 years. At baseline, 545 patients (84.4%) were taking PDN, 460 (71.2%) antimalarials and 316 (48.8%) immunosuppressants. Demographic and clinical characteristics of patients are reported in table 2.

**Table 1** Definitions of remission according to clinical, serological and therapeutic status allowed

| | Disease activity | | | Treatment | |
|---|---|---|---|---|---|
| | SLEDAI-2K | | | | |
| | Clinical | Serological | PGA | PDN | Antimalarials, biologics, immunosuppressants* |
| PDN ≤5 mg/day | Regardless | Regardless | Regardless | ≤5 mg/day | Regardless |
| PGA <0.5 | Regardless | Regardless | <0.5 | Regardless | Regardless |
| cSLEDAI=0 | 0 | Regardless | Regardless | Regardless | Regardless |
| PDN ≤5 mg/day plus PGA <0.5 | Regardless | Regardless | <0.5 | ≤5 mg/day | Regardless |
| cSLEDAI=0 plus PGA <0.5 | 0 | Regardless | <0.5 | Regardless | Regardless |
| cSLEDAI=0 plus PDN ≤5 mg/day | 0 | Regardless | Regardless | ≤5 mg/day | Regardless |
| cSLEDAI=0 plus PDN ≤5 mg/day plus PGA <0.5 | 0 | Regardless | <0.5 | ≤5 mg/day | Regardless |

*Stable well-tolerated doses.
cSLEDAI, clinical systemic lupus erythematosus Disease Activity Index 2000; PDN, prednisone equivalent; PGA, physician global assessment.

**Table 2** Demographic and clinical characteristics of lupus patients in the whole cohort and in patients with cSLEDAI=0 for at least 1 year

| | | cSLEDAI=0 for at least 1 year | | | |
|---|---|---|---|---|---|
| | Whole cohort 646 (100) | Overall 548 (84.8) | PGA <0.5 concordant with cSLEDAI=0 353 (64.4) | PGA <0.5 discordant with cSLEDAI=0 195 (35.6) | P value |
| Female | 585 (90.6) | 502 (77.8) | 324 (64.5) | 178 (35.5) | ns |
| Age at baseline, years | 40.6±12.1 | 40.7±12.2 | 39.8±12.3 | 42.4±12.0 | 0.015 |
| SLE duration ≤2 years | 159 (24.6) | 139 (21.5) | 107 (77.0) | 52 (37.4) | ns |
| Disease duration, years | 9.2±6.7 | 8.9±6.8 | 9.1±6.8 | 8.7±6.9 | ns |
| Damage | | | | | |
| SDI baseline | 0.55±0.96 | 0.52±0.94 | 0.50±0.96 | 0.55±0.91 | ns |
| SDI increase during FU | 0.43±0.74 | 0.36±0.67 | 0.35±0.68 | 0.37±0.67 | ns |
| SDI increase during FU | 206 (31.9) | 149 (27.2) | 92 (26.1) | 57 (29.2) | ns |
| GC related SDI | 88 (13.6) | 69 (12.6) | 40 (11.3) | 29 (14.9) | ns |
| GC possibly related SDI | 43 (6.7) | 33 (6.0) | 21 (5.9) | 12 (6.2) | ns |
| GC independent SDI | 101 (15.6) | 64 (11.7) | 43 (12.2) | 21 (10.8) | ns |
| Lupus flare during FU | | | | | |
| Muco-cutaneous | 243 (37.6) | 189 (34.5) | 93 (26.3) | 44 (22.6) | ns |
| Musculoskeletal | 249 (38.5) | 213 (38.9) | 97 (27.5) | 70 (35.9) | 0.040 |
| Serositic | 45 (7.0) | 37 (6.8) | 22 (6.2) | 6 (3.1) | ns |
| Neurological | 36 (5.6) | 28 (5.1) | 12 (3.4) | 8 (4.1) | ns |
| Glomerulonephritis | 202 (31.3) | 149 (27.2) | 76 (21.5) | 36 (18.5) | ns |
| Haematological | 166 (25.7) | 138 (25.2) | 81 (22.9) | 39 (20.0) | ns |
| Therapy during follow-up | | | | | |
| Cumulative PDN, grams | 11.25±14.55 | 9.87±13.45 | 8.64±11.40 | 12.10±16.31 | 0.004 |
| Immunosuppressants | 316 (48.9) | 250 (45.6) | 150 (42.5) | 100 (51.3) | 0.048 |
| Antimalarials | 460 (71.2) | 394 (71.9) | 248 (70.3) | 146 (74.9) | ns |
| Belimumab | 48 (7.4) | 32 (4.9) | 19 (59.4) | 13 (40.6) | ns |
| Previous treatments | | | | | |
| Immunosuppressant ever | 381 (59.0) | 306 (55.8) | 199 (56.4) | 107 (54.9) | ns |
| Mycophenolate Mofetil | 197 (30.5) | 150 (27.4) | 103 (29.2) | 47 (24.1) | ns |
| Cyclophosphamide | 144 (22.3) | 111 (20.3) | 75 (21.2) | 36 (18.5) | ns |
| Azathioprine | 200 (31.0) | 158 (28.8) | 105 (29.7) | 53 (27.2) | ns |
| Cyclosporine A | 110 (17.0) | 83 (15.2) | 56 (15.9) | 27 (13.8) | ns |
| Methotrexate | 90 (13.9) | 67 (12.2) | 32 (9.1) | 35 (17.9) | 0.002 |
| Antimalarials | 483 (74.8) | 409 (74.6) | 267 (75.6) | 142 (72.8) | ns |
| Rituximab | 31 (4.8) | 18 (3.3) | 11 (3.1) | 7 (3.6) | ns |
| Belimumab | 41 (6.4) | 29 (5.3) | 15 (4.2) | 14 (7.2) | ns |
| IV Ig | 28 (4.3) | 19 (3.5) | 10 (2.8) | 9 (4.6) | ns |
| Plasmapheresis | 26 (4.0) | 20 (3.7) | 11 (3.1) | 9 (4.6) | ns |
| Previous lupus manifestations, ever | | | | | |
| Muco-cutaneous | 412 (63.8) | 342 (62.4) | 214 (60.6) | 128 (65.6) | ns |
| Musculoskeletal | 436 (67.5) | 371 (67.7) | 230 (65.2) | 141 (72.3) | ns |
| Serosal | 125 (19.4) | 98 (17.9) | 65 (18.4) | 33 (16.9) | ns |
| Neurological | 103 (15.9) | 82 (15.0) | 39 (11.0) | 43 (22.1) | 0.001 |
| Glomerulonephritis | 262 (40.6) | 206 (37.6) | 148 (41.9) | 58 (29.7) | 0.005 |
| Haematological | 245 (37.9) | 211 (38.5) | 139 (39.4) | 72 (36.9) | ns |
| Anti-dsDNA antibodies | 490 (75.9) | 412 (75.2) | 259 (73.4) | 153 (78.5) | ns |
| Anti-SSA/SSB antibodies | 275 (42.7) | 229 (41.9) | 145 (41.2) | 84 (43.3) | ns |
| Anti-U1RNP antibodies | 135 (20.9) | 107 (19.6) | 76 (21.6) | 31 (15.9) | ns |
| Anti-Sm antibodies | 93 (14.4) | 74 (13.5) | 45 (12.7) | 29 (14.9) | ns |
| Antiphospholipid antibodies | 258 (39.9) | 220 (40.1) | 140 (39.7) | 80 (41.0) | ns |
| Antiphospholipid syndrome | 94 (14.6) | 80 (14.6) | 51 (14.4) | 29 (14.9) | ns |

Data are reported as number (%) or mean±SD.
anti-dsDNA, anti double-stranded DNA; anti-U1RNP, anti-(U1) ribonucleoprotein; cSLEDAI, clinical SLE Disease Activity Index 2000; FU, follow-up; GC, glucocorticoids; IV Ig, intravenous immunoglobulines; ns, not significant; PDN, prednisone; PGA, physician global assessment; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; SLE, systemic lupus erythematosus.

**Table 3** Number (%) of patients in remission according to different definitions

| | PDN ≤5 mg/day | PGA <0.5 | cSLEDAI=0 | PGA <0.5 plus PDN ≤5 mg/day | cSLEDAI=0 plus PGA <0.5 | cSLEDAI=0 plus PDN ≤5 mg/day | cSLEDAI=0 plus PDN ≤5 mg/day plus PGA <0.5 |
|---|---|---|---|---|---|---|---|
| Unremitted patients | 73 (11.3) | 164 (25.4) | 98 (15.2) | 194 (30.0) | 170 (26.3) | 147 (22.8) | 199 (30.8) |
| At least 1 year remission | 573 (88.7) | 482 (74.6) | 548 (84.8) | 452 (70.0) | 476 (73.7) | 499 (77.2) | 447 (69.2) |
| At least 2 years remission | 512 (79.3) | 377 (58.4) | 456 (70.6) | 352 (54.5) | 369 (57.1) | 409 (63.2) | 346 (53.6) |
| At least 3 years remission | 425 (65.8) | 264 (40.9) | 327 (50.6) | 248 (38.4) | 254 (39.3) | 294 (45.5) | 243 (37.6) |
| At least 4 years remission | 345 (53.4) | 173 (26.8) | 250 (38.7) | 166 (25.7) | 167 (25.9) | 220 (34.1) | 161 (24.9) |
| 5 years remission | 222 (34.4) | 84 (13.0) | 119 (18.4) | 81 (12.5) | 82 (12.7) | 107 (16.6) | 80 (12.4) |

cSLEDAI, clinical SLE Disease Activity Index 2000; PDN, prednisone equivalent; PGA, physician global assessment; SLE, systemic lupus erythematosus.

### Prevalence of remission using different definitions

According to the seven different definitions, remission lasting at least 1 year was achieved by a proportion of patients ranging between 69.2% and 88.7% and two consecutive year remission by more than 50% of patients (table 3).

### Overlapping and agreement among different definitions

The overlapping of the three major items included in the DORIS definitions of remission (cSLEDAI=0, PGA <0.5, PDN ≤5 mg/day) is shown in figure 1.

The agreement was poor between PDN ≤5 mg/day and other definitions except for cSLEDAI=0 plus PDN ≤5 mg/day (κ=0.622) (online supplementary table S1).

The agreement between cSLEDAI=0 and PGA <0.5 was good (κ=0.697). Patients with PGA <0.5 usually had a concomitant cSLEDAI=0, but not vice versa.

When PDN ≤5 mg/day was added to PGA <0.5 or cSLEDAI=0 there was a good agreement with PGA <0.5 alone (κ=0.921) or cSLEDAI=0 alone (κ=0.837), respectively, since patients in remission according to cSLEDAI=0 or PGA <0.5 were often taking a dosage of PDN ≤5 mg/day.

When PGA <0.5 was added to cSLEDAI=0 and/or PDN ≤5 mg/day the prevalence of unremitted patients increased. As it was very uncommon that a patient fulfilled PGA <0.5 and PDN ≤5 mg/day, but not cSLEDAI=0, a very high agreement was observed between PGA <0.5 plus PDN ≤5 mg/day and cSLEDAI=0 plus PDN ≤5 mg/day plus PGA <0.5 (κ=0.981).

### Comparison between cSLEDAI=0 and PGA <0.5

The mean time spent in remission was significantly shorter when remission was defined according to PGA <0.5 compared with cSLEDAI=0 (2.1±1.7 vs 2.63±1.7 years, p<0.001).

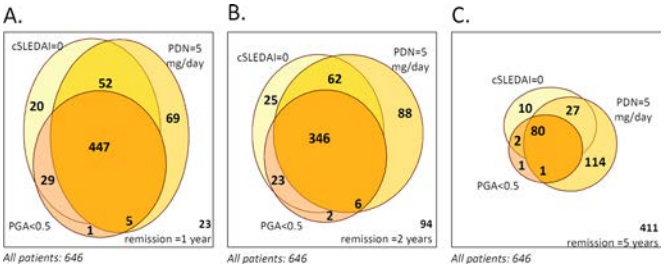


**Figure 1** Venn diagram representing the number of patients fulfilling the three single major remission items (PGA <0.5; cSLEDAI=0; PDN ≤5 mg/day) and their combination, during a 5-year follow-up. (A) Patients achieving at least 1-year remission. (B) Patients with at least two consecutive years in remission. (C) Patients achieving prolonged remission (five consecutive years). cSLEDAI, clinical SLE Disease Activity Index 2000; PGA, physician global assessment; PDN, prednisone-equivalent; SLE, systemic lupus erythematosus.

cSLEDAI=0 was observed in 548/646 patients (84.8%), while concomitant cSLEDAI=0 and PGA ≥0.5 were observed in 195/548 patients (35.6%) (table 2), meaning a loss of remission of 1.8 year/patient when PGA <0.5 was added to cSLEDAI=0.

To understand the reason why some patients had PGA ≥0.5 despite cSLEDAI=0, we compared patients who could not be defined as remitted with those showing no change in remission duration after adding PGA <0.5 to cSLEDAI=0 (table 2). Among patients with cSLEDAI=0, no difference in prevalence, extent or type of damage accrual was observed between those displaying PGA <0.5 or PGA ≥0.5. Patients with PGA ≥0.5 despite cSLEDAI=0 had more frequently musculoskeletal activity/involvement and were more commonly treated with immunosuppressants and/or higher dose of glucocorticoids during follow-up; moreover, they had less frequently previous glomerulonephritis and more commonly previous neurological involvement compared with patients with PGA <0.5 plus cSLEDAI=0 (table 2).

Among patients with musculoskeletal activity, no significant difference was observed in the proportion of patients with PGA ≥0.5 despite cSLEDAI=0 in patients who developed Jaccoud-like arthropathy compared with the overall cohort, confirming that damage was not scored as activity.

Among 195 patients with PGA ≥0.5 and cSLEDAI=0, 126 (64.6%) were on PDN ≤5 mg/day achieving cSLEDAI=0 plus PDN ≤5 mg/day remission; 155/195 (79.5%) were in lupus low disease activity state (LLDAS) according to Franklyn's definition (SLEDAI-2K≤4 and PGA ≤1 and PDN ≤7.5 mg/day).[17] Forty patients did not fulfil LLDAS definition due to a PGA >1 (n=21) and/or PDN intake >7.5 g/day (n=33).

### Patients with no residual disease activity (cSLEDAI=0 and/or PGA <0.5) on PDN >5 mg/day

When PDN ≤5 mg/day was added to cSLEDAI=0, 112 (17.3%) patients did not meet remission criteria. This may be due to a longer time needed for glucocorticoid tapering after resolution of the disease manifestations, leading to prolonged PDN intake (>5 mg/day) despite cSLEDAI=0. Accordingly, the difference in the proportion of patients in cSLEDAI=0 and cSLEDAI=0 plus PDN ≤5 mg/day decreased over time, as the longer the remission duration the higher the probability of minimising/withdrawing PDN therapy.

Overall, these patients lost 1.5 year/patient in remission. Among them, patients with renal or serosal involvement lost 2 years/patient in remission, those with haematological manifestations 1.5 year/patient in remission, and patients with articular, cutaneous, constitutional involvement 1.3 year/patient in remission, suggesting that time to reduce PDN ≤5 mg/day after achieving cSLEDAI=0 was different depending on the specific SLE manifestation.

**Table 4** ORs (95% CI) and goodness-of-fit of different definitions of remission in predicting damage accrual by Poisson regression*

| | PDN ≤5 mg/day | PGA <0.5 | cSLEDAI=0 | PGA <0.5 plus PDN ≤5 mg/day | cSLEDAI=0 plus PGA <0.5 | cSLEDAI=0 plus PDN ≤5 mg/day | cSLEDAI=0 plus PGA <0.5 plus PDN ≤5 mg/day |
|---|---|---|---|---|---|---|---|
| Five consecutive year remission | 0.620 (0.430 to 0.894) *0.010* | 0.377 (0.293 to 0.595) *<0.0001* | 0.382 (0.260 to 0.561) *<0.0001* | 0.363 (0.227 to 0.581) *<0.0001* | 0.397 (0.251 to 0.626) *<0.0001* | 0.411 (0.275 to 0.614) *0.0001* | 0.374 (0.234 to 0.599) *<0.0001* |
| Four consecutive year remission | 0.391 (0.245 to 0.625) *<0.0001* | 0.226 (0.130 to 0.394) *<0.0001* | 0.173 (0.105 to 0.286) *<0.0001* | 0.294 (0.177 to 0.487) *<0.0001* | 0.243 (0.140 to 0.424) *<0.0001* | 0.251 (0.155 to 0.405) *<0.0001* | 0.296 (0.176 to 0.496) *<0.0001* |
| Three consecutive year remission | 0.912 (0.601 to 1.386) *0.668* | 0.427 (0.281 to 0.649) *<0.0001* | 0.512 (0.342 to 0.767) *0.0001* | 0.430 (0.278 to 0.665) *<0.0001* | 0.459 (0.302 to 0.698) *<0.0001* | 0.548 (0.363 to 0.828) *0.0004* | 0.438 (0.284 to 0.676) *<0.0001* |
| Two consecutive year remission | 0.858 (0.566 to 1.300) *0.471* | 0.560 (0.396 to 0.793) *0.0001* | 0.454 (0.319 to 0.647) *<0.0001* | 0.495 (0.341 to 0.718) *<0.0001* | 0.514 (0.359 to 0.737) *<0.0001* | 0.497 (0.345 to 0.717) *<0.0001* | 0.479 (0.327 to 0.702) *<0.0001* |
| 1-year remission | 0.952 (0.611 to 1.484) *0.828* | 0.808 (0.590 to 1.107) *0.185* | 0.766 (0.548 to 1.071) *0.119* | 0.764 (0.554 to 1.053) *0.101* | 0.857 (0.629 to 1.166) *0.326* | 0.888 (0.642 to 1.227) *0.471* | 0.800 (0.583 to 1.098) *0.167* |
| AIC | 1132.83 | 1100.62 | 1082.90 | 1105.87 | 1103.96 | 1101.13 | 1106.59 |
| BIC | 1159.66 | 1127.44 | 1109.72 | 1132.70 | 1130.78 | 1127.96 | 1133.42 |
| P value | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* |

Significant variables are given in grey cells.
Number of patients in the analysis: 646.
*SDI increase during follow-up (range 0–4) was considered the dependent variable in Poisson regression. The best performance in predicting damage accrual was identified with the lowest AIC and BIC.
AIC, Akaike information criterion; BIC, Bayesian information criterion; cSLEDAI, clinical SLE Disease Activity Index 2000; PDN, prednisone; PGA, physician global assessment; SLE, systemic lupus erythematosus.

When PDN ≤5 mg/day was combined with PGA <0.5, 57 (8.8%) patients lost 1.4 year/patient in remission.

Among patients with PGA <0.5 or cSLEDAI=0, no significant difference in the prevalence, extent and type of damage accrual was observed between patients on PDN ≤5 mg/day and those on PDN >5 mg/day (data not shown).

### Damage accrual

Over the five consecutive year follow-up, 206 (31.9%) patients developed damage; 280 new damage events were observed, corresponding to 0.43 damage event per 5 years/person.

The prevalence and the extent of damage significantly decreased as the time spent in remission increased with all the definitions (table 4).

In Poisson regression, remission according to cSLEDAI=0 had the best fitness over all other remission models, showing the lowest AIC and BIC (table 4). When PGA was considered (alone or in combination with cSLEDAI=0 and/or PDN ≤5 mg/day) AIC and BIC increased, thereby indicating less fitness.

At the Poisson multivariate analysis, remission lasting at least two consecutive years was an independent negative predictor of damage accrual according to all the definitions of remission, except for PDN ≤5 mg/day that required at least four consecutive years (table 5); age, vasculitis, high glucocorticoid doses and antiphospholipid antibody syndrome were independent predictors of new damage. Due to the possible impact of Centre variability on PGA evaluation, we performed a Poisson regression analysis adjusted for data source, obtaining similar results (data not shown).

Since the different components of remission definitions might have diverse effects depending on whether continuous or cumulative time periods are considered, we also evaluated the cumulative proportion of follow-up spent in remission during the 5-year period. By regression analysis, we found that 50% of the follow-up was the shortest duration of remission which resulted protective against new damage when remission was defined as PGA <0.5 or cSLEDAI=0, whereas at least 75% of the follow-up was needed when remission was defined as PDN ≤5 mg/day (online supplementary table S2).

When we tested potential definitions of remission that resulted to be protective against damage accrual after at least two consecutive years, we found a similar positive predictive value against damage across all the definitions used (figure 2). In particular, adding PGA <0.5 and/or PDN ≤5 mg/day to cSLEDAI=0 did not improve its positive predictive value, meaning that the ability of cSLEDAI=0 in identifying patients without damage progression is not increased by the concomitant fulfilment of the other items. Addition of PDN ≤5 mg/day to cSLEDAI=0 increased the specificity of cSLEDAI=0 in predicting no damage accrual while maintaining a higher sensitivity compared with the definitions including PGA <0.5 (figure 2 and table 6).

## DISCUSSION

Our aim was to explore the performance of the three major items included in the DORIS definition of remission (cSLEDAI=0, PGA <0.5 and PDN ≤5 mg/day) in capturing a remission status and in predicting damage accrual.

First, we showed that adding PGA <0.5 to cSLEDAI=0 led to loss of remission in a relevant proportion of patients, without any significant improvement in its predictive value against damage. Additionally, it did not shorten the time spent in remission required to hinder damage accrual (at least two consecutive years for all remission definitions).

Interestingly, more than one-third of our patients in sustained cSLEDAI=0 spent over 1.7 year with PGA ≥0.5. These patients had more frequently previous neurological involvement, which may imply difficult attribution of neurological events to SLE activity,[18] thereby causing discrepancy between cSLEDAI and PGA. They also showed more frequently musculoskeletal involvement yet not classifiable as arthritis according to

**Table 5** Multivariate Poisson regression analysis: predictors of damage accrual over the follow-up (OR, 95% CI, p value)

| | PDN ≤5 mg/day | PGA <0.5 | cSLEDAI=0 | PGA <0.5 plus PDN ≤5 mg/day | cSLEDAI=0 plus PGA <0.5 | cSLEDAI=0 plus PDN ≤5 mg/day | cSLEDAI=0 plus PGA <0.5 plus PDN ≤5 mg/day |
|---|---|---|---|---|---|---|---|
| Five consecutive year remission | 0.498 (0.325 to 0.762) *0.0013* | 0.448 (0.278 to 0.721) *0.001* | 0.467 (0.311 to 0.702) *<0.0001* | 0.431 (0.264 to 0.705) *0.001* | 0.470 (0.292 to 0.757) *0.002* | 0.499 (0.325 to 0.767) *0.002* | 0.442 (0.270 to 0.722) *0.001* |
| Four consecutive year remission | 0.534 (0.325 to 0.877) *0.013* | 0.322 (0.183 to 0.567) *<0.0001* | 0.230 (0.138 to 0.385) *<0.0001* | 0.400 (0.238 to 0.670) *0.001* | 0.338 (0.192 to 0.596) *<0.0001* | 0.305 (0.186 to 0.500) *<0.0001* | 0.389 (0.229 to 0.661) *<0.0001* |
| Three consecutive year remission | 0.885 (0.567 to 1.382) *0.59* | 0.500 (0.328 to 0.764) *0.001* | 0.595 (0.393 to 0.900) *0.014* | 0.485 (0.313 to 0.754) *0.001* | 0.527 (0.345 to 0.805) *0.003* | 0.590 (0.387 to 0.899) *0.0014* | 0.490 (0.315 to 0.760) *0.0019* |
| Two consecutive year remission | 0.878 (0.562 to 1.370) *0.56* | 0.631 (0.444 to 0.896) *0.010* | 0.531 (0.371 to 0.759) *0.001* | 0.554 (0.381 to 0.805) *0.002* | 0.574 (0.400 to 0.826) *0.003* | 0.543 (0.376 to 0.785) *0.001* | 0.532 (0.363 to 0.781) *0.001* |
| 1-year remission | 1.022 (0.642 to 1.627) *0.92* | 0.868 (0.632 to 1.194) *0.386* | 0.915 (0.652 to 1.284) *0.606* | 0.845 (0.610 to 1.171) *0.313* | 0.913 (0.668 to 1.248) *0.566* | 1.072 (0.770 to 1.494) *0.679* | 0.877 (0.636 to 1.208) *0.422* |
| Age at baseline | 1.031 (1.019 to 1.042) *<0.001* | 1.029 (1.017 to 1.040) *<0.001* | 1.032 (1.021 to 1.044) *<0.001* | 1.029 (1.017 to 1.041) *<0.001* | 1.029 (1.017 to 1.041) *<0.0001* | 1.033 (1.033 to 1.045) *<0.001* | 1.029 (1.018 to 1.041) *<0.001* |
| Previous GC pulses | *ns* | 1.441 (1.100 to 1.888) *0.008* | 1.369 (1.044 to 1.795) *0.023* | 1.452 (1.109 to 1.901) *0.007* | 1.447 (1.105 to 1.895) *0.007* | 1.457 (1.112 to 1.908) *<0.006* | 1.463 (1.117 to 1.915) *0.006* |
| Previous vasculitis | *ns* | 1.660 (1.175 to 2.346) *0.004* | 1.689 (1.195 to 2.387) *0.003* | 1.656 (1.171 to 2.341) *0.004* | 1.677 (1.187 to 2.370) *0.003* | 1.666 (1.181 to 2.350) *<0.004* | 1.666 (1.179 to 2.355) *0.004* |
| Cumulative GC dose at baseline | 1.008 (1.001 to 1.016) *0.025* | *ns* | 1.008 (1.000 to 1.015) *0.036* | 1.007 (1.000 to 1.014) *0.043* | *ns* | 1.008 (1.001 to 1.015) *0.023* | 1.007 (1.000 to 1.042) *0.046* |
| aPL syndrome | 1.377 (1.008 to 1.882) *0.044* | *ns* | 1.383 (1.016 to 1.882) *0.039* | *ns* | *ns* | *ns* | *ns* |
| AIC | 1033.68 | 1031.74 | 1014.99 | 1032.56 | 1032.52 | 1020.78 | 1031.48 |
| BIC | 1136.52 | 1107.74 | 1090.01 | 1108.56 | 1108.52 | 1096.79 | 1107.49 |
| P value | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* |

Variables with a p<0.2 at the univariate analysis were entered in the multivariate analysis: duration of remission (categorical variable with six levels) and the following characteristics: sex, age, disease duration, baseline SDI, cumulative GC dose at baseline, previous GC pulses, previous cyclophosphamide treatment, previous neurological and renal involvement, previous vasculitis, antiphospholipid syndrome. cSLEDAI, clinical SLE Disease Activity Index 2000. Significant variables are given in grey cells. The table reports only variables with at least one p<0.05 at the multivariate analysis for at least one remission definition.
Goodness-of-fit of different models according to the seven definitions of remission are also reported.
AIC, Akaike Information Criterion; aPL, antiphospholipid antibody; BIC, Bayesian Information Criterion; GC, glucocorticoid; ns, not significant; PDN, prednisone-equivalent; PGA, physician global assessment; SDI, Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index.

SLEDAI, leading to higher PDN intake. It should be noted that non specific symptoms (i.e., arthralgias, stiffness, fatigue) are not considered in cSLEDAI, but can increase the PGA without impacting on damage accrual. PGA has a large interobserver and intraobserver variability because it reflects a complex physician-perceived global evaluation not always related to the real disease activity.[19 20] Therefore, patients in cSLEDAI=0 but PGA ≥0.5 should be carefully evaluated in order to distinguish lupus residual activity from other conditions.

Since additional treatment is not always needed in patients with cSLEDAI=0 despite PGA ≥0.5, and since these items showed overlapping sensitivity, specificity, positive and negative predictive value against damage accrual (table 6 and figure 2), adding PGA <0.5 to cSLEDAI=0 could lead to overtreatment in controlled trials as well as in clinical practice when a treat-to-target (T2T) approach is adopted.[21] In fact, T2T should aim at the most reachable target that positively influences patient prognosis. We observed that cSLEDAI=0 was the definition of remission easiest to achieve in real life, being able to predict damage accrual with a consistent degree of accuracy. Noteworthy, cSLEDAI=0 alone or in combination with other items conferred a similar protection against damage accrual, provided remission lasted at least 2 years (table 5), therefore, posing a rationale for the use of cSLEDAI=0 in spite of composite indexes. Importantly, cSLEDAI=0 was already shown to be a feasible target in the BLISS trials, bearing the highest discriminatory capability in capturing remission compared with the DORIS definition, lupus low disease activity state (LLDAS) and D/E in all British Isles Lupus Activity Group (BILAG) domains.[22] Additionally, cSLEDAI=0 had the same performance of the SLE Responder Index-4 (SRI-4) in showing the superiority of belimumab over placebo, while displaying a better correlation with PGA<0.5 than SRI-4 or LLDAS.[22 23] Hence, it could be speculated that adding PGA <0.5 could hamper the results of randomised trials aiming at remission, as patients achieving cSLEDAI=0 at 52 weeks can still have PGA ≥0.5 which will not affect their outcome.

Since definitions of low disease activity (LDA) use the same items included in definitions of remission (cSLEDAI/SLEDAI-2K, PGA, PDN) although with less stringent cut-offs, a patient fulfilling remission definition is often fulfilling LDA status as well.[17 24 25] Notably, in this study, we found that around two thirds of patients with cSLEDAI=0 and PGA ≥0.5 were in LDA and/or in remission according to cSLEDAI=0 and PDN ≤5 mg/day. Since a patient can lose remission definition only due to PGA ≥0.5 while maintaining LDA, adding PGA to definitions including cSLEDAI=0 and/or PDN ≤5 mg/day leads to a consistent overlap between the definitions of LDA and remission.[11 19 24]

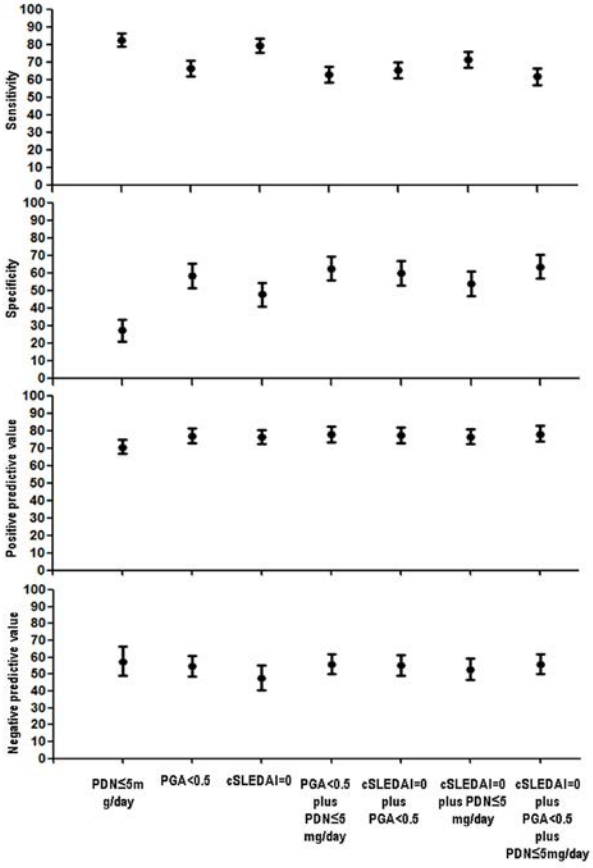


**Figure 2** Sensitivity, specificity, positive and negative predictive values of different remission definitions in identifying patients who will not accrue damage throughout the follow-up. Values and their 95% CI are depicted. Remitted is defined as patients with at least two consecutive year remission and unremitted is defined as patients with remission lasting less than 2 years. cSLEDAI, clinical SLE Disease Activity Index; PGA, physician global assessment; PDN, prednisone equivalent; SLE, systemic lupus erythematosus.

On the other hand, 40 out of the 195 patients in cSLEDAI=0 with PGA ≥0.5 did not fulfil LLDAS definition, due to glucocorticoid daily doses and/or PGA over the LLDAS thresholds. This might infer that a significant proportion of patients could have fulfilled simplified remission criteria (cSLEDAI=0), that is, without lupus manifestations, and still not be in a desirable state of remission, due to a suboptimal HRQoL or to a higher glucocorticoid intake. Nevertheless, patients in cSLEDAI=0 irrespective of PDN dosage and PGA accrued significantly less damage than those with cSLEDAI >0 during the follow-up.

A remission lasting at least 50% of the follow-up was protective against damage accrual in our cohort when remission was defined according to PGA <0.5 or c-SLEDAI=0. Notably, this duration corresponds to 2.5 cumulative years in our study where the follow-up was 5 years, in keeping with the results of our analysis of continuous-time periods.

We confirmed a good agreement between cSLEDAI=0 and PGA <0.5 in our real-life cohort. Therefore, a simplified remission definition based on cSLEDAI=0 can be reasonably used in observational studies where complete serological data, PGA and BILAG are not routinely assessed.[21] In the cSLEDAI=0 remission definition, the exclusion of a cut-off for PDN could imply that high dose glucocorticoids may mask disease activity, thus allowing patients not in real remission to be defined as remitted. Nevertheless, we found that adding PDN ≤5 mg/day to cSLEDAI=0 does not increase the performance against damage of cSLEDAI=0 in the short/medium term (5 years), as shown by the high overlap between confidence intervals. Of note, the specificity, sensitivity and predictive values of cSLEDAI=0 were not significantly improved on addition of other items, with a substantially modest prognostic value of all different definitions of remission in predicting damage-free status (table 6). These observations further support cSLEDAI=0 to be considered the first target to achieve in order to prevent damage accrual, while cSLEDAI=0 plus PDN ≤5 mg/day could be considered the best target in the medium/long term, since it has been widely demonstrated that even low dose of PDN lead to damage accrual in the longer run.[4 16 26 27]

We observed that the majority of patients in remission according to cSLEDAI=0 for at least 1 year were on PDN ≤5 mg/day, some being off-steroids (data not shown), while almost half of them were still on immunosuppressive maintenance therapy. This finding reflects the current clinical practice of tapering glucocorticoids before immunosuppressive agents which exert a greater protective effect against flare occurrence in remitted patients.[28] Accordingly, in a recent study by Mathian *et al*,[29] a relevant proportion of remitted patients who discontinued glucocorticoid therapy experienced a flare during 1-year follow-up, but only 26% of them were on immunosuppressive maintenance therapy at the time of glucocorticoid discontinuation.

There are some limitations in our study that it is a retrospective analysis of prospective collected data, with the majority of patients being Caucasian and follow-up limited to 5 years. Moreover, other relevant outcomes, for example, HRQoL, were not systematically assessed. On the other hand, this is a multicentre real-life

**Table 6** Sensitivity, specificity, positive and negative predictive values of different remission definitions in identifying patients without damage accrual throughout the follow-up

| | PDN ≤5 mg/day | PGA <0.5 | cSLEDAI=0 | PGA <0.5 plus PDN ≤5 mg/day | cSLEDAI=0 plus PGA <0.5 | cSLEDAI=0 plus PDN ≤5 mg/day | cSLEDAI=0 plus PGA <0.5 plus PDN ≤5 mg/day |
|---|---|---|---|---|---|---|---|
| Sensitivity | 82.4 (78.5 to 85.9) | 66.2 (61.6 to 70.6) | 79.2 (75.1 to 83.0) | 62.6 (57.8 to 67.1) | 65.1 (60.4 to 69.5) | 71.2 (66.8 to 75.4) | 61.6 (56.9 to 66.2) |
| Specificity | 27.4 (21.5 to 34.0) | 58.2 (51.2 to 65.0) | 47.6 (40.7 to 54.6) | 62.5 (55.5 to 69.1) | 59.6 (52.6 to 66.3) | 53.9 (46.8 to 60.8) | 63.5 (56.5 to 70.0) |
| PPV | 70.5 (66.4 to 74.4) | 76.9 (72.3 to 81.1) | 76.1 (71.9 to 80.0) | 77.8 (73.1 to 82.1) | 77.2 (72.6 to 81.4) | 76.5 (72.1 to 80.5) | 78.0 (73.3 to 82.3) |
| NPV | 57.5 (48.6 to 66.0) | 55.0 (48.9 to 61.1) | 47.9 (40.6 to 55.3) | 55.8 (49.9 to 61.6) | 55.2 (49.2 to 61.2) | 52.9 (46.4 to 59.4) | 56.0 (50.2 to 61.7) |

Values and their 95% CI are reported.
cSLEDAI, clinical SLE Disease Activity Index; NPV, negative predictive value; PDN, prednisone-equivalent; PGA, physician global assessment; PPV, positive predictive value; SLE, systemic lupus erythematosus.

observational cohort where patients were closely followed-up and homogeneously treated according to current recommendations.

In conclusion, cSLEDAI=0 was the most achievable definition of remission and showed a good performance in terms of damage prediction, while addition of PGA <0.5 to cSLEDAI=0 was not relevant in identifying patients who would develop damage, thus submitting cSLEDAI=0 as the most advisable target in a short-to-mid-term follow-up.

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**Patient consent for publication** Not required.

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CLINICAL SCIENCE

# Targeting JAK/STAT pathway in Takayasu's arteritis

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## ABSTRACT

**Objective** Takayasu's arteritis (TAK) is a large vessel vasculitis with important infiltration of proinflammatory T cells in the aorta and its main branches, but its aetiology is still unknown. Our work aims to explore the involvement of Janus Kinase/Signal Transducers and Activators of Transcription (JAK/STAT) signalling pathway in proinflammatory T cells differentiation and disease activity of TAK.

**Methods** We analysed transcriptome and interferons gene signatures of fluorescence-activated cell sorting (FACS-sorted) CD4+ and CD8+ T cells from healthy donors (HD) and in 25 TAK (median age of 37.6 years including 21 active TAK with National Institutes of Health (NIH) score >1). Then we tested, in vitro and in vivo, the effects of JAK inhibitors (JAKinibs) in TAK.

**Results** Transcriptome analysis showed 248 and 432 significantly dysregulated genes for CD4+ and CD8+ samples between HD and TAK, respectively. Among dysregulated genes, we highlighted a great enrichment for pathways linked to type I and type II interferons, JAK/STAT and cytokines/chemokines-related signalling in TAK. We confirmed by Real Time Reverse Transcription Polymerase Chain Reaction (RT-qPCR) the upregulation of type I interferons gene signature in TAK as compared with HD. JAKinibs induced both in vitro and in vivo a significant reduction of CD25 expression by CD4+ and CD8+ T cells, a significant decrease of type 1 helper T cells (Th1) and Th17 cells and an increase of Tregs cells in TAK. JAKinibs also decreased C reactive protein level, NIH score and corticosteroid dose in TAK patients.

**Conclusions** JAK/STAT signalling pathway is critical in the pathogenesis of TAK and JAKinibs may be a promising therapy.

## Key messages

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

- Takayasu's arteritis (TAK) inflammation is driven by Th1 and Th17 cells in peripheral blood and inflamed tissues. Molecular mechanisms underlying the differentiation of proinflammatory T cells in TAK are unknown.

**What does this study add?**

- JAK/STAT and downstream signalling pathways like interferons, cytokines and chemokines-related pathways are critical in the immunopathology of TAK.
- JAK inhibitors (JAKinibs) are effective both in vitro and in vivo to reduce T cells activation, to restore T cells homeostasis and to decrease systemic inflammation in patients.

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

- JAKinibs may represent a new promising avenue for the treatment of TAK.

## INTRODUCTION

Takayasu's arteritis (TAK) is a large vessel vasculitis (LVV) preferentially affecting the aorta and its main branches.[1] Vessel inflammation induces wall thickening, fibrosis and stenosis that can lead to complete occlusion of the artery. Although TAK has a worldwide distribution, the disease is known to be more common in young women mostly in the second or the third decade of life. Patients with TAK have a high morbidity rate as 50% will relapse and experience a vascular complication within 10 years from diagnosis.[2 3]

There are several lines of evidence showing that TAK inflammation is mediated by T cells and macrophages, predominantly in the adventitia and media layers.[4 5] CD4+ T cells activation is driven by Th1 and Th17 cells in peripheral blood and inflamed tissues.[5–9] Neovessels and adventitial vasa vasorum are the main sites where circulating leucocytes are recruited into the vascular bed.[8] Molecular mechanisms underlying the differentiation of proinflammatory T cells in TAK are essentially unknown.

Currently, TAK is mainly treated with non-specific corticosteroids,[1] which are associated with potential side effects, especially when used for a long-time course. To develop more efficient treatments against this persistent inflammation, physicians need a deeper understanding of the disease and its mechanisms.

One of these could be the JAK/STAT signalling cascade, which is a central biological pathway aiming at transferring ligands/receptors signals from the extracellular medium to the nucleus, ultimately leading to the transcription of numerous downstream genes. This pathway is involved in many critical downstream immune functions, such as cytokines, chemokines and interferons signalling. Inhibition of JAK kinases using JAK inhibitors (JAKinibs) represents an efficient way to dampen these downstream pathways. Different first-generation JAKinibs exist and differ by their specificities: either JAK1/JAK2 for Ruxolitinib/Baricitinib or JAK1/JAK3 for Tofacitinib. They suppress effector T cells (ie, Th1 and Th17) and reduce the secretion of proinflammatory cytokines.[5 10 11]

**Table 1** Characteristics of patients with TAK

| Parameters | TAK (n=25) |
|---|---|
| **Demographic features** | |
| Median age (IQR), years | 37.6 (27.1–50) |
| Origin | |
| Europe | 14 (56%) |
| North Africa | 7 (28%) |
| South Africa | 1 (4%) |
| West Indies | 1 (4%) |
| Middle East | 1 (4%) |
| Female gender | 18 (72%) |
| **Clinical features** | |
| Median time from symptoms to diagnosis (IQR), months | 12 (3;24) |
| Newly diagnosed | 13 (52%) |
| Hypertension | 14 (56%) |
| Fever | 4 (16%) |
| Asthenia | 12 (48%) |
| Carotydodynia | 11 (44%) |
| Stenosis/Occlusion* | 21 (84%) |
| Aneurysm | 9 (36%) |
| Retinopathy | 3 (12%) |
| Stroke | 2 (8%) |
| Mainly affected vessels | |
| Aorta | 17 (68%) |
| Iliofemoral arteries | 6 (24%) |
| Supra-aortic arteries | 18 (72%) |
| Elevated CRP | 13 (52%) |
| Level of CRP (IQR) (mg/L) | 23 (10–52) |
| NIH score >1† | 21 (84%) |
| Numano classification | |
| I | 1 (4%) |
| II | 1 (4%) |
| III | 3 (12%) |
| IV | 3 (12%) |
| V | 17 (68%) |
| **Treatments** | |
| Low dose corticosteroids (5 mg/day) | 12 (48%) |
| Methotrexate | 4 (16%) |
| Azathioprine | 1 (4%) |

*All TAK had stenosis and arterial wall thickening. However, 21 out of 25 had severe arterial stenosis or occlusion.
†Considered as active TAK patients.
CRP, C reactive protein; TAK, Takayasu's arteritis.

Here, we aim to explore the involvement of JAK/STAT signalling pathway in proinflammatory T cells differentiation and disease activity of TAK.

## METHODS

### Study population

The study population consisted of 25 TAK (13 newly and 12 formerly diagnosed) with a median age of 37.6 years (IQR (27.1–50)) fulfilling the American College of Rheumatology criteria.[12] Demographic, clinical and treatments characteristics of TAK are indicated in table 1. Overall, 21 out of 25 had an NIH score >1 and were considered as active TAK. Blood samples from 37 sex-matched and age-matched healthy donors (HD) were obtained from the Établissement Français du Sang (Hôpital de la Pitié-Salpêtrière, Paris) and used as controls. Three active TAKs were treated with JAKinibs. Their medical history, treatments and outcome are presented in online supplementary table S1.

### Patient and public involvement

Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

### Transcriptomic data of CD4+ and CD8+ T cells

CD3+ T cells were isolated from PBMCs of active TAK or HD by negative isolation using Dynabeads Untouched Human T Cells Kit (ThermoFisher). CD4+ cells were then isolated from previously sorted T cells by positive selection using Dynabeads CD4+ isolation kit. CD8+ cells were isolated from the previous CD4- fraction by positive selection using Dynabeads CD8 +isolation kit. Cell purity was ≥95%. Total RNA from CD4+ and CD8+ T cells was extracted using the NucleoSpin RNA kit (Macherey-Nagel). Total RNA was quantified by an NanoDrop ND-1000 spectrophotometer. Samples with RNA concentration <20 ng/µL were excluded (5 HD CD4+, 6 HD CD8+, 2 TAK CD4+ and 4 TAK CD8+). For quality control, RNA dilution was performed using Agilent RNA 6000 Nano Kit and 1 µL of the sample was run on the Nano chip using an Agilent 2100 electrophoresis bioanalyzer. The quality of total RNA was assessed using the electropherogram's profile and the calculated RNA integrity number (RIN). All samples showed RINs between 7.3 and 9.3. For Illumina Beadarrays, cRNA samples were prepared using Illumina TotalPre-96 RNA Amp kit (LifeTechnologies) and hybridised to Human HT-12 v4 Beadarrays. Then, raw IDAT files were processed using illuminaio R package and concatenated into a single text file. Data were further background-corrected using limma R package and interchip batch effects were removed using ComBat method from sva R package. The following samples numbers remained and were analysed: 37 CD4+ T cells and 34 CD8+ T cells from HD, and 25 CD4+ T cells and 14 CD8+ T cells from active TAK. Details on pathways enrichment analysis and on network graphs are available in the online supplementary material and methods.

### Type I interferons gene signature

We compiled from several publications a gene signature to evaluate the type I interferons-specific activity in our transcriptomes.[13–17] This signature, called interferons signature genes (ISG), is defined by the eight following type I-specific interferons response genes: LY6E, HERC5, IFI44L, ISG15, MX1, MX2, EPSTI1 and RSAD2. To quantify such signature activity, we used two different methods. First, we analysed this signature at the transcriptome level using a specialised R package called gene set variation analysis (GSVA)[18] allowing to compute an enrichment score for each patient involved in the dataset. Then, differential analysis of the presented groups was performed using the limma R package to assess for significance. We also performed RT-qPCR on the ISG on FACS-sorted CD4+ and CD8+ T cells coming from HD or TAK PBMCs. For each patient independently, these two T cells populations were then pooled in a single Eppendorf tube before being frozen into Trizol. To perform actual RT-qPCR, we used SuperScript VILO cDNA Synthesis Kit (Invitrogen). After Ct acquisition, individual gene data were normalised to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene expression. Then, each gene expression (for every sample and group) was individually normalised to the mean of HD group.

### T cells culture and flow cytometry analysis

For in vitro experiments, PBMCs for either HD or TAK were cultured in 48 wells plates at 1 million cells/mL using R10 culture

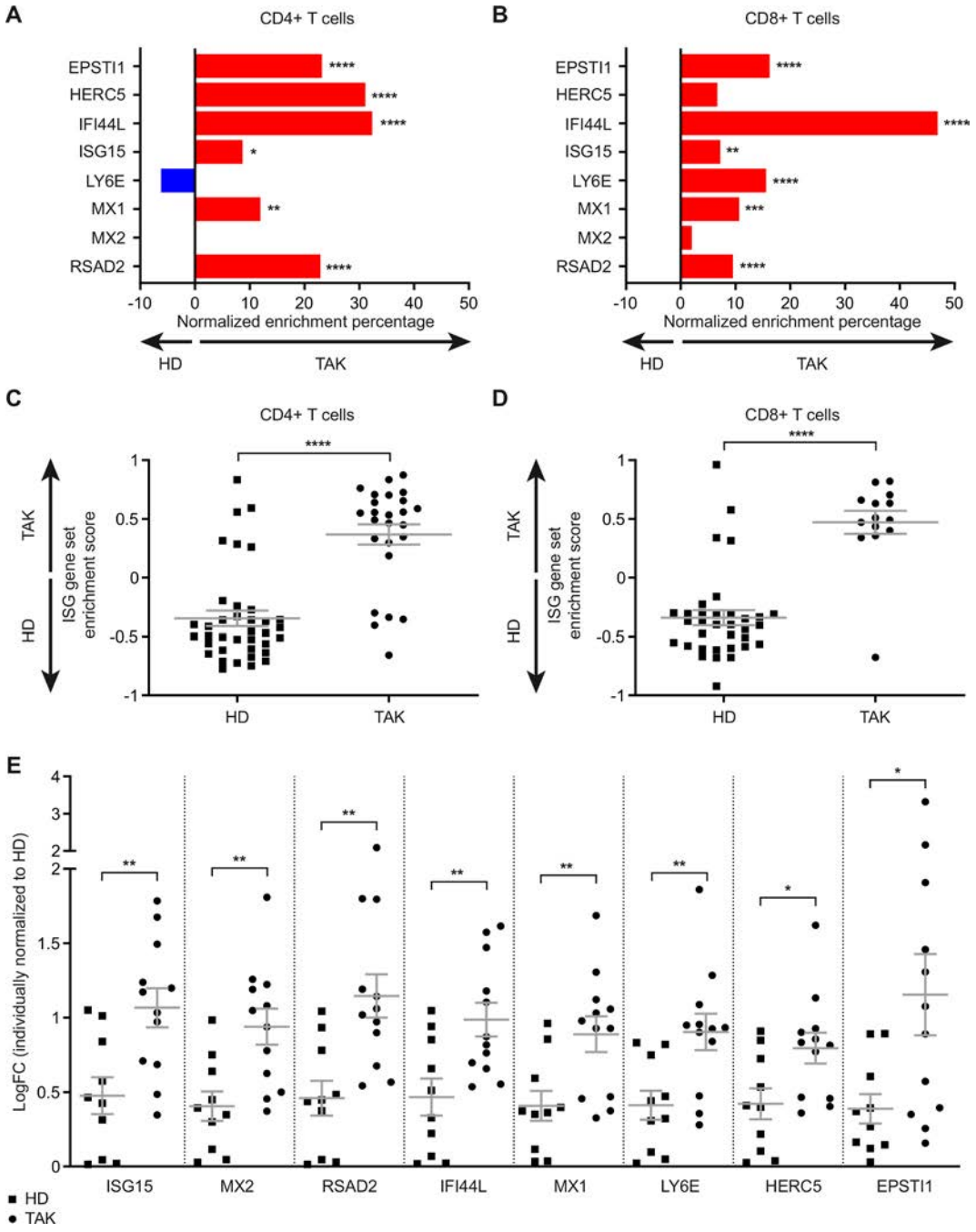


**Figure 1** Upregulation of type I IFN signaling-related gene signature in TAK. (A, B) A type I IFN signaling-specific gene signature was analysed in TAK and HD transcriptomic samples of FACS-sorted CD4+ T cells (A) or CD8+ T cells (B). Normalised fold changes are relative to the absolute maximal fold change available within the full differential transcriptome analysis between HD and TAK. P values were corrected using the Benjamini-Hochberg procedure. (C, D) Then, the signature was analysed as a set of genes using GSVA R package and subsequent enrichment scores were computed for each HD and TAK sample of FACS-sorted CD4 +T cells (C) or CD8+ T cells (D). Then, standard t-tests were performed to compare the enrichment scores between HD and TAK. (E) The same gene signature was tested by RT-qPCR in FACS-sorted T cells from HD and TAK. Normalised LogFC were computed from the raw Ct values using standard ΔΔCt transformation method. Differences between HD and TAK were assessed using standard t-tests. *p-value <0.05, **p-value <0.01, ***p-value <0.001, ****p-value <0.0001. HD, healthy donors; IFN, interferon; ISG, interferons signature genes; TAK, Takayasu's arteritis.

medium (Roswell Park Memorial Institute (RPMI) supplemented with BSA, L-glutamine and Penicillin/Streptomycin, all from Gibco) for 5 days, concomitantly with either phosphate-buffered saline (PBS), Ruxolitinib (1 µM) or Tofacitinib (1 µM). Samples were antibody-stained following standard protocol with anti-CD3-APC-AlexaFluor750 (UCHT1, Beckman Coulter), anti-CD4-PerCP (VIT4, Miltenyi Biotec), anti-CD8-AlexaFluor700 (B9.11, Beckman Coulter), anti-CD25-phycoerythrin (PE) (4E3, Miltenyi Biotec), anti-IFNγ-fluorescein isothiocyanate (FITC) (45–15, Miltenyi Biotec), anti-interleukin-17A (IL-17A)-allophycocyanin (APC) (eBio17B7, ThermoFisher), anti-FoxP3-APC (PCH101, eBioscience) and anti-Ki-67-BV650 (B56, BD Biosciences). 'Fluorescence Minus One' controls were used to ensure reliable gating. For pSTAT5A analysis, cells were

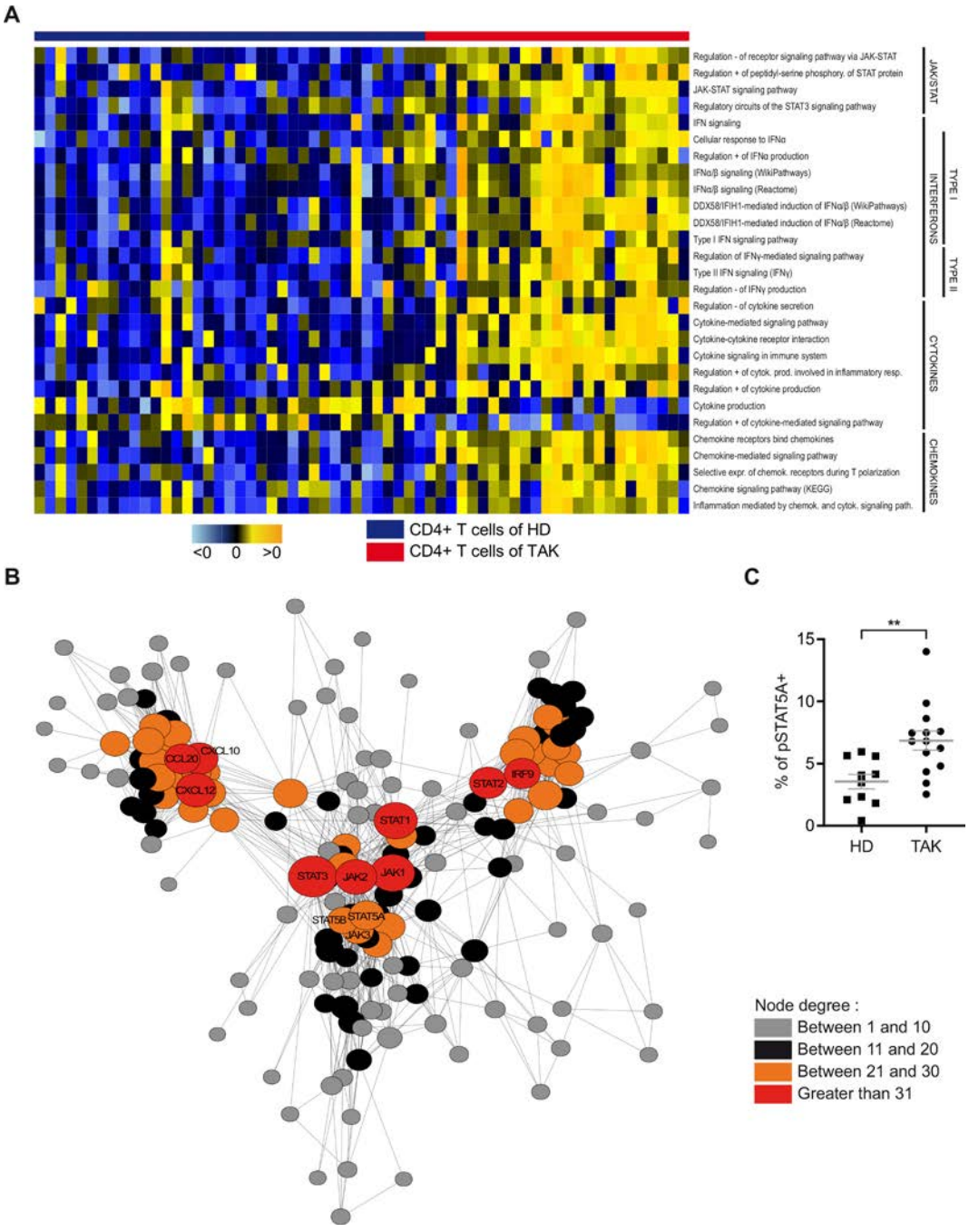


**Figure 2** JAK/STAT signalling pathway efficiently separates CD4+ T cells from HD and TAK. (A) Heatmap showing selected SEPs (with Benjamini-Hochberg-adjusted p-value <0.05) between HD and TAK CD4+ T cells. (B) Using the genes contained in the pathways presented in (A) and the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database, a genes network was constructed. Nodes were coloured according to their degree (number of connections) and eventually annotated with their gene name. (C) Measurement by flow cytometry of pSTAT5A percentage of expression by CD4+ T cells in HD and TAK. Standard t-test was used. **p-value <0.01. HD, healthy donors; SEPs, significantly enriched pathways; TAK, Takayasu's arteritis.

fixed using paraformaldehyde (PFA) (2% PFA final) for 30 min in the dark at room temperature. Next, cells were centrifuged and resuspended in 4°C absolute methanol for 30 min. Finally, cells were washed in PBS/FCS 10% and standard staining was performed during 45 min at room temperature in the dark with anti-CD3-APC-AlexaFluor750 (UCHT1, Beckman Coulter), anti-CD4-ECD (SFCI12T4D11, Beckman Coulter), anti-CD8A-AlexaFluor700 (B9.11, Beckman Coulter) and anti-pSTAT5-FITC (SRBCZX, ThermoFisher).

## RESULTS

### Interferon gene signature in T cells of TAK

First, we performed differential transcriptome analysis between HD and TAK (with corticosteroids ≤10 mg/day and no immunosuppressants) for both CD4+ and CD8+ T cells. We obtained 248 significantly dysregulated genes (adjusted p≤0.05) for CD4+ samples (148 and 100 enriched in HD and TAK, respectively) and 432 for CD8+ samples (254 and 178 enriched in HD and TAK, respectively). Among dysregulated genes, we

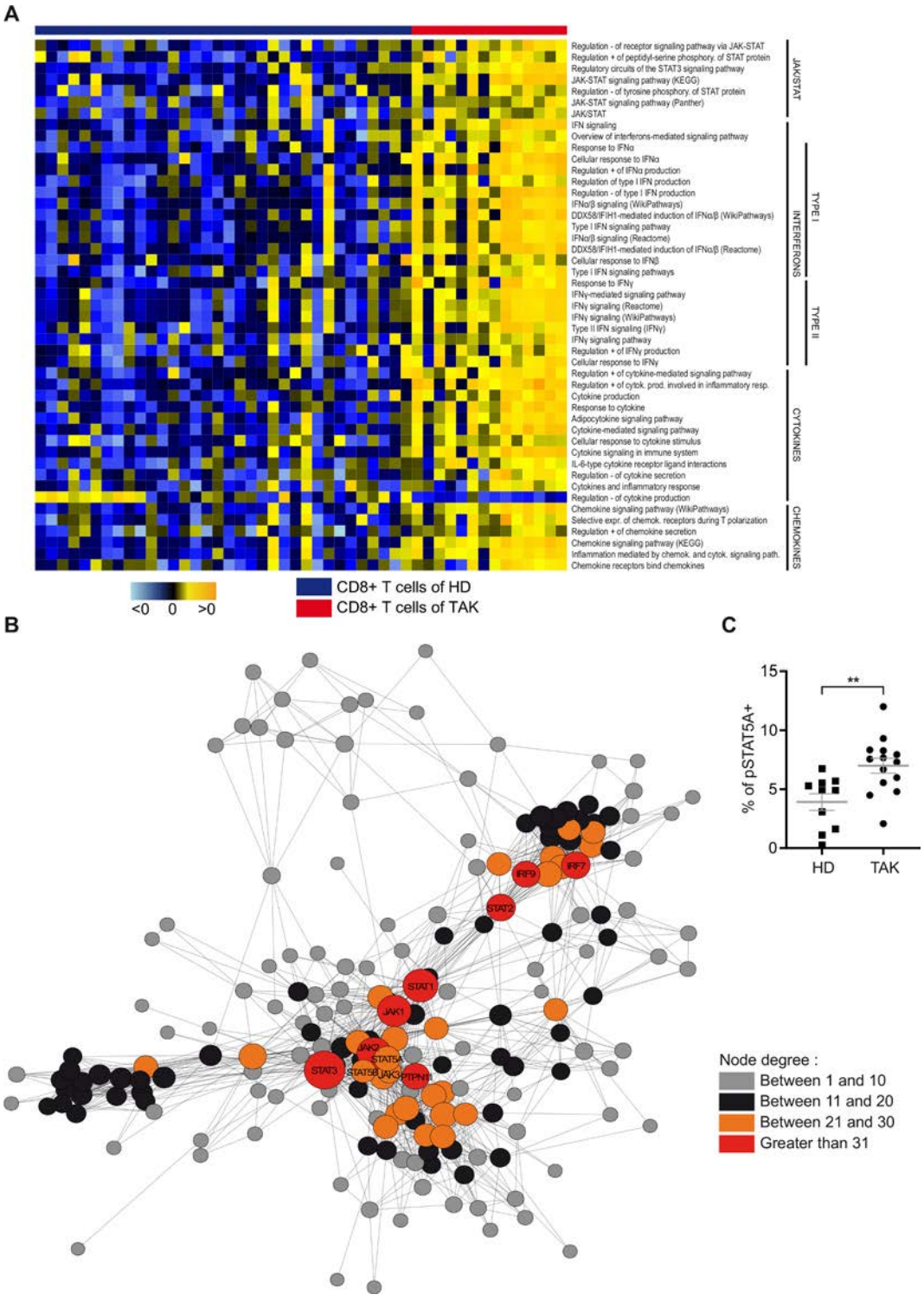


**Figure 3** JAK/STAT signalling pathway efficiently separates CD8+ T cells from HD and TAK. (A) Heatmap showing selected SEPs (with Benjamini-Hochberg-adjusted p-value <0.05) between HD and TAK CD8+ T cells. (B) Using the genes contained in the pathways presented in (A) and the STRING database, a genes network was constructed. Nodes were coloured according to their degree (number of connections) and eventually annotated with their gene name. (C) Measurement by flow cytometry of pSTAT5A percentage of expression by CD8+ T cells in HD and TAK. Standard t-test was used. **p-value <0.01. HD, healthy donors; SEPs, significantly enriched pathways; TAK, Takayasu's arteritis.

highlighted a great enrichment for genes related to T cells activation/differentiation pathways (CCR2, CCR4, CCR6, CXCR5, RORC, and IL2RA), cytokines/chemokines (IL8, IL15, IL17F, IL19, IL22, IL24, IL33, CCL3L1, CCL3L3, CCL14, CCL19, and CXCL15), type I interferons (IFIT1, IFIT3, ISG20, interferon alpha-5 (IFNA5), IFIH1, ADAR, IFNA1, IFNA7, and IFI27) and type II interferons (IFNGR1, CXCL10, GBP5 and ICAM1) in both CD4+ and CD8+ T cells of TAK.

To further investigate interferons-related genes in TAK, we evaluated the type I interferons activity using a specific gene signature called ISG. We plotted the individual genes' normalised enrichment percentages between HD and TAK for

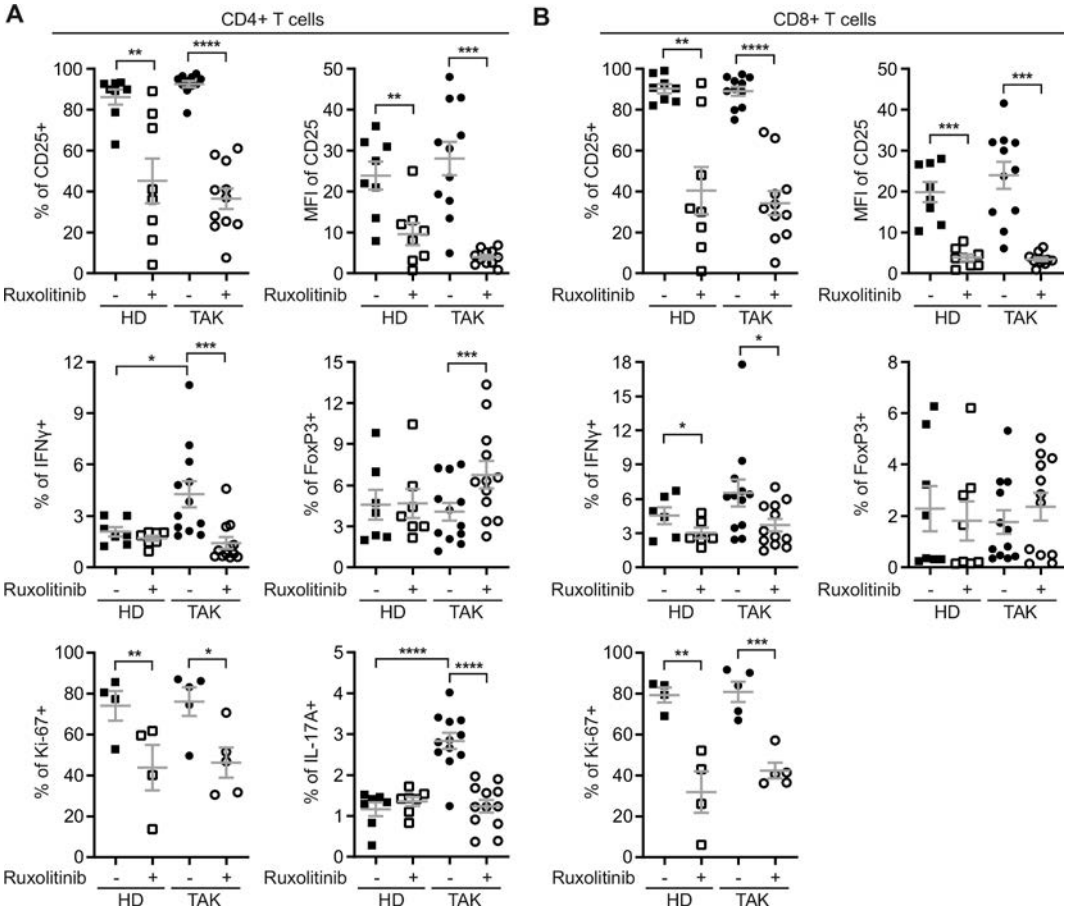


**Figure 4** Ruxolitinib reduces global T cells activation, Th1/Th17 polarisation and promote increase of Tregs in TAK. PBMCs from HD and TAK were cultured for 5 days in complete RPMI medium with or without Ruxolitinib. Cells were washed and surface (CD25) and intracellular (FoxP3, IFNγ, IL-17A and Ki-67) markers were antibody stained and analysed by flow cytometry in CD4+ (A) and CD8+ (B) T cells. Percentage of CD25 denotes CD25 +cells, including CD25low and CD25high cells. For comparisons of untreated versus treated groups (within the same medical condition), paired t-tests were used. For comparisons of HD versus TAK groups, standard t-tests were used. *p-value <0.05, **p-value <0.01, ***p-value <0.001, ****p-value <0.0001. HD, healthy donors; IFNγ, interferon-γ; IL-17, interleukin-17; TAK, Takayasu's arteritis.

CD4+ (figure 1A) or CD8+ (figure 1B) cells. We observed a major enrichment of genes composing this ISG toward TAK (six significant genes out of eight for both CD4+ ranging from 8.67% to 32.36% of the greatest gene enrichment and CD8+ samples ranging from 7.22% to 46.91% of the greatest gene enrichment). Then, we confirmed this observation by calculating individual ISG enrichment scores using GSVA R package[18] that we compared between HD and TAK using limma R package. This analysis method was applied on CD4+ (figure 1C) and CD8+ (figure 1D) cells from HD and TAK. In both situations, we observed a significant enrichment of the ISG in TAK as compared with HD, which tends to be even greater in CD8+ samples.

Next, we FACS-sorted CD4+ and CD8+ T cells from either HD or TAK PBMCs and pooled the two populations before performing RT-qPCR using the ISG. This allowed us to quantify the ISG expression regarding real CD3+ T cells without other non-T cells contaminants (figure 1E). We confirmed the upregulation of the ISG in TAK versus HD in T cells. Together, our results strongly demonstrate that type I interferons-related genes expression is greatly upregulated in TAK.

### JAK/STAT pathway signature efficiently separates CD4+ and CD8+ T cells between HD and TAK

Next, we performed a pathways enrichment analysis between HD and TAK, for CD4+ and CD8+ samples separately. For CD4+ T cells, among the 2286 significantly enriched pathways (SEPs) between HD and TAK (adjusted p-value ≤0.05), 996 and 1290 were enriched in HD and in TAK, respectively. For CD8+ T cells, among the 2772 SEPs between HD and TAK, 756 and 2016 were enriched in HD and in TAK, respectively. To reduce the complexity of the results, we generated a network of SEPs linked together according to their degree of overlap. We noticed an important enrichment of pathways linked to interferons (especially type I interferons), JAK/STAT- and cytokines/chemokines-related signalling pathways among the ones greatly upregulated in TAK, both for CD4+ (see online supplementary figure S1A) and CD8+ (see online supplementary figure S1B) T cells. Then, we decided to focus on these particular biological functions and extracted the most representative pathways that we plotted on a heatmap (individual adjusted p-value ≤0.05), for either CD4+ (figure 2A) or CD8+ (figure 3A) samples. The 2286 SEPs in CD4 +TAK versus HD and the 2772 SEPs in CD8+ TAK versus HD shared 1127 common pathways (respectively about 49.3%

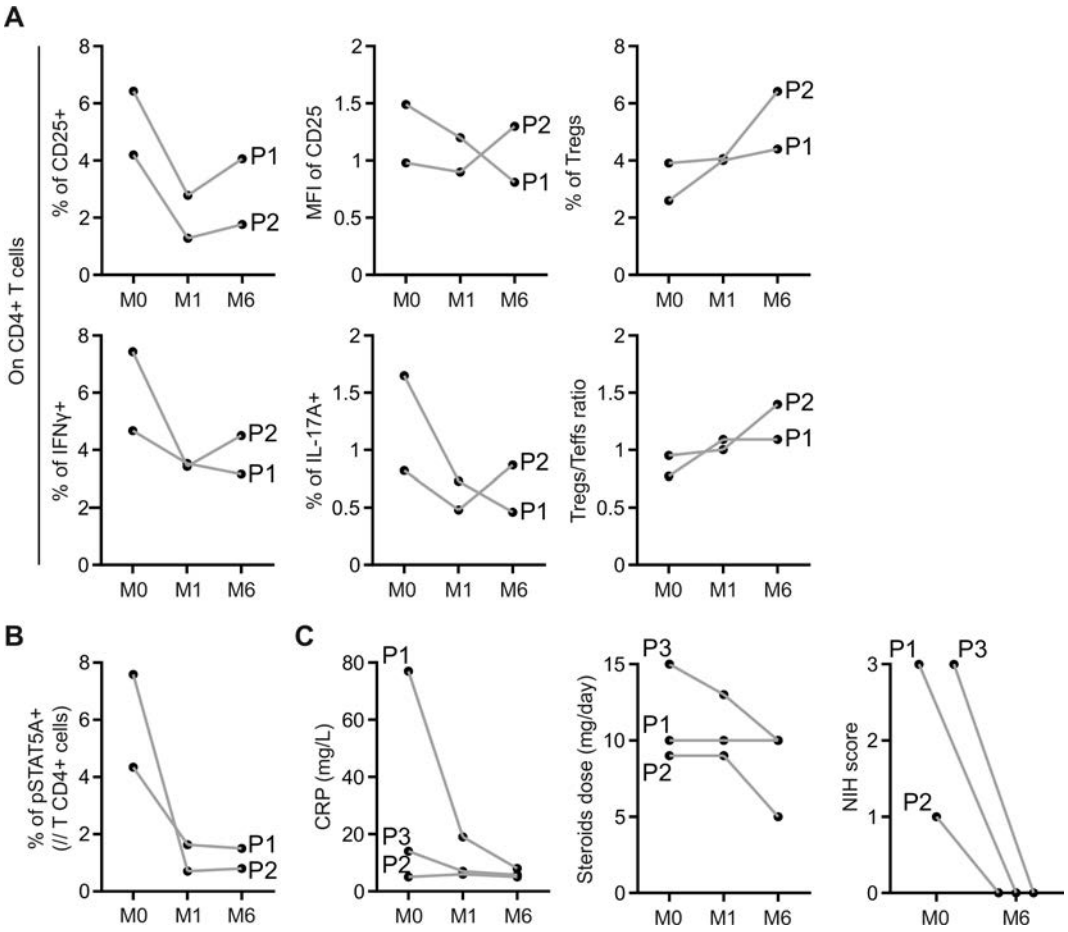


**Figure 5** Targeting JAK/STAT pathway with JAKinibs improve TAK. (A) Flow cytometry analysis of surface (CD25) and intracellular (FoxP3, IFNγ and IL-17A) markers on PBMCs CD4+ T cells coming from in vivo untreated (M0) and treated (M1 for month 1, M6 for month 6) paired TAK. (B) Measurement by flow cytometry of pSTAT5A percentage of expression by CD4+ T cells in in vivo untreated and treated paired TAK. (C) Fresh blood dosage of CRP levels (left) in untreated or treated paired TAK, concomitant corticosteroids doses administrated to patients under JAKinibs treatment (middle) and NIH score before/after JAKinibs treatment (right). CRP, C reactive protein; IFNγ, interferon-γ; IL-17, interleukin-17; TAK, Takayasu's arteritis.

and 40.7% of each group pathways), indicating the proximity of the upregulated pathways in CD4+ and CD8+ T cells in TAK.

We then created a network of all the genes contained in the SEPs presented in the heatmaps using the STRING interaction database, for CD4+ (figure 2B) and for CD8+ (figure 3B) T cells. Among the highest degree nodes, we found for both CD4+ and CD8+ samples JAK1, JAK2, STAT1, STAT2, STAT3 and IRF9 with more than 30 connections. Other genes of this degree category were specific to only one group: CCL20, CXCL10 and CXCL12 for CD4+ samples and Tyrosine-Protein Phosphatase Non-Receptor Type 11 (PTPN11) and Interferon Regulatory Factor 7 (IRF7) for CD8+ samples. Among genes with 22–30 connections, we found JAK3, STAT5A and STAT5B in both CD4+ and CD8+ samples networks. Interestingly, these genes are mainly involved in JAK/STAT signalling pathway, either *via* interferons or cytokines/chemokines upstream stimuli, thus confirming the crucial implication of the JAK/STAT signalling pathway in T cells-mediated physiopathology of TAK. As the STAT5A gene mRNA was significantly upregulated in both CD4+ (p-value ≈0.0011) and CD8 +samples (p-value ≈$6.85e^{-8}$) of TAK versus HD, we also showed by flow cytometry a significantly increased proportion of pSTAT5A+ cells in TAK as compared with HD for CD4+ (figure 2C) and CD8+ samples (figure 3C). Together, our results clearly establish that JAK/STAT pathway is critical in T cells for the immunopathology of TAK, which provides us with a rationale to target JAK/STAT in TAK.

### JAKinibs reduce T cells activation, Th1 and Th17 polarisation and promote increase of Tregs in TAK

Next, we aimed to study the in vitro effect of JAKinibs on PBMCs from HD or TAK cultured for 5 days with or without ruxolitinib (figure 4) or tofacitinib (see online supplementary figure S5). Ruxolitinib treatment induced a significant reduction of CD25 expression by CD4+ (figure 4A) and CD8+ (figure 4B) T cells, in percentage of CD25+ cells (CD25low and CD25high) and MFI (Mean Fluorescence Intensity) in HD and TAK. Furthermore, TAK showed a significant increase in CD4+IFNγ+ Th1 and CD8+IFNγ+ cells that was abrogated by ruxolitinib (figure 4). Ruxolitinib also induced an increase of CD4+ tregs in TAK, which was not observed in HD (figure 4A) nor for CD8+ Tregs (figure 4B). Furthermore, while TAK showed a marked increase in CD4+IL-17A+ Th17 cells as compared with HD, ruxolitinib abrogated this polarisation (figure 4A). Ruxolitinib also decreased Ki-67 expression in CD4+ (figure 4A) and CD8+ (figure 4B) T cells from HD and TAK as compared with untreated. Representative dot plots for several markers are shown in online supplementary figure S2 for CD4+ and in

online supplementary figure S3 for CD8+ T cells. We observed similar trends for CD69 expression in CD4+ and CD8+ T cells from HD and TAK that did not reach significance for every group (see online supplementary figure S4). Finally, we obtained akin results as presented in figure 4 using tofacitinib (see online supplementary figure S5). Together, these results confirm the in vitro effect of JAKinibs on T cells activation/differentiation in TAK, by restoring the balance between effector and regulatory T cells.

### Targeting JAK/STAT pathway with JAKinibs improves TAK

Next, we treated three TAK with JAKinibs as detailed in figure 5 and online supplementary table S1. Their previous treatment (ie, corticosteroids) was not modified on JAKinibs introduction. Using flow cytometry (available for two patients), we found that JAKinibs treatment decreased the CD25 expression by CD4 +T cells and increased Tregs percentages after 6 months of treatment (M6), with a visible effect as soon as 1 month of treatment (M1) as compared with baseline (M0) (figure 5A). We also observed that Th1 and Th17 cells were decreased and that the Tregs/Teffs ratio increased (figure 5A) with JAKinibs, thus confirming the reduction of CD4 +effector T cells activation/differentiation in vivo. We also confirmed a reduction of pSTAT5A expression among CD4 +T cells as soon as M1 with JAKinibs (figure 5B). The treatment effectively decreases systemic inflammation, as we found a reduction of C reactive protein level under JAKinibs in two out of three treated TAK at M6 (figure 5C). JAKinibs treatment also allowed corticosteroids dose reduction in two out of three patients (figure 5C) and led to a reduction of NIH activity score to 0 for all treated patients at M6 (figure 5C) as compared with M0.

## DISCUSSION

We identified for the first time the JAK/STAT signalling pathway as a central biological function linking imbalance between effector (Th1 and Th17) and regulatory T cells in TAK. Using transcriptome analysis, we showed that JAK/STAT, interferons and cytokines/chemokines-related genes and pathways were markedly upregulated in TAK in both CD4+ and CD8+ T cells.

We demonstrated that interferons signalling is greatly implicated in the physiopathology of TAK. Type I (IFNα/β) and type II (IFNγ) both trigger JAK/STAT signalling and activate STAT1.[19] IFNα/β and IFNγ pathways were significantly upregulated in TAK, as well as the target genes of STAT1, STAT2 and STAT3, supporting the critical role of T cells in the disease process. Using transcriptome and RT-qPCR, we highlighted the upregulation of a type I interferons-specific signature in TAK. A large body of evidence already implicates type I interferons in the development of auto-immune and autoinflammatory diseases such as rheumatoid arthritis,[13–16] systemic lupus erythematosus[15 17] or systemic sclerosis.[15] Mendelian autoinflammatory disorders associated with upregulation of type I interferons signalling also present severe inflammation and autoimmunity.[20] In LVV, Zhang *et al* previously showed in a GCA mice model that IFNα, IFNγ, STAT1, STAT2 and STAT4, but also specific downstream-associated genes were significantly upregulated as compared with controls.[10]

Type I and II cytokines signal through the JAK/STAT pathway and are major drivers of LVV as they enhance Th1 and Th17 cell responses.[6] Although the relative contribution of Th1 versus Th17 cells is the subject of ongoing research in LVV, these proinflammatory subsets are dominant infiltrates in the vascular walls, producing IFNγ and IL-17 to drive the systemic and vascular manifestations of TAK. Meanwhile, corticosteroids which preferentially target innate cytokines, such as IL-1β, IL-12, and IL-6,[21] have little effects on tissue-residing T cells in LVV.[22] They suppress Th1 cytokines but spare Th17 cytokines in patients with TAK.[6] Thus, alternative therapeutic approaches targeting all pathogenic effector T cells are required in LVV.

We demonstrated here that JAKinibs can be used to dampen and eventually control overwhelming autoinflammatory responses in TAK. Our in vitro data showed that JAKinibs lead to reduced Th1/Th17 cells differentiation and increased proportion of Tregs in TAK as compared with HD. We also confirmed in vivo that JAKinibs could efficiently regulate autoinflammatory responses and restore T cells homeostasis in TAK. This is consistent with previous works in GCA mice showing that tofacitinib reduced quantities of IL-17A, IFNγ and IL-21 mRNAs in vascular lesions as compared with untreated mice.[10] Tofacitinib also suppressed multiple effector T cell lineages commitment (significant decrease of RORγc, T-bet and Bcl6 mRNAs) and their subsequent cytokines secretion (IFNγ, IL-17A and IL-21 mRNAs), but also global T cells expansion, inflammation-associated microangiogenesis and hyperplasia.

Both baricitinib and ruxolitinib were used to treat our TAK patients. In vitro experiments pointed out similar effects with ruxolitinib (anti-JAK1/2) and tofacitinib (anti-JAK1/3). One can conclude that any JAKinib would have an equivalent effect on T cells in the treatment of TAK. However, baricitinib seems to be more efficient than ruxolitinib to increase Tregs proportion among human primary T cells, through preservation of JAK3/STAT5 signalling pathway.[23] These results raise the question of whether a specific JAKinib should be preferentially indicated in the TAK treatment.

Together, our findings bring a strong rationale for considering JAKinibs as a new potent therapy in TAK, used to dampen deleterious immune responses and overactivated signalling pathways. JAK/STAT signalling pathway appears as a central biological function linking major imbalance between effector (Th1/Th17) and regulatory T cells in TAK. Nevertheless, further investigations are needed to assess their precise role in TAK and to determine which JAKinib should preferentially be used.

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**Data availability statement** Data are available on reasonable request. Transcriptome data from FACS-sorted CD4+ and CD8+ T cells from healthy donors and Takayasu's arteritis-affected patients are available on request to Pr. DS: david.saadoun@upmc.fr. They are presented as deidentified patient tabular data (rows = normalised gene expression and columns = patients) in a plain text format. Reuse is permitted if data are not altered in any way and if original authors are correctly cited in the subsequent publications. Raw CEL files were standardised and normalised using limma R package and standard methods (notably RMA normalisation).

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TRANSLATIONAL SCIENCE

# Blood-based test for diagnosis and functional subtyping of familial Mediterranean fever

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## ABSTRACT

**Background and objective** Familial Mediterranean fever (FMF) is the most common monogenic autoinflammatory disease (AID) worldwide. The disease is caused by mutations in the *MEFV* gene encoding the inflammasome sensor Pyrin. Clinical diagnosis of FMF is complicated by overlap in symptoms with other diseases, and interpretation of genetic testing is confounded by the lack of a clear genotype–phenotype association for most of the 340 reported *MEFV* variants. In this study, the authors designed a functional assay and evaluated its potential in supporting FMF diagnosis.

**Methods** Peripheral blood mononuclear cells (PBMCs) were obtained from patients with Pyrin-associated autoinflammation with an FMF phenotype (n=43) or with autoinflammatory features not compatible with FMF (n=8), 10 asymptomatic carriers and 48 healthy donors. Sera were obtained from patients with distinct AIDs (n=10), and whole blood from a subset of patients and controls. The clinical, demographic, molecular genetic factors and other characteristics of the patient population were assessed for their impact on the diagnostic test read-out. Interleukin (IL)-1β and IL-18 levels were measured by Luminex assay.

**Results** The ex vivo colchicine assay may be performed on whole blood or PBMC. The functional assay robustly segregated patients with FMF from healthy controls and patients with related clinical disorders. The diagnostic test distinguished patients with classical FMF mutations (M694V, M694I, M680I, R761H) from patients with other *MEFV* mutations and variants (K695R, P369S, R202Q, E148Q) that are considered benign or of uncertain clinical significance.

**Conclusion** The ex vivo colchicine assay may support diagnosis of FMF and functional subtyping of Pyrin-associated autoinflammation.

## Key messages

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

- Familial Mediterranean fever (FMF) is the most common monogenic autoinflammatory disease (AID), affecting an estimated 150 000 patients.
- More than 340 disease-associated variants in *MEFV*, the causal gene in FMF, have been reported.
- FMF diagnosis is primarily clinical, and further supported by review of ethnic origin, family history and genetic information.
- Diagnosis delay is common in FMF, and complicated by incomplete clinical presentation and overlap in symptoms with other periodic fever syndromes.

**What does this study add?**

- The study reports and validates a functional diagnostic test that discriminates FMF over healthy controls and related AIDs.
- The ex vivo colchicine test identifies two mechanistic subtypes of Pyrin-associated AID.

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

- The test may help to expedite FMF diagnosis and timely initiation of colchicine therapy.

## INTRODUCTION

Monogenic autoinflammatory diseases (AIDs) are a rapidly expanding group of genetically diverse but phenotypically overlapping inflammatory disorders caused by primary dysfunction of the innate immune system.[1–3] They are also referred to as 'periodic fever syndromes' because many of these diseases feature recurrent fevers and episodes of systemic or organ-specific inflammation. They can cause significant morbidity and even mortality. A clinical challenge is that efficient diagnosis is hampered by overlapping clinical features and non-specific symptoms that are shared by patients suffering from diseases with distinct aetiologies. Moreover, patients suffering from AIDs with similar underlying mechanisms, who respond to particular therapies, may present with atypical or even distinctive symptoms.[4] While genetic testing is widely implemented for AID diagnosis, interpretation of genetic results is often challenging, which has even become more complex with the availability of next-generation sequencing allowing multiple genes to be tested simultaneously and generating an increasing number of variants, frequently of unknown significance.[5 6] Thus, there clearly is a growing need for new or improved tools to diagnose these diseases.



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With an estimated 150 000 patients, FMF is considered the most common monogenic AID worldwide, mainly affecting populations originating from the Mediterranean basin.[7] Since its suggested use in 1972, the microtubule polymerisation inhibitor colchicine has become the gold standard for treatment in FMF, with an overall non-responder rate of only 5%–10%.[8 9] For patients who are resistant or intolerant to colchicine, anti-IL-1 therapy is a safe and effective alternative.[10–13] Colchicine prevents not only FMF attacks but also disease-associated complications such as amyloid A amyloidosis, a severe manifestation with poor prognosis.[9] However, it is crucial to establish a timely and correct diagnosis of FMF before committing to daily, lifelong treatment. Current FMF diagnosis is primarily clinical, and further supported by review of ethnic origin, family history and genetic information.[7] Robust and timely clinical diagnosis of FMF is complicated by significant overlap in symptoms and the clinical presentation of other AIDs, most of which do not respond to colchicine therapy. Additionally, interpretation of genetic testing may prove challenging with around 340 disease-associated variants in *MEFV*, the gene mutated in FMF patients, being reported in the Infevers database to date.[14–16] Many of these variants are common in the general public, but there are also a number of rare variants of unknown pathogenicity. While in silico tools can be useful in predicting pathogenicity, care should be taken when used for clinical interpretation. Some of the most common *MEFV* mutations (M694V, M694I and M680I) are predicted as benign/non-deleterious by two such programmes, PolyPhen and SIFT, while having the most severe clinical consequences.[17 18] In silico prediction for *MEFV* variants may be hampered by the fact that amino acids that cause human disease are often present as a wild-type allele in primates,[19] but also by the incomplete understanding of the pathophysiological mechanisms underlying FMF. Acknowledging that the difficulties in linking genotype and phenotype in FMF are caused by an incomplete understanding of the molecular pathogenic mechanism underlying FMF, a recently reported consensus-driven pathogenicity classification was able to classify most variants in three genes causing other AID (*MVK*, *NLRP3* and *TNFRSF1A*), but almost half of the *MEFV* variants (42.4%) could not be classified or were classified as 'variants of uncertain significance'.[5]

*MEFV* was identified as the causal gene of FMF in 1997.[14 15] More recently, it was established that Pyrin, the protein encoded by *MEFV,* senses inactivation of RhoA GTPase, resulting in formation of an inflammasome that activates the protease caspase-1 and drives production of interleukin (IL)-1β and IL-18.[20–28] In a prior study, we reported that in contrast to wild-type Pyrin, which requires microtubules to activate the inflammasome pathway, FMF-associated Pyrin mutants engage the inflammasome pathway independently of microtubules.[24] Here, we report that microtubule-independent activation of the Pyrin inflammasome in the ex vivo colchicine assay is specific to FMF alleles, allowing discrimination from healthy individuals and patients suffering from Pyrin-associated AIDs that are distinct from FMF and other AIDs, including pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA) and mevalonate kinase deficiency (MKD) that also have been associated with altered Pyrin inflammasome activation. Technical optimisation showed that the ex vivo colchicine assay may be performed using a small volume of human whole blood to support convenient and straightforward diagnosis of FMF. Finally, we provide an extensive validation of the ex vivo colchicine assay in a distinct population of patients suffering from FMF (n=43) and Pyrin-associated AID that is distinct from FMF (n=8). We show that the functional assay correlates with the *MEFV* genotype, and that the diagnosis of FMF almost perfectly coincides with the recently published consensus pathogenicity classification with some notable exceptions. This test thus aids in and provides further support for the pathogenicity classification of specific *MEFV* variants.

## MATERIALS AND METHODS

### Human whole blood

Peripheral venous blood specimens were collected from healthy individuals as well as from patients with FMF using EDTA-coated Vacutainer tubes. Whole blood was used either fresh or after overnight storage at room temperature in the dark. Whole blood was seeded, 200 $\mu$L per 96-well, and maintained in a 5% $CO_2$ incubator at 37°C.

### Human PBMC isolation

Peripheral venous blood specimens were collected from healthy individuals as well as from patients suffering from FMF, PAPA or MKD. Human PBMCs were isolated from blood collected in EDTA-coated Vacutainer tubes followed by Ficoll-Hypaque density gradient centrifugation. After isolation, PBMCs were stored in liquid nitrogen for later usage. On thawing, PBMCs were allowed to recover for 1 hour at 37°C in culture medium consisting of Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum (FBS). Following cell viability determination, cells were seeded at a density of $2.5\times10^5$ per 96-well and maintained in a 5% $CO_2$ incubator at 37°C.

### Reagents and stimulation

Activation of the Pyrin inflammasome was performed by stimulating PBMCs or whole blood with *Clostridium difficile* toxin A (TcdA; 1 µg/mL; Enzo Life Sciences) alone, or with a combination of colchicine (1 µM; Sigma) and TcdA (1 µg/mL; Enzo Life Sciences). PBMC samples were incubated for 5 hours, while whole blood tests were incubated for 24 hours.

### Cytokine analysis

Human IL-1β and IL-18 cytokine levels were determined in cell culture supernatants by magnetic bead-based multiplex assay using Luminex technology (Bio-Rad). The IL-1β and IL-18 ratios were calculated by dividing the cytokine level of the combined colchicine TcdA treatment by the cytokine level of the treatment with TcdA alone. GraphPad Prism V.6.0 software was used for data analysis.

### Statistics

To evaluate the predictive accuracy of the functional assay, a receiver operating characteristic (ROC) curve was generated using GraphPad Prism V.7.01 software. The area under curve (AUC), sensitivity and specificity were calculated with the latter two being used to determine the Youden index. For the analysis of variance, a linear model of the form y=µ+*MEFV* genotype+gender+origin+age+error was fitted to the IL-1β ratio and IL-18 ratio data of the patients. The term *MEFV* genotype was constructed as a factor product of all 15 genotype variants having wild type, homozygous and heterozygous as levels. Significances of the *MEFV* genotype, gender, origin and age effects were assessed by an F test. A 2×2 table summarising the outcome of the assay and the presence or absence of a particular clinical parameter was generated, followed by a Fisher's exact test to assess potential correlations of the clinical parameters of the patient cohort presented in online supplementary table 1 and

the ex vivo colchicine assay. To examine the potential correlation between the assay and the clinical response to colchicine, a regression analysis was performed, followed by a Fisher's unprotected least significant difference (LSD) test at the 5% significance level. For all tests, $p<0.05$ was considered statistically significant.

### Ethical approval information

All patients and controls provided written informed consent for participation in the study, in accordance with ICH/GCP guidelines. Treating physicians provided information regarding the *MEFV* genotype, symptoms, treatment, age and gender for patients with FMF (see online supplementary table 1). Patients or the public were not involved in the design, or conduct, or reporting or dissemination plans of our research.

## RESULTS

### A colchicine challenge assay to support diagnosis of FMF

We previously described the biochemical principle of a functional assay that may support diagnosis of FMF.[24] The Pyrin inflammasome pathway is activated by toxin A from *Clostridium difficile*, resulting in the release of significant amounts of proinflammatory cytokines IL-1β and IL-18 from intoxicated monocytes and macrophages. The microtubule polymerisation inhibitor colchicine prohibits Pyrin inflammasome activation in cells expressing wild-type Pyrin. Contrastingly, cells harbouring the common and clinically severe FMF allele $MEFV^{M694V}$ engaged the Pyrin inflammasome and secreted IL-1β and IL-18 in the presence of colchicine despite inhibition of microtubule dynamics.[24] We hypothesised that determining the ratio of the released cytokines in the presence versus absence of colchicine may provide a robust and fast functional read-out to support functional stratification and diagnosis of FMF.[24]

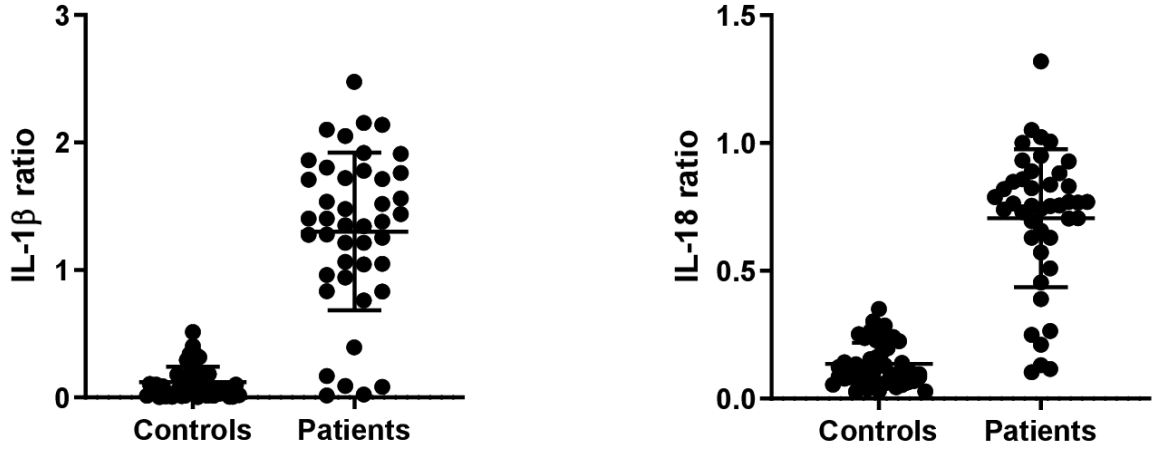
We performed a validation study in order to assess whether this functional test may indeed support sensitive stratification of a wider spectrum of patients with FMF differentiated according to genetic makeup, age, sex and geographical location. Additionally, we set out to compare its selectivity against healthy individuals and across a spectrum of related AID. The study group consisted of 43 patients with FMF and 8 patients with Pyrin-associated AID that was not compatible with FMF. Patients were enrolled in four hospitals located in Italy (Bari—21; Rome—8) and Belgium (Antwerp—7; Ghent—15). The median age was 20 years (2–86), and 63% patients were male. Along with the *MEFV* genotype, clinical and therapeutic characteristics of the patient group are described in online supplementary table 1. The control group consisted of 48 donors who were enrolled in three different locations (Bari—9; Rome—7; Ghent—32). Part of the blood donations for the control group were through the Red Cross, who did not pass on information regarding age and sex. Microtubule dependency was first tested for the control and FMF patient groups with both IL-1β and IL-18 ratios being used as a read-out (figure 1A). As expected,[24] patients with FMF and healthy controls secreted IL-1β and IL-18 equally in response to *Clostridium difficile* toxin A (TcdA) alone, but contrary to patients with FMF, the cytokine ratios for healthy donors were low because of inhibition of wild-type Pyrin by colchicine (figure 1A). To evaluate the effectiveness and accuracy of the functional test, the receiver operating characteristic (ROC) curve was generated for both parameters and the area under curve (AUC) was calculated (figure 1B).[29 30] With an AUC of 0.93 and 0.96, respectively, both parameters performed well in discriminating FMF from control samples. The Youden index was determined to establish the most appropriate cut-off value to differentiate the diseased from the non-diseased (figure 1C).[29] This analysis resulted in respective cut-off values of 0.64 and 0.37 for the IL-1β and IL-18 ratio, maximising the specificity while maintaining a sensitivity at 0.86 for both read-outs. Inclusion in the cohort analysis of the eight patients with the prevalent *MEFV* R202Q, E148Q and P369S variants that presented with autoinflammatory features not compatible with FMF resulted in an AUC of 0.88 for both the IL-1β and IL-18 ratios, and a somewhat lower sensitivity of 0.77 for the assay (see online supplementary figure S1). In conclusion, these results suggest that the ex vivo colchicine assay can be deployed to discriminate patients with FMF from other Pyrin-associated AIDs and a control population.

### Ex vivo colchicine assay discriminates two mechanistic subtypes of FMF that correlate with pathogenicity of *MEFV* variants

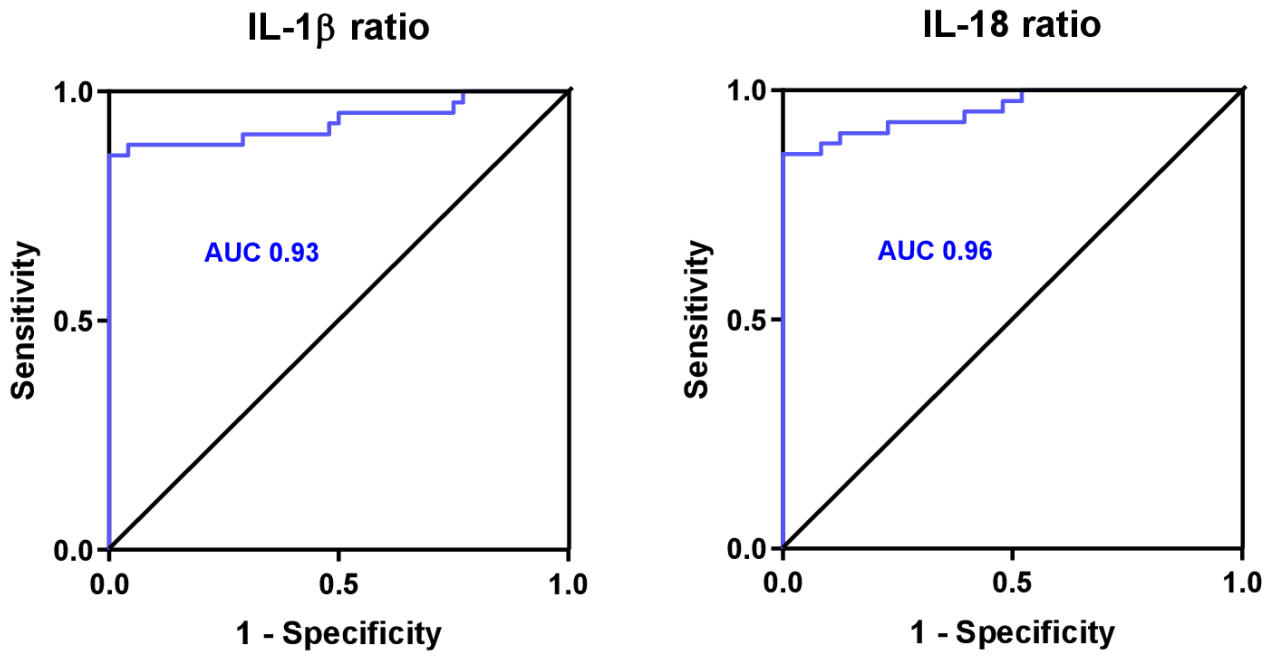
The statistical parameters of the ROC curve demonstrate that the ex vivo colchicine assay may support the reliable identification of patients with FMF. Consistent with our previous findings,[24] colchicine enhanced TcdA-induced IL-1β secretion in PBMCs of most patients with FMF as reflected by IL-1ß ratios>1 (figure 2A). Interestingly, early studies[31–33] similarly reported that colchicine upregulated IL-1ß secretion and pro-IL-1ß transcript levels in lipopolysaccharide (LPS)-stimulated PBMCs, while downregulating tumour necrosis factor (TNF)-α and IL-6 levels. Further work is needed to understand the molecular mechanisms by which colchicine modulates inflammatory cytokine secretion. We next performed an analysis of variance in order to explore the epidemiological and clinical factors that correlate with the measured IL-1β and IL-18 responses in the patient group (figure 2A,B). This analysis showed that the most important contributor to the variation in the IL-1β and IL-18 ratios among patients with Pyrin-associated AID is the *MEFV* genotype, while age has a minor, but statistically significant effect. The effects of gender and the location where the samples were collected were not significant (figure 2B). The clinical parameters presented in online supplementary table 1, but amyloidosis (that did not occur in the patient cohort) were also assessed for potential correlations with the ex vivo colchicine assay by using a Fisher's exact test. This analysis showed a lack of correlation with chest pain, abdominal pain and arthritis, with p values for these three parameters corresponding to $p=0.346$, $p=0.467$ and $p=0.366$, respectively. However, fever was significantly correlated with the ex vivo colchicine test ($p=0.017$). Notably, a regression analysis followed by a Fisher's unprotected least significant difference test also showed a significant correlation between the ex vivo colchicine assay and the clinical response to colchicine (figure 2B). A possible explanation for this correlation is that the ex vivo colchicine assay primarily selects for patients with classical FMF mutations, the majority of whom shows a favourable clinical response to colchicine therapy (see online supplementary table 1).

Given the key role of the *MEFV* genotype, IL-1β and IL-18 ratios of patients and controls were clustered and plotted according to the *MEFV* genotype (figure 2C). Notably, this analysis showed that patients with disease-penetrant *MEFV* mutations (M694V, M694I, M680I, E148Q/R761H) are clearly separated from controls, whereas the functional response of patients with other *MEFV* variants (K695R, P369S, R202Q, E148Q) fully coincided with the controls (figure 2C). The M694V mutation located in exon 10 is considered to be the most pathogenic

A




B

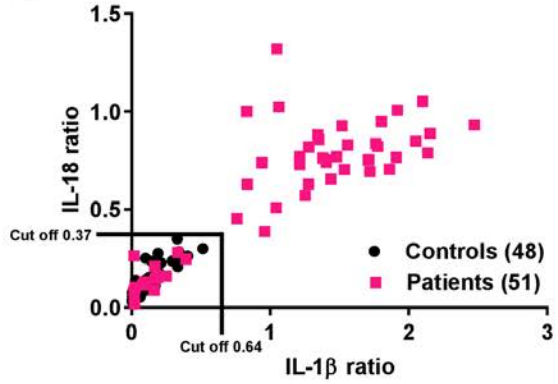



C

| Parameter | Cut-off point | Sensitivity | Specificity |
|---|---|---|---|
| IL-1β ratio | 0.64 | 0.86 | 1 |
| IL-18 ratio | 0.37 | 0.86 | 1 |

**Figure 1** Diagnosis of familial Mediterranean fever (FMF) using a functional assay. (A) Peripheral blood mononuclear cells from healthy donors (n=48) and patients with FMF (n=43) were treated for 5 hours with either *Clostridium difficile* toxin A (TcdA) alone or with TcdA in combination with colchicine before culture supernatants were analysed for interleukin (IL)-1ß and IL-18, and the TcdA+colchicine over TcdA ratio for each cytokine was calculated. Data are combined from multiple experiments. (B) For both parameters, the receiver operating characteristic (ROC) curve was calculated, as well as the area under curve (AUC). (C) For both parameters, the Youden index was calculated to determine the most appropriate cut-off point, given the sum of sensitivity and specificity being maximum.

A




B

| p-value | IL-1β ratio | IL-18 ratio |
|---|---|---|
| *MEFV* genotype | <0.001 | <0.001 |
| sample origin | 0.158 | 0.075 |
| gender | 0.489 | 0.485 |
| age | 0.018 | 0.010 |
| clinical response to colchicine | 0.04 | 0.016 |

C

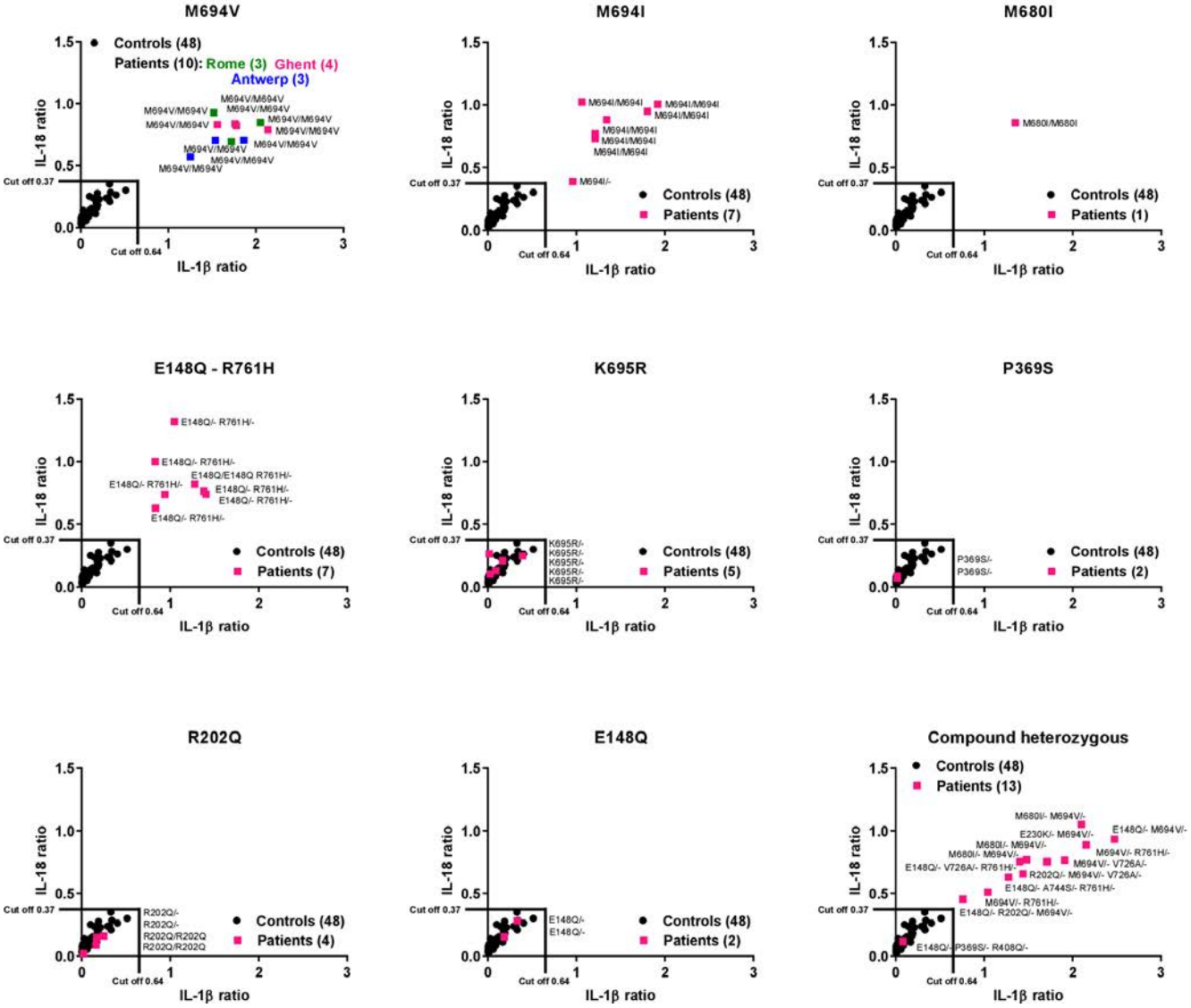



**Figure 2** Functional stratification of patients with Pyrin-associated autoinflammatory disease correlates with *MEFV* genetic variants. (A) Combined representation of interleukin (IL)-1β and IL-18 ratios of the ex vivo colchicine assay with peripheral blood mononuclear cells from healthy donors (n=48) and the patient group composed of patients with *MEFV* gene variants that presented with either a familial Mediterranean fever (FMF) phenotype (n=43) or with autoinflammatory features not compatible with FMF (n=8). Cut-off points as determined by the Youden index are indicated. (B) Analysis of variance for the patient group represented by the p value of the F test. Regression analysis for potential correlation between the assay and clinical response to colchicine tested at 5% significance level. (C) Representation of the functional assay with patient data being separated based on *MEFV* variants.

FMF allele causing severe disease, both in patients that are homozygous and compound heterozygous for M694V.[34–36] The functional test described here thus objectively identifies these patients as patients with FMF, in agreement with the consensus classification (see online supplementary table 2). Patients with the M694V mutation were enrolled at three different locations (Rome, Ghent and Antwerp), all of them responding with a clear induction of Pyrin inflammasome activity in the presence of colchicine in the ex vivo colchicine test. The outcome of the test is thus independent of the location where the sample was collected. The M694I and M680I alleles also map to exon 10 and are associated with a more severe phenotype.[35] Patients expressing these disease alleles also were clearly separated from the controls in the ex vivo colchicine assay (figure 2C), demonstrating that ex vivo colchicine testing allows identification of FMF patients with classical *MEFV* mutations. Likewise, the functional assay confirmed the pathogenic classification of variant R761H (or E148Q/R761H). Interestingly, the K695R variant—positioned adjacent to M694V in exon 10—did not cluster with the classical exon 10 FMF alleles in the functional ex vivo colchicine assay, suggesting that the functional effect of this mutation on the Pyrin inflammasome differs from the other tested disease-associated exon 10 mutations. In addition to K695R, the exons 2 and 3 *MEFV* variants in our cohort that are considered benign (R202Q) or of uncertain clinical significance (P369S and E148Q) clustered with the control population in the functional ex vivo colchicine assay (figure 2C and online supplementary table 2). All but one compound heterozygote patient in our cohort harboured at least one of the classical penetrant exon 10 FMF alleles (M680I, M694V, M694I or R761H), and were objectively reported as FMF by the ex vivo colchicine assay. The single compound heterozygous patient in our cohort that was diagnosed with variants of only uncertain clinical significance clustered with the control population in the functional assay. We also analysed PBMC from a cohort of family members of patients (n=10) to examine whether the ex vivo colchicine assay is able to identify asymptomatic carriers of FMF alleles. All nine tested carriers of penetrant exon 10 disease mutations (eight heterozygous carriers for M694V and one for M694I) clustered together with FMF patients, whereas the carrier of prevalent *MEFV* variant E148Q clustered with the healthy donor control group that lacks *MEFV* mutations (see online supplementary file 2). Together, these results highlight the clear correlation between the results of the ex vivo colchicine assay and the consensus pathogenicity classification of *MEFV* gene variants.

### Ex vivo colchicine assay distinguishes FMF from both healthy and diseased controls

We previously demonstrated that patients afflicted with cryopyrin-associated periodic syndrome and juvenile idiopathic arthritis were classified separately from patients with FMF by the ex vivo colchicine assay.[24] To further assess specificity of the assay, we evaluated the response of patient groups suffering from AIDs of which the pathophysiological mechanisms have been linked to deregulated activation of the Pyrin inflammasome. A first group of patients was diagnosed with PAPA syndrome, a dominantly inherited autoinflammatory disorder caused by mutations in the CD2-binding protein 1 (CD2BP1) that is predominantly mediated by granulocytes.[37] CD2BP1 and its murine orthologue, proline-serine-threonine phosphatase interacting protein (PSTPIP1), are adaptor proteins that interact with several proteins involved in cytoskeletal organisation and inflammatory processes, including Pyrin. The mutations in *PSTPIP1* underlying PAPA syndrome trigger hyperphosphorylation and markedly increased binding to Pyrin.[38] Consistent with the ex vivo colchicine assay being highly specific to FMF, results from the test showed that PAPA patients clustered separately from patients with FMF, thus confirming that the ex vivo colchicine assay supports reliable discrimination of patients with PAPA and FMF (figure 3A and online supplementary table 3A). Encouraged by these findings, we next examined the response of patients suffering from mevalonate kinase deficiency (MKD)/hyper-immunoglobulin D syndrome (HIDS), another inflammatory disease that has been suggested to be associated with defective geranylgeranylation of RhoA GTPase and Pyrin inflammasome

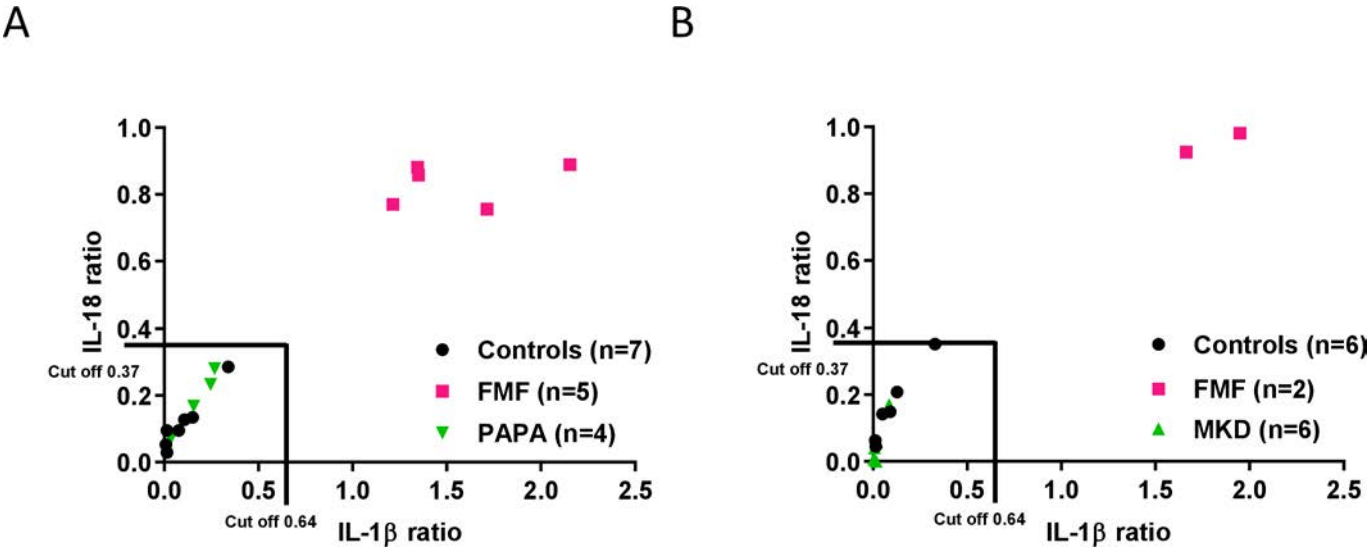



**Figure 3** Functional stratification of familial Mediterranean fever (FMF) patients from healthy donors, pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA), and mevalonate kinase deficiency (MKD) patients. Peripheral blood mononuclear cells from controls, patients with FMF, and patients with PAPA (A) or patients with MKD (B) were treated for 5 hours with either *Clostridium difficile* toxin A (TcdA) alone or TcdA in combination with colchicine before culture supernatants were analysed for interleukin (IL)-1ß and IL-18, and the TcdA+colchicine over TcdA ratio for each cytokine was calculated.

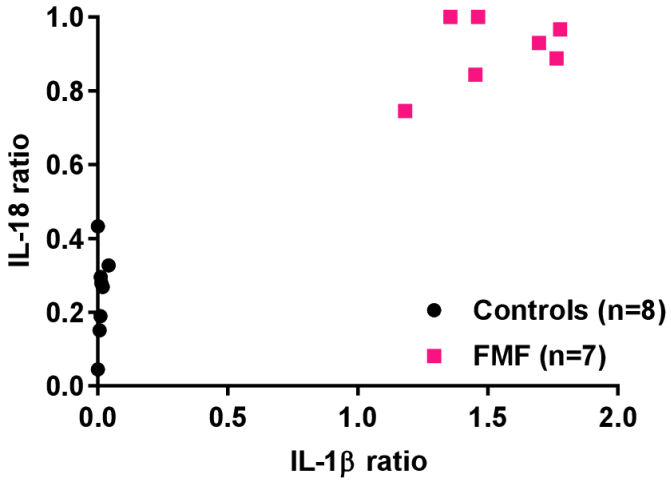



**Figure 4** Functional familial Mediterranean fever (FMF) screening in human whole blood. Fresh, undiluted whole blood from controls (n=8) and patients with FMF(n=7) was treated for 24 hours either with *Clostridiumdifficile*toxin A (TcdA) alone or TcdA in combination with colchicine before culture supernatants were analysed for interleukin (IL)-1ß and IL-18, and the TcdA+colchicine over TcdA ratio for each cytokine was calculated. Results are combined from four independent experiments.

activation.[20 39 40] MKD/HIDS is caused by mutations in the *MVK* gene that target mevalonate kinase activity in the cholesterol and isoprene biosynthesis pathways.[41–43] Although the molecular aetiology of MKD/HIDS is still debated, it is clear that reduced MVK activity leads to build-up of mevalonic acid, and a shortage of cholesterol, vitamins and other products of the isoprenoid biosynthesis pathway, which cause uncontrolled release of IL-1β through incompletely understood mechanisms. However, we found that the response of patients with PAPA in the ex vivo colchicine assay resembled that of healthy controls, with both markedly segregating from a panel of patients with FMF with classical *MEFV* mutations (figure 3B and online supplementary table 3B). Together, these results demonstrate that the ex vivo colchicine assay is able to highly specifically stratify patients with FMF from healthy donors and patients suffering from related AIDs.

### Functional FMF testing is feasible in human whole blood

Although a well-established sampling method in laboratory testing of disease-specific activity for inflammatory disorders, the isolation of PBMC is a labour-consuming and time-consuming activity that requires specialised laboratory equipment that is not commonly available in clinical laboratories. Moreover, PBMC purification requires larger amounts of blood draws compared with direct whole blood analysis. In order to facilitate broad adoption of ex vivo colchicine testing in routine screening of patients suspected of FMF, we explored whether our findings with purified PBMC could be replicated in whole blood testing. Indeed, ex vivo colchicine challenge of undiluted whole blood showed a clear segregation of both the IL-1β and IL-18 ratios for FMF patients with classical *MEFV* mutations and healthy donors (figure 4 and online supplementary table 4), establishing that the functional assay can be conveniently performed on fresh blood, bypassing the need for PBMC isolation. Notably, we confirmed that the assay also works in whole blood stored overnight, although this procedure came with significantly increased background levels for IL-18, which limited the sensitivity of IL-18 ratio determination in both control and FMF samples (data not shown). Regardless, we demonstrated here that functional screening of FMF alleles based on ex vivo colchicine challenge is a technically robust procedure that may support FMF screening based on purified PBMC as well as whole blood.

## DISCUSSION

A consensus-driven pathogenicity classification was recently proposed to support the urgent need for clear guidelines and uniform diagnosis of FMF across the world.[5] An unforeseen outcome of this study was the large number of *MEFV* variants that were classified as 'variants of uncertain significance' or 'unsolved pathogenicity', demonstrating the urgent need for insight in the functional impact of these *MEFV* variants on Pyrin function. A functional test might shed more light on the deleterious effect of specific variants and aid in a more straightforward diagnosis of the disease as exemplified by clinical experience in XIAP (X-linked inhibitor of apoptosis) deficiency.[44] Here, we presented and validated a robust functional assay that is able to specifically stratify patients with FMF from healthy controls, as well as from patients suffering from distinct Pyrin-associated autoinflammation and related AIDs. We demonstrated that the secretion ratios of IL-1β and IL-18 can be used together to increase the robustness of the ex vivo colchicine assay, although it remains possible to rely on a single cytokine for reading out results. When whole blood is used for testing, we noted that IL-1β outperforms IL-18, especially in samples that have been stored overnight prior initiation of the test.

We showed that the primary variable determining the outcome of the ex vivo colchicine assay is the *MEFV* genotype. While we noted that age may have a minor contribution, this may be related to the fact that patients with the most severe FMF mutations are symptomatic at a younger age. Within the FMF patient population, M680I, M694V, M694I and V726A are the most common disease-associated pathogenic mutations.[45–47] The ex vivo colchicine assay clearly classifies patients carrying the M694V, M680I or M694I mutations as patients with FMF, thus supporting the validity of the test. Unfortunately, no conclusion could be drawn for the V726A mutation because only patients harbouring this mutation in a compound heterozygous state participated in our study.

The consensus agreement is that the E148Q variant in exon 2 is a highly prevalent *MEFV* variant of 'uncertain clinical significance'. Results from the ex vivo colchicine assay support this assessment by showing that the functional response of patients with the E148Q variant resembles that of healthy donors expressing wild-type Pyrin, contrary to patients with disease-penetrant FMF mutations such as M694V. Moreover, colchicine was not beneficial in patients with E148Q variants included in this study, further supporting the classification of these patients as Pyrin-associated periodic fever that is distinct from FMF (see online supplementary tables S1–S2).

R202Q is another heavily debated variant. Akin to E148Q, R202Q is located in exon 2 and highly prevalent in control populations. The current consensus classifies this variant as benign.[48] Functional evaluation in the ex vivo colchicine assay showed that the R202Q Pyrin variant responded similarly to wild-type Pyrin. Furthermore, unlike patients with classical FMF mutations, none of the R202Q-bearing patients in our study benefitted from colchicine therapy (see online supplementary table 1).

P369S is an exon 3 variant for which limited genetic and clinical data are currently available.[48] The variant can be present by itself or as part of a complex (E148Q-P369S-R408Q)[49] as is the case for one of the compound heterozygous patients in our

patient cohort. Similar to E148Q and R202Q, P369S is highly common (2%–3% of the overall population) and is therefore to be rather considered a polymorphism. In addition, the mild phenotype and incomplete penetrance that have been reported for patients with P369S variants matches with the consensus agreement that this variant should be classified as one of 'uncertain significance'.[45] Notably, the ex vivo colchicine assay corroborates this conclusion by showing that the variant clearly segregated from disease-penetrant FMF mutations.

We also evaluated the R761H variant in the present study. In our patient population, this exon 10 variant was always present in combination with exon 10 mutation M694V or the exon two variant E148Q. Both the E148Q and R761H variants are usually considered low penetrance alleles, although they have been associated with FMF in patients from a recently described novel endemic area in southeastern Italy.[50] Interestingly, patients with compound heterozygous E148Q-R761H alleles responded similarly to patients with disease-penetrant FMF mutations in the ex vivo colchicine test. Given that E148Q does not cause FMF as discussed above, we conclude that the R761H mutation renders Pyrin activation independent of microtubule dynamics, similarly to the disease-penetrant FMF mutations M680I, M694V and M694I.

The K695R allele, positioned adjacent to the most common pathogenic M694V mutation in exon 10, recently made the switch from 'uncertain significance' to 'likely pathogenic' based on the consensus agreement of an expert team.[5 7] Our results from the ex vivo colchicine test, however, show that the functional response of this mutation clearly differs from that of classical exon 10 FMF mutations, including the adjacent M694 disease alleles, thus suggesting that Pyrin inflammasome activation in AID patients with K695R alleles may differ mechanistically from that in FMF patients with classical exon 10 mutations. Further research is required to understand how Pyrin inflammasome signalling is deregulated in patients with the K695R mutation.

Notably, we observed a significant correlation between the ex vivo colchicine assay and the clinical response to colchicine ($p<0.05$), possibly because the ex vivo colchicine assay primarily selects for patients with classical FMF mutations, the majority of whom shows a favourable clinical response to colchicine therapy. At first sight, this may appear paradoxical because identification of FMF alleles by the ex vivo colchicine assay is based on the inability of high colchicine concentrations (in the range of 0.1–1 $\mu$M) to inhibit inflammasome activation by FMF-associated Pyrin mutants.[24] However, plasma concentrations of colchicine that are therapeutically effective in patients with FMF (<4 ng/mL or <0.01 µM)[51] fail to robustly inhibit TcdA-induced secretion of IL-1ß and IL-18 from TcdA-stimulated PBMCs of healthy donors (data not shown). This suggests that colchicine likely exerts its therapeutic benefit in patients with FMF through other, yet incompletely understood mechanisms that clearly warrant further investigation,[8 51 52] and emphasises that the functional response in the ex vivo colchicine assay should not be interpreted as a predictive marker of the clinical response to colchicine therapy.

Regardless, the ex vivo colchicine assay presented here supports straightforward functional stratification of patients with FMF. Moreover, the test may enable in-depth mechanistic studies of the many prevalent and rare *MEFV* variants and mutations to examine whether and how they impact Pyrin function to promote Pyrin-associated AID.

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**Contributors** HVG, GP, AI, BO, JF, FDB, JD, FH, GC, PP and ML designed the study; HVG, LH, PHVS, TA, JJ and IC performed experiments; HV, MV, GP, AI, BO, JJ, IC, JF, FDB, JD, FH, GC, PP and ML analysed data. HVG and ML wrote the manuscript with input from all authors; GP, AI, BO, PJ, KYV, RJ, VS, AS, JF, FDB, JD, FH, GC and PP provided critical reagents and samples; ML oversaw the project.

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**Competing interests** None declared.

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

**Patient consent for publication** Not required.

**Ethics approval** The research protocol was approved by the local ethics committee of Ghent University Hospital (joint Ghent-Antwerp protocol 2012_593), the local ethical board for human experimentation of Policlinico di Bari (2770-27/2/19), the protocols of Bambino Gesù Children's Hospital Rome (1770/2019), and the ethical committee of UMC Utrecht (protocol 16-349). Approval for the use of 'blood products unsuitable for transfusion' was obtained with the Red Cross (CG20161219B).

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CLINICAL SCIENCE

# Treat-to-target study for improved outcome in polyarticular juvenile idiopathic arthritis

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## ABSTRACT

**Background** Juvenile idiopathic arthritis is one of the most prevalent chronic inflammatory diseases in children. Evidence suggests that early effective treatment minimises the burden of disease during childhood and in further life. We hypothesise that a guided treat-to-target (T2T) approach is superior to routine care in polyarticular juvenile idiopathic arthritis (pJIA) in terms of reaching a clinical remission after 12 months of treatment.

**Methods** Patients with early and active pJIA were enrolled. Targets for treatment were the following: Recognisable Juvenile Arthritis Disease Activity Score (JADAS) improvement after 3 months, acceptable disease at 6 months, minimal disease activity at 9 months and as primary endpoint remission after 12 months. Initially, patients received methotrexate. Failure to meet a defined target required treatment modification at the specified intervals. The choice of biologics was not influenced by the protocol. Finally, T2T patients were compared with a cohort of matched controls of patients with pJIA with unguided therapy documented by BIKER.

**Results** Sixty-three patients were enrolled. Treatment targets after 3/6/9 and 12 months were reached by 73%/75%/77% and 48% of patients. Fifty-four patients completed the protocol. Compared with matched controls, on T2T guidance significantly more patients reached JADAS remission (48% vs 32%; OR 1.96 (1.1–3.7); p=0.033) and JADAS minimal disease activity (JADAS-MDA) (76% vs 59%; OR 2.2 (1.1–4.4); p=0.028). Patients from the T2T cohort received a biologic significantly more frequent (50% vs 9% after 12 months; OR 9.8 (4.6–20.8); p<0.0001).

**Conclusion** The T2T concept was feasible and superior to unguided treatment. High rates of patients reached JADAS-MDA and JADA remission after 12 months. Approximately half of the patients achieved their therapy goals without a biologic.

## Key messages

**What is already known about this subject?**
- A treat-to-target approach is successfully used in the treatment of rheumatoid arthritis.
- In juvenile idiopathic arthritis (JIA), it is known that an early response to treatment is associated with better outcome.

**What does this study add?**
- This study tested the treat-to-target approach for polyarticular JIA in clinical practice and compared it with unguided treatment for polyarticular JIA.
- It could be shown that patients with polyarticular JIA with targeted treatment strategy reached Juvenile Arthritis Disease Activity Score (JADAS) remission and JADAS minimal disease activity, and also more patients received biologics compared with an unguided treatment strategy.

**How might this impact on clinical practice or future developments?**
- A treat-to-target strategy can be easily implemented in routine care of JIA, with benefits for the patients.

## INTRODUCTION

Over the last decades the outcome of patients with polyarticular juvenile idiopathic arthritis (pJIA) has improved significantly due to the availability of more efficacious antirheumatic therapies and improved treatment strategies.[1–5] Guidelines for the treatment of juvenile idiopathic arthritis (JIA) exist in Germany and other countries.[6–8] However, inadequate standardisation and poor penetration of therapies and recommendations in clinical practice may result in late or inadequate treatment. The standard of care in the management of rheumatoid arthritis currently is considered to include early diagnosis with prompt initiation of disease-modifying antirheumatic drugs (DMARDs), tight control monitoring of disease activity, and treatment adjustments aiming at the target of clinical remission or at least low disease activity.[9] Also in patients with JIA early DMARD treatment is associated with better disease control and outcome, such as drug-free remission in early adulthood.[10] An early response to treatment is associated with a better outcome.[11 12] This supports the concept of a 'window of opportunity' for JIA, which suggests that the long-term disease process can be altered by early successful disease control. This can be achieved by setting targets to monitor sufficient treatment response and using a step-up design, if targets are failed (treat-to-target (T2T)).[13] Guided treatment aims at monitoring disease activity at defined intervals with predetermined treatment targets and steps to be followed in case of failure to reach the target.

This open-label intervention study was designed to examine the T2T principle in a routine clinical setting and not to test or compare specific treatments. Thus, standard of care was to be maintained. According to national and international guidelines,



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all patients started with methotrexate (MTX). The choice of the biologic within the approved spectrum was an independent decision of the treating paediatric rheumatologist.

## METHODS

### Patients

In six German centres for paediatric rheumatology, a total number of 63 patients with early pJIA (disease duration <12 months) were recruited for this study. Inclusion criteria were the following: diagnosis of pJIA according to International League of Associations for Rheumatology criteria (seropositive, seronegative pJIA and extended oligoarthritis),[14] active disease with a baseline Juvenile Arthritis Disease Activity Score (JADAS) 10 of greater than 5.4 (inacceptable disease[15]), age 2–16 years and written informed consent of patient and parents/legal guardian to participate in the study (informed consent).

The study was performed in compliance with the Declaration of Helsinki. The study protocol was registered at the German clinical trials register, DRKS (Deutsches Register Klinischer Studien (German registry for clinical trials)), DRKS-ID: DRKS00010764.

As a control cohort, biologic-naive patients from the German biologics in JIA register (BIKER)[16] were selected, who also had a short disease duration of no more than 12 months, had an active disease at therapy start and started with MTX as their first DMARD between 2005 and 2011. These patients were matched to the study patients 3:1, matching criteria were JIA category, baseline JADAS and gender.

**Table 1** Baseline patient characteristics of T2T cohort and matched controls from BIKER

| | T2T screened patients, N=63 | T2T patients completing protocol, N=54* | BIKER matched controls, N=162 | P value † |
|---|---|---|---|---|
| Gender, female, n (%) | 47 (74.6) | 42 (77.8) | 126 (77.8) | 1.0 |
| Age a treatment start, years, mean (SD) | 9.4 (4.8) | 9.1 (4.8) | 8.8 (4.5) | 0.68 |
| Disease duration, years, mean (SD) | 0.5 (0.7) | 0.36 (0.2) | 0.4 (0.22) | 0.24 |
| JIA category | | | | |
| Rheumatoid factor – PA, n (%) | 49 (77.8) | 44 (81.6) | 132 (81.6) | 1.0 |
| Rheumatoid factor + PA, n (%) | 8 (12.7) | 6 (11) | 18 (11) | 1.0 |
| Extended oligo JIA, n (%) | 3 (4.7) | 4 (7.4) | 12 (7.4) | 1.0 |
| Enthesitis-associated arthritis, n (%) | 2 (3.2) | 0 | 0 | |
| Psoriatic arthritis | 1 (1.6) | 0 | 0 | |
| Number of active joints, mean (SD) | 10.0 (7.2) | 9.9 (7.5) | 11.2 (9.7) | 0.37 |
| Physician-assessed disease activity VAS, cm, mean (SD); 0–10 | 5.5 (1.8) | 5.6 (1.8) | 5.9 (2.1) | 0.34 |
| Patient-assessed disease activity VAS, cm, mean (SD); 0–10 | 5.4 (2.4.) | 5.3 (2.2) | 4.5 (2.5) | 0.0625 |
| CHAQ-DI, mean (SD); 0–3 | 0.99 (0.77) | 0.92 (0.77) | 0.81 (0.65) | 0.31 |
| ESR, mm/hour mean (SD) | 25.1 (23.9) | 25.5 (25.0) | 28.3 (20.8) | 0.42 |
| CRP, mg/L, mean (SD) | 16.1 (23.9) | 16.0 (24.5) | 19.4 (28.0) | 0.43 |
| JADAS10, mean (SD); 0–40 | 19.3 (5.0) | 19.2 (5.2) | 19.0 (5.4) | 0.81 |
| Systemic steroids baseline, n (%) | 37 (63) | 33 (61) | 60 (37) | 0.003 |

Matching 1:3 with the following criteria: JIA category, baseline JADAS and gender.
*Only data of patients treated according to study protocol are shown.
†Comparing T2T patients who completed the protocol and matched control, p-values <0.05 were considered significant.
CHAQ-DI, Childhood Health Assessment Questionnaire Disability Index; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; JADAS, Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis; PA, polyarthritis; T2T, treat-to-target; VAS, Visual Analogue Scale.

### Study design

This was an open single-arm multicentre study investigating a T2T strategy.

All patients started MTX at the baseline visit in a dose of 10–15 mg/m$^2$ per week subcutaneously or orally as prescribed by the investigator. Concomitant treatment as non-steroidal anti-inflammatory drugs, bridging with systemic steroid or intra-articular steroids were allowed at the discretion of the treating investigator. The first assessment of treatment effectiveness was scheduled after 12 weeks. The required target was a JADAS improvement defined as a decrease in JADAS10 as validated by Horneff and Becker.[17] If the target was not met, a biologic should be started (online supplementary table 1S). The decision, which biologic was started and whether MTX was continued or not was the responsibility of the treating investigator and made in a shared decision with parents and patients after informing them of the options. Further effectiveness evaluations were scheduled after 24 and 36 weeks. Targets were set more rigorous with treatment duration requiring JADAS acceptable disease activity (ADA), defined as JADAS10 $\leq$5.4 at week 24 and JADAS minimal disease activity (MDA), defined as JADAS10 <3.8, at week 36.[15] If targets were not met, a modification of treatment, meaning either start of a biologic or switching to an alternative biologic was mandatory. Again the choice of treatment remained with the investigator, the only requirement being, that a treatment approved for the diagnosis in the approved dosing was used. The final assessment after 48 weeks determined if the study objective of JADAS remission was met.

### Outcomes

Parent-reported or patient-reported outcomes included a global assessment of disease activity on a 10 cm Visual Analogue Scale (Pat VAS) and the functional status assessed by the Childhood Health Assessment Questionnaire Disability Index (CHAQ-DI; range 0–3). Physician-reported outcomes comprised the number of joints with swelling, range of motion limitations, tenderness or pain with motion, erythrocyte sedimentation rate (ESR) or C reactive protein (CrP) levels, as well as the physician's global assessment of the patient's disease activity (PGA) on a 10 cm VAS. Disease activity was additionally assessed by the JADAS10, calculated as a sum of the number of active joints up to a maximum of 10, the PGA, the Pat VAS and normalised to a 0–10 scale either ESR[18] or CrP[19] with a range from 0 to 40. The JADAS is recommend for the assessment and monitoring of disease activity as well as for the definition of a target to treat to.[19–22]

The primary outcome was percentage of patients reaching JADAS remission, defined as JADAS10 <1 at month 12. The secondary outcome measures were percentage of patients reaching JADAS MDA at months 9 and 12, JADAS ADA at months 6, 9 and 12 and JADAS improvement at months 3, 6, 9 and 12.[15 17]

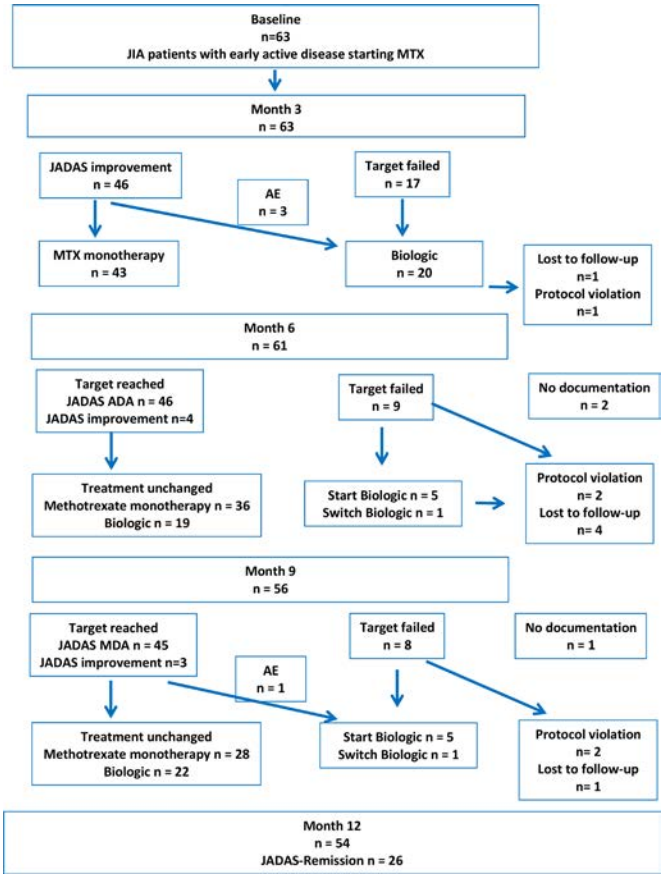


**Figure 1** Patient flow. ADA, acceptable disease activity; AE, adverse event; JADAS, Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis; MDA, minimal disease activity; MTX, methotrexate.

### Statistical analyses

Mean values and SD were calculated for quantitative variables. Demographic and baseline characteristics were summarised by descriptive statistics. Efficacy and safety analyses were performed and the cohort completing the study according to protocol was compared with the matched control cohort. An intention-to-treat analysis was not performed because the assessment of the guided treatment protocol would not have been meaningful, if patients not adhering to protocol were included. Tests were two sided, and p-values $<0.05$ were considered statistically significant. Frequencies were compared using the $\chi^2$ test or Fisher's exact test as appropriate. Data were entered in an Access 2010 database and analysed with Excel 2010 (Microsoft, Redmond, Washington, USA) or IBM SPSS V.23.

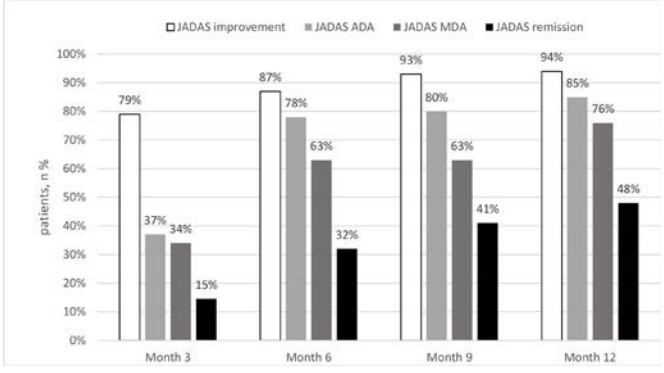


**Figure 2** Response rates at months 3, 6, 9 and 12. ADA, acceptable disease activity; JADAS, Juvenile Arthritis Disease Activity Score; MDA, minimal disease activity.

## RESULTS

Sixty-three patients were enrolled in the current study of whom 54 completely adhered to the protocol who finally were compared with 162 matched control patients selected from BIKER. Baseline patient characteristics of enrolled patients and matched controls from BIKER are shown in table 1.

All patients had highly active disease with a JADAS10 >10 and started MTX treatment at baseline and had the first follow-up documentation at month 3. According to the criteria for JADAS improvement, 46 patients had reached the target for month 3, the remaining 17 did not reach the target and a biologic was introduced (figure 1). A further three patients switched to a biologic because of intolerance of MTX treatment. Biologics used were etanercept (ETA) in 11 patients, tocilizumab (TOC) in 4 patients, adalimumab (ADM) in 3 and golimumab in 2 patients. In all, 43 patients remained on MTX monotherapy.

After 6 months, 61 patients were assessable. By then 46 patients had reached the target of JADAS ADA, 4 patients who had started a biologic at month 3 showed considerable JADAS improvement and 9 patients had failed to reach the target. While 54 patients continued their treatment (36 patients remained on MTX monotherapy and 19 patients continued the treatment they had initiated at month 3), 5 patients newly started a biologic treatment (ETA n=3, ADM n=1, TOC n=1) and one patient switched biologic from ETA to TOC.

At month 9, 56 patients could be evaluated according to protocol. Of the 56 patients, 43 patients reached the required target of JADAS MDA, and a further 4 patients who had started a biologic at month 6 had significant JADAS improvement and 8 patients failed to reach the target. Altogether 49 patients remained on their treatment with 27 on MTX monotherapy. While five patients newly started a biologic (ETA n=3, ADM n=1, TOC n=1), one patient switched from TOC to ETA. Of the patients starting a biologic, one patient had reached the month 9 target, but had to discontinue MTX due to intolerance.

Altogether, nine patients could not be evaluated for the final analysis. Six patients were lost to follow-up. Patients with protocol violation were also not considered for the outcome analysis at month 12 and are described here: In one patient, MTX was discontinued because of AE at month 3, but no biologic was started. Two patients (one was also lost to follow-up) had started a biologic (ETA, ADM) at month 3 and did not show JADAS improvement at month 6, but were not switched to another biologic. Two further patients failed to reach the month 9 target but treatment was not modified accordingly. Of the five patients not following the protocol, one patient reached the target of JADAS remission at month 12, the other four had JADAS scores of 5, 7, 9 and 12, respectively. (figure 2)

### Outcome at month 12

After 1 year of treatment, 54 patients were assessable and had been treated according to protocol. Of these, 27 patients still received MTX monotherapy and 27 patients were on biologics.

The target of JADAS remission was reached by 48% (n=26) of patients, 16 patients with MTX monotherapy (59%) and 10 patients treated with biologics (37%). In all, 76% (n=41) of the patients reached JADAS MDA and 85% (n=46) JADAS ADA.

Of the patients remaining on MTX monotherapy, 23 (85%) reached JADAS MDA and 15 (56%) reached JADAS remission.

### Comparison with patients with unguided treatment

The JADAS outcome parameters at month 12 of the patients treated according to the T2T protocol were compared with patients with early active pJIA documented in the BIKER registry, who were biologic naive and started MTX within the first year of JIA onset. Patients were matched in a ratio of 1:3 using JIA category, gender and baseline-JADAS as criteria. The baseline characteristics of the 162 patients from BIKER are shown in table 1. Apart from higher concomitant systemic steroid use in the T2T cohort, there were no significant differences. The proportion of patients receiving intra-articular steroids at baseline was numerically but not significantly lower in the T2T cohort (n=12 (22%) vs n=59 (36%) in the control cohort (p=0.07)). Patients from BIKER had slightly more active joints at baseline, while the patients of the T2T cohort were slightly older and had a slightly higher CHAQ-DI at treatment start (table 1).

After 12 months of treatment, significantly more patients from the T2T cohort compared with the BIKER cohort (JADAS remission: n=52; 32%, JADAS MDA: n=96; 59%) had reached JADAS remission (OR 1.96; 95% CI: 1.05 to 3.68; p=0.033) and JADAS MDA (OR 2.2, 95% CI: 1.08 to 4.36; p=0.028) compared with patients from the T2T cohort. The proportion of patients reaching JADAS ADA in the BIKER cohort (n=119; 73%) was not significantly lower than that in the T2T cohort (p=0.068) (figure 3).

Compared with 9% of patients in the BIKER cohort, a significantly higher ratio (50%) of patients in the T2T cohort received biologic treatment at month 12 (OR 9.8; 95% CI: 4.6 to 20.8; p<0.0001). Also, fewer patients in the T2T cohort did receive systemic steroids after 12 months (5.6% vs 21.6%, p=0.007).

### Safety

Altogether, 88 adverse events (AEs) were reported in 51 patients, of which 3 were serious AEs (SAEs) in 3 patients. In detail, the SAEs were norovirus gastroenteritis in a patient treated with TOC and MTX, Perthes disease and severe anaemia in MTX-treated patients. Most common AEs were infectious events (n=26), mainly of the upper airways (n=12), bronchitis (n=2) and gastroenteritis (n=3). MTX-related gastrointestinal symptoms (n=20) and elevation of liver enzymes (n=10) were also frequent events. AEs for both cohorts according to treatment are shown in table 2.

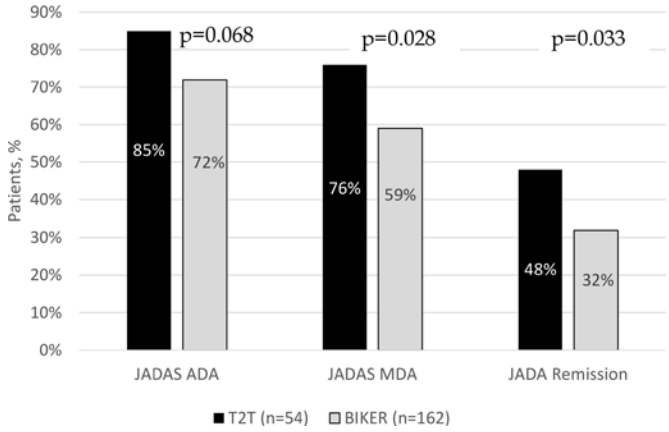


**Figure 3** JADAS ADA, MDA and remission rates after 12 months of treatment compared between T2T study cohort and matched controls. ADA, acceptable disease activity; JADAS, Juvenile Arthritis Disease Activity Score; MDA, minimal disease activity; T2T, treat-to-target.

**Table 2** Safety

| n/number of pts (% of pts) | T2T cohort MTX only, n=63 | T2T cohort biologic exposed, n=27 | BIKER Control cohort MTX only, n=162 | BIKER Control cohort biologic exposed, n=15 |
|---|---|---|---|---|
| AE | 69/45 (71) | 19/14 (52) | 104/61 (38) | 14/7 (47) |
| Serious AE | 2/2 (3.2) | 1/1 (3.7) | 1/1 (0.6) | 0 |
| Infectious AE | 18/17 (27) | 9/9 (33) | 36/22 (14) | 2/2 (13) |
| Uveitis | 2/2 (3.2) | 0 | 3/3 (1.9) | 0 |
| Gastrointestinal AE | 21/20 (32) | 4/4 (15) | 38/30 (18.5) | 2/2 (13) |
| Transaminases elevated | 11/10 (16) | 1/1 (3.7) | 12/12 (7.4) | 0 |

AEs are according to treatment and cohort. Gastrointestinal events were nausea, vomiting and abdominal pain.

## DISCUSSION

The ongoing development of effective treatment options for pJIA has led to a situation where remission of disease or at least MDA can be reached in a high percentage of patients. Also the concept of a window of opportunity[10 11] suggests that early treatment of pJIA alters the disease course. Hence, the aim of any treatment for pJIA should be early reduction of disease activity. To reach this goal in clinical practice, a guided standardised T2T concept seems a promising approach. A Dutch randomised single-blinded study with a T2T design in three different treatment arms (sequential DMARD monotherapy (sulfasalazine or MTX), combination therapy MTX + prednisolone or combination therapy MTX + ETA) with a step-up option within the treatment arm also showed promising response rates after 1 year with about 47%–62% of patients reaching inactive disease regardless of initial treatments.[23]

This T2T study showed that patients benefit from a tightly controlled T2T strategy. Significantly more patients reached MDA or remission in comparison to the control group. Interestingly, significantly more patients were treated with biologics to reach the target of JADAS remission/MDA. Although in the T2T cohort more patients initially received systemic steroids, steroid use was significantly lower in the T2T cohort after 12 months compared with the control cohort, further supporting this concept.

Another approach is an early aggressive treatment as tested in the multicentre, prospective, double blind, randomised, placebo-controlled TREAT trial, where patients after diagnosis of pJIA were either treated with ETA + MTX + oral steroids or with MTX monotherapy including a step-up option in case of insufficient response. MTX was given at a comparably high dosage of 0.5 mg/kg/week subcutaneously in both arms. While there was a trend toward a higher rate of patients in the combination therapy arm reaching the primary endpoint of clinical inactive disease at month 12 of induction, the difference was not statistically significant. In the extension of the TREAT, patients were treated as per provider's discretion. In this cohort, prolonged periods of clinically inactive disease could be observed in the majority of patients during follow-up regardless of the initial treatment arm with more than 50% of patients receiving biologics.[24 25] When looking at the data of our T2T study, it is remarkable that over half of the T2T patient cohort reached JADAS MDA on MTX monotherapy. This observation justifies the step-up regimen used here since biologics were not necessary to reach the target in every case. It seems important to start treatment early in the disease course, irrespective whether using initially a step-up

design or an aggressive therapy. With the step-up approach, overtreatment might be avoided.

It would be very interesting to be able to distinguish between patients showing a sustainable good response to MTX and patients needing biologics early in the disease course. It remains to be shown, if the patients who do not show a sufficient or sustained response to MTX might benefit from initial treatment with biologics.

The validated JADAS score for measuring disease activity was chosen, because it is an easy, time-efficient and flexible method to guide therapeutic interventions aimed to pursue tight disease control. Different validated levels of disease activity, i.e. for improvement of JADAS, ADA, MDA and remission are available, which are useful to gradually tighten the treatment goals.[15 17]The CARRA (Childhood Arthritis and Rheumatology Research Alliance) protocols[8] use the physician global assessment, ability to taper/discontinue steroids as well as a not clearly defined 'patient much improved' statement as criteria for treatment success. The recently published American College of Rheumatology guidelines[26] and the recently revised German consensus-based treatment guidelines for JIA [27] both recommend the JADAS10 to assess disease activity.

The T2T strategy used in this study has been shown to be applicable in clinical routine care. Such a standardised approach to treatment is transparent and easy to implement in clinical routine practice. The treating physician/paediatric rheumatologist is not influenced in the choice of the approved biologics and differences in known safety profiles as well as approval status and application can and should be taken into account. For a successful treatment, an early diagnosis and referral to a paediatric rheumatologist is of great importance.

Limitations of this study are the non-controlled and non-blinded approach. The comparison with a more or less historic cohort may pose a bias, in as far as physicians at present might be more generous in using biologics than in the past.

Also this analysis ended after the initial 12 months, long-term data regarding rates of patients remaining in remission and rates of patients who could successfully discontinue treatment are not available. Also the question of tapering or discontinuing JIA treatment in case of remission is not addressed by this study. Larger controlled studies are needed to address these issues.

## CONCLUSION

A guided T2T strategy with early escalation of therapy was superior to unguided treatment in pJIA. Significantly more patients achieved JADAS MDA and JADAS remission after 12 months of treatment. Approximately half of the patients achieved their therapy goals without the use of a biologic. This approach is feasible and easy to implement in routine clinical care.

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TRANSLATIONAL SCIENCE

# BCP crystals promote chondrocyte hypertrophic differentiation in OA cartilage by sequestering Wnt3a

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## ABSTRACT

**Objective** Calcification of cartilage with basic calcium phosphate (BCP) crystals is a common phenomenon during osteoarthritis (OA). It is directly linked to the severity of the disease and known to be associated to hypertrophic differentiation of chondrocytes. One morphogen regulating hypertrophic chondrocyte differentiation is Wnt3a.

**Methods** Calcification and sulfation of extracellular matrix of the cartilage was analysed over a time course from 6 to 22 weeks in mice and different OA grades of human cartilage. Wnt3a and ß-catenin was stained in human and murine cartilage. Expression of sulfation modulating enzymes (HS2St1, HS6St1) was analysed using quantitative reverse transcription PCR (RT-PCR). The influence of BCP crystals on the chondrocyte phenotype was investigated using quantitative RT-PCR for the marker genes Axin2, Sox9, Col2, MMP13, ColX and Aggrecan. Using western blot for β-catenin and pLRP6 we investigated the activation of Wnt signalling. The binding capacity of BCP for Wnt3a was analysed using immunohistochemical staining and western blot.

**Results** Here, we report that pericellular matrix sulfation is increased in human and murine OA. Wnt3a co-localised with heparan sulfate proteoglycans in the pericellular matrix of chondrocytes in OA cartilage, in which canonical Wnt signalling was activated. In vitro, BCP crystals physically bound to Wnt3a. Interestingly, BCP crystals were sufficient to induce canonical Wnt signalling as assessed by phosphorylation of LRP6 and stabilisation of β-catenin, and to induce a hypertrophic shift of the chondrocyte phenotype.

**Conclusion** Consequently, our data identify BCP crystals as a concentrating factor for Wnt3a in the pericellular matrix and an inducer of chondrocyte hypertrophy.

## Key messages

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

► It is known that basic calcium phosphate (BCP) based calcification of cartilage is associated with osteoarthritis (OA) severity and is a result from hypertrophic differentiation of chondrocytes. However, it is unknown whether the calcification is an epiphenomenon due to the hypertrophic differentiation or has itself an impact on the differentiation process.

**What does this study add?**

► This study shows how canonical Wnt signalling is influenced by BCP crystals and describes how OA cartilage gets primed to react to canonical Wnt signals.

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

► The interference with canonical Wnt signalling as a therapeutic option is currently tested. This study helps to understand the various levels of Wnt signalling influence in OA cartilage. The interference with mineralisation or sulfation might also be a potential target for OA treatment.

## INTRODUCTION

Osteoarthritis (OA) is a progressive joint disease, which is associated with severe pain and impairment of movement. OA comprises aspects of a degenerative disease including progressive structural changes in joint tissues, especially in the articular cartilage, which is associated with cartilage fibrillation and erosions, accompanied by chondrocyte hypertrophic differentiation and changes in the extracellular matrix (ECM) composition. OA-induced changes in the cartilage partially resemble endochondral ossification during embryonic development.[1]

One prominent family of morphogens that regulate chondrocyte differentiation during endochondral ossification as well as in OA cartilage are the Wnts.[2] Several studies have shown that canonical Wnt signalling plays an important role in regulating chondrocyte differentiation during OA.[2–7] Wnts are known to bind to and be stabilised by heparan sulfate (HS) proteoglycans (PG) in the ECM of cartilage.[8] Modifications of the glycosaminoglycan (GAG) chains of HSPGs influence signal transduction by modulating the binding capacity of these morphogens.[9–11] Various enzymes are involved in the maturation of HS-GAG chains, including multiple glycosyltransferases, sulfotransferases and an epimerase. Interestingly, the sulfation of HS-GAG chains has been shown to regulate the activation of canonical Wnt signalling. Thus, 6-O-sulfation of HS-GAG chains has been found to be necessary for the activation of canonical Wnt signalling,[12 13] whereas 2-O-sulfation of HS-side chains seems to deactivate canonical Wnt signalling.[14–16] There is increasing evidence of the importance of HSPG in

the regulation of cartilage degeneration during OA as various knockout mice (eg, syndecan-4 and decorin) are protected from OA induction.[17–19] Furthermore, an increase or alterations in ECM sulfation have been linked to cartilage protective effects in OA.[18 20–22] These data indicate that the composition of ECM strongly influences the chondrocyte phenotype in OA cartilage.

Another important change in cartilage during OA, which is directly associated with the hypertrophic differentiation of chondrocytes, is the mineralisation of the pericellular matrix.[23 24] Basiccalcium phosphate (BCP) based cartilage matrix mineralisation has been shown to be part of the pathogenic changes associated with the terminal, hypertrophic differentiation of diseased chondrocytes in OA.[23–26] In healthy articular cartilage, most chondrocytes maintain a stable resting phenotype and do not proliferate. In contrast, in osteoarthritis chondrocytes undergo ectopic hypertrophic differentiation, typically close to areas of mineralised cartilage matrix and near sites of surface lesions.[27 28] This hypertrophic differentiation can be monitored by the change in the expression profile from high levels of Col2a1 and Aggrecan in healthy chondrocytes, to production of hypertrophic marker genes such as MMP13 and Col10a1.

It remains unclear, however, whether BCP crystals themselves contribute to the shift of the chondrocyte phenotype towards hypertrophy or are a by-product of hypertrophic differentiation. The effects of BCP crystals on chondrocytes have been linked mostly to inflammatory pathways activating the inflammasome.[29–31] Here, we hypothesised that BCP crystals actively contribute to the loss of the chondrocyte phenotype by directly interfering with canonical Wnt signalling in OA cartilage.

## METHODS

### Chondrocyte culture

C28/I2 chondrocytes (SCC043, Merck-Millipore, Darmstadt, Germany) were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 1 mM sodium pyruvate and penicillin (10 000 U/mL)/streptomycin (10 mg/mL).

### Isolation and cultivation of murine primary chondrocytes

Murine chondrocytes of articular knee cartilage were isolated from 5-day to 8-day old mice. Cartilage was digested overnight at 37°C using 1 mg/mL collagenase (Worthington) diluted in the chondrocyte culture medium composed of DMEM containing 10% FBS, 1 mM sodium pyruvate and penicillin (10 000 U/mL)/streptomycin (10 mg/mL) at 37°C in a humidified atmosphere containing 5% carbon dioxide. After filtration of the suspension, chondrocytes were washed and resuspended in culture medium. All experiments were performed using freshly isolated, (sub-) confluent P0 chondrocytes.

### Micro pellet cultures

Wild-type murine chondrocytes amounting to $0.5\times10^5$ were mixed with 0.05 or 0.1 ng/mL BCP crystals and centrifuged at 400 g for 10 min in a 15 mL Falcon cup. The micro pellets were cultured for 2 days in the Falcon tube. The next day the pellets were moved to a 96 well plate for 24 hours. After that they were fixated with 4% formaldehyde and embedded in paraffin. Pellets were cut in 5 µm sections for immunohistological staining.

### Human cartilage samples

Human OA articular cartilage was obtained from patients undergoing joint replacement for knee OA after obtained consent. Healthy cartilage samples were taken from the Department of Forensic Medicine during autopsies of young patients without macroscopic signs of OA or joint trauma. OA patients and healthy controls are not age matched. Full thickness samples were dissected from OA areas of articular cartilage of OA patients.

### Murine samples

The NPP1-mutant ttw/ttw (tiptoe walking) mouse has been described by Okawa *et al* (1998). Destabilisation of the medial meniscus (DMM)-based induction of OA in wild-type (wt) mice was performed as described by Glasson *et al*.[32] Joints of wild type and ttw/ttw mice were harvested at the age of 8, 15 and 22 weeks and frontal sections were taken through the entire joint.

### Sample preparation and histological staining

Hind legs were dissected and fixed in freshly prepared 4% paraformaldehyde in phosphate buffered saline (PBS, pH 7.4, 137 mM NaCl, 2.7 mM KCl, 1.4 mM Na2HPO4, 1.4 mM KH2PO4) at 4°C for 24 hours. Subsequently, the bones were embedded in Technovit for Safranin-O/von Kossa staining or decalcified in 10% EDTA (Sigma, Taufkirchen, Germany), pH 7.4, dehydrated through a graded series of ethanol solutions and embedded in paraffin. Sections (5 µm) were cut through the long axis of each tibia in a frontal plane, were deparaffinised and histochemically stained. Osteoarthritic changes were evaluated by staining with Safranin-Orange. To examine sulfated glycoproteins, the samples were first kept for 3 min in 0.1 M hydrochloric acid (HCl) pH 1 and then stained with 1% Alcian blue 8 GX (Sigma Taufkirchen, Germany) in 0.1 M HCl, pH 1.0 for 30 min at room temperature (RT). Cartilage scoring was performed as described using the OARSI Score (Pritzker *et al*, 2006). Von Kossa stainings of knee sections and human cartilage samples were performed to assess the calcification.

### RNA extraction, cDNA synthesis, real-time RT-PCR

Total RNA was extracted from cells and cells or cartilage explants using TRIzol reagent (Invitrogen). Total RNA of 1 ng from each sample was reverse transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) using oligo dT primers. Quantitative PCR was performed with SYBR Green I (SG) asymmetrical cyanine dye (SYBR) green using Applied Biosystems PRISM 7900HT (Thermo Scientific). Primer sequences are listed in online supplementary tables 1 and 2. Absolute quantification was carried out using standard curves. Target gene expression was normalised to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

### Imunofluorescence stainings

Paraffin sections from human and murine OA cartilage sections were rehydrated. For antigen retrieval for the primary antibody against β-catenin (1:70, Cell Signaling #9562) or Wnt3a (1:100, Abcam #ab28472). Sections were pre-treated with 0.02% HCl and then incubated with pepsin (0.25 mg/mL in 0.02% HCl) for 45 min at 37°C. For Aggrecan (1:200, Abcam #ab36861), Col2A1 (H-300) (1:300, Santa Cruz #sc-28887), ColX (1:300, Abcam #ab58632) antigen retrieval was performed with trypsin. For MMP13 (1:300, Abcam #ab39012) antigen retrieval was performed with citrate buffer at pH6. Free epitopes were blocked with 4% bovine serum albumin (BSA) in PBS for 1 hour at RT. Cartilage sections were stained Alexa Fluor 555 (Thermo Scientific) was applied as secondary antibody. Sections were fixed with Roti-Mount FluorCare DAPI. For heparatinase treatment paraffin sections were pre-incubated with 0.5 mU heparinase (Amsbio 100703) per section 100 µl in 25 mM Tris (pH 8.0)

in 50 mM sodium acetate and 1 mM calcium chloride ($CaCl_2$) for 2 hours at 37°C. Control IgG stainings were performed for each antibody staining and served as an internal control for antibody specificity.

### BCP crystal preparation

Sterile, pyrogen-free BCP crystals were synthesised as previously described.[33] The nature of BCP crystals was checked before and after sterilisation by X-ray diffraction and infrared spectroscopy. X-ray diffraction patterns were recorded with Co-Kalpha (λ=1.78892 Å) using Inel CPS 120 diffractometer operating at 45 kV and 28 mA. Infrared spectra were obtained over the 4000 to 400 cm-1 range, using Nicolet FT-IR 5700 spectrometer with KBr pellet. Hydroxyapatite (HA) and octacalciumphosphate (OCP) crystal sizes and Ca/(p+CO3) ratios were determined as previously and were for HA 1.1±0.3 µm and 1.56 and for OCP crystals 1.5±0.5 and 1.33, respectively. Crystals were suspended in sterile PBS and dispersed by brief sonication. All crystals were determined to be endotoxin free (<0.01 EU/10 mg) by Limulus amebocyte cell lysate assay.

### Protein binding to crystals

BCP crystals of 0.2 to 2 mg were incubated with 100 ng/mL recombinant Wnt3a (R&D) for 1 hour at RT. BCP crystals of 1 mg was incubated with 0, 50 or 100 ng/mL Wnt3a (R&D) or GFP (R&D). The crystals were washed twice with PBS. SDS-PAGE loading buffer was mixed with crystals and subsequently loaded on a SDS-PAGE for western blotting.

### SDS-PAGE and western blotting

After treatment, cells were washed once in ice-cold PBS containing phosphatase and protease inhibitors (Roche). Total cell extracts were obtained by scraping the cells in extraction buffer (10 mM Hepes, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM DTT, and 0.05% NP-40, pH 7.9) and leaving the lysates on ice for 30 min. The protein extracts were run on an SDS-PAGE and transferred to a nitrocellulose membrane (GE Healthcare). Following primary antibodies were used: β-catenin (1:1000; Cell Signaling Technology), p-LRP6 (1:1000 Cell Signaling #2568), LRP6 (1:1000 Cell Signaling #3395), GAPDH (1:1000 Cell Signaling #2118). Paraffin sections were incubated with 0.5 mU heparitinase (amsbio 100703) per section 100 µl in 25 mM Tris (pH 8.0) in 50 mM sodium acetate and 1 mM CaCl2 for 2 hours at 37°C.

### Statistics

All data were presented as mean±SEM. Data comparing two groups were anaylsed by a t-test for statistical significance. Data with more than two groups were analysed by a one-way analysis of variance (ANOVA) following a Dunnett's test as post hoc test in case of a statistically significant ANOVA result. Data analyses were performed using GraphPad Prism V.6.00 for Windows (GraphPad Software, La Jolla, California, USA, www.graphpad.com). Statistical significance was determined at level of $p \leq 0.05$.

## RESULTS

### Increased activation of canonical Wnt signalling in calcified OA cartilage is accompanied by an increased 6-O-sulfation of ECM

As healthy cartilage is not calcified, we used Safranin-Orange/von Kossa staining of human cartilage samples to score OA severity and evaluate the amount of calcification in the cartilage. We found a 25-fold increase in calcification around chondrocytes from healthy to OARSI 1 to 3 and 2.9-fold increase from OARSI 1 to 3 to OARSI 4 to 5 (figure 1A). In healthy cartilage, the calcification was barely present in the deep cartilage layer, but was increasingly found with rising OA severity. At the same time, an increase in pericellular ECM sulfation was observed with increasing OA severity. We observed an about twofold increase from healthy to OARSI 1 to 3 and again to OARSI 4 to 5. (figure 1B) Chondrocytes undergo hypertrophic differentiation during OA progression. Wnt3a is known to induce hypertrophic differentiation in chondrocytes. Wnt3a was detected in histological sections using immunohistological straining. We found Wnt3a to be significantly upregulated within the pericellular matrix in osteoarthritic cartilage proportionally with OA severity (figure 1C). The intracellular effector of canonical Wnt signalling as well as the downstream target of canonical Wnt signalling is β-catenin (figure 1D). We stained human cartilage sections with an antibody against β-catenin. β-catenin was also detected at higher levels in chondrocytes proportionally with OA grade, suggesting activation of canonical Wnt signalling with increasing OA severity.

As it is known that the posttranslational modification of HS side chains of proteoglycans can be modified during OA and that Wnt3a binds preferentially to HS side chains, we treated human cartilage sections with heparitinase before staining again for Wnt3a (figure 1E). Following the heparitinase treatment we found a twofold reduction of Wnt3a staining from the pericellular matrix of chondrocytes. This finding indicates that at least part of Wnt3a was bound to the HS side chains of proteoglycans.

To investigate the source of the increased sulfation in the pericellular matrix, we analysed the messenger RNA expression of HS 2-O and 6-O sulfotransferases in OA cartilage samples (figure 1F). We observed no change in HS2ST1 expression with increasing OA severity. Interestingly, there was a threefold increase in the HS6ST1 expression in OA samples with OARSI grades 1 to 2 compared with healthy samples and a sixfold increase in OARSI 3 to 4 samples compared with the healthy controls. However, no difference in the expression of HS6ST1 was seen in end-stage OA cartilage with an OARSI score of 5 to 6 as compared with normal. This finding may suggest an increase in 6-O HS-sulfation during early and medium stages of OA, promoting the activation of canonical Wnt signalling but not in end-stage disease.

### Cartilage calcification in NPP1 null mice results in increased ECM calcification and activation of canonical Wnt signalling

The Npp1-mutant ttw/ttw mouse has been described by Okawa *et al*.[34] The loss of function mutation in the NPP1 gene induces postnatal development of progressive intervertebral ankylosis, peripheral joint hyperostosis, arterial and articular cartilage calcification and increased vertebral cortical bone formation in these mice.[34] Radiographic analyses of ttw/ttw knee joints in comparison to wild type knee joints in a time course from 8 to 22 weeks revealed typical OA-like bone changes that progressed with increasing age of the mice. We found osteophyte formation, exostosis and changes in the subchondral bone in 22-week-old ttw/ttw mice. Furthermore, ttw/ttw mice showed a narrowed joint space at week 15 and week 22. These morphological changes were not visible in X-rays of wild type knee joints (figure 2A). Safranin-Orange/von Kossa stainings showed already at 8 weeks the breakdown of the tidemark in ttw/ttw mice, with increased calcification of the deep cartilage layer and superficial cartilage layer at the late time point. This calcification around the chondrocytes was not observed in the wt control knees (figure 2A).

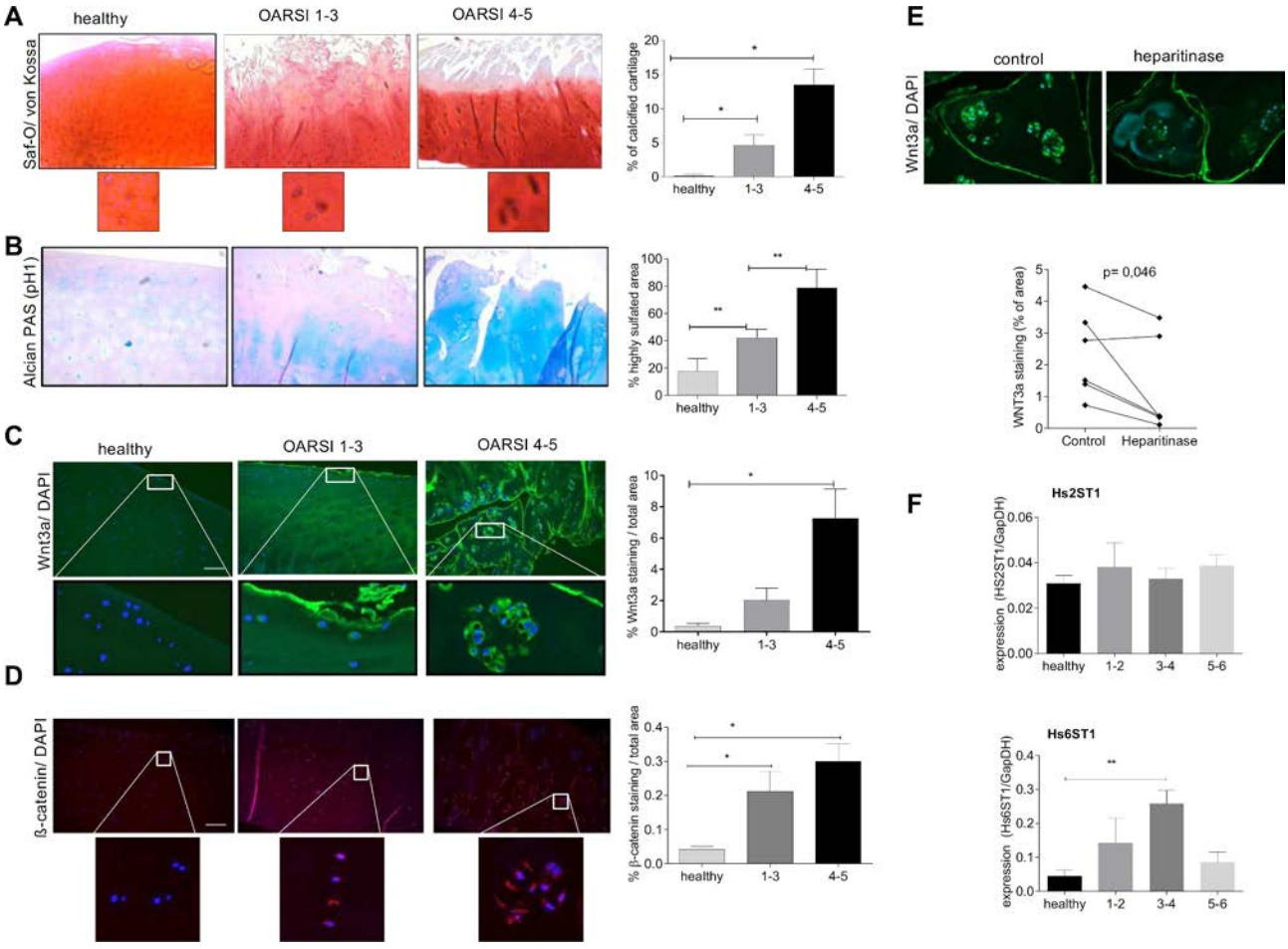


**Figure 1** Increased activation of canonical Wnt signalling in calcified OA cartilage is accompanied by an increased 6-O-sulfation of ECM: (A) Representative images of safranin-O/von Kossa staining in human cartilage samples of increasing OA grade, with higher magnification pictures from representative calcified areas. The quantification of calcification from the whole section is shown in the graph (healthy vs OARSI 1 to 3 95% CI: −10.86 to 2.009 and healthy vs OARSI 4 to 5 95% CI: −19.45 to −7.130 (p=0.0002), one-way ANOVA p=0.0003, F (2, 13)=15.78). (B) Representative alcian/PAS stainings pH 1 of human OA cartilage with increasing OA grade. The quantification of blue staining is shown in the graph (healthy vs OARSI 1 to 3 95% CI: −45.00 to −3.666 (p=0025) and healthy vs OARSI 4 to 5: 95% CI: −83.13 to −38.94 (p=0.0003), one-way ANOVA (p=0.0004) F (2, 7)=29.20). (C) Representative Wnt3a staining of human cartilage (Wnt3a: green, DAPI: blue). Quantification of green staining is shown in the graph (healthy vs OARSI 1 to 3: 95% CI: −0.3310 to −0.01168 (p=0.0345) and healthy vs OARSI 4 to 5 95% CI: −0.4099 to −0.1055 (p=0.002), one-way ANOVA (p=0.002) F (2, 26)=7.944). (D) β-catenin staining of human cartilage (β-catenin: red, DAPI: blue). Quantification of red staining is shown in the graph (healthy vs OARSI 1 to 3 95% CI: −5.773 to 2.492 and healthy vs OARSI 4 to 5 95% CI: −11.13 to −2.618 (p=0.001), one-way ANOVA (p=0.003) F (2, 18)=8.474). (E) Wnt3a immunostaining can be removed from sections using heparitinase (Wnt3a: green, DAPI: blue, control=2.36%±0.57% and heparitinase treated=1.27%± 0.61%, paired t-test: p=0.046, n=6)). (F) No change in HS2ST1 expression with increasing OA grade (n=18 patients) (one way ANOVA not significant). 6-O-sulfation promoting enzyme HS6ST1 is increased with low and medium grade OA samples (healthy vs OARSI 1 to 2 95% CI: −0.2667 to −0.06968, healthy vs OARSI 3 to 4 95% CI: −0.3816 to −0.04521 (p=0.0130) and healthy vs OARSI 5 to 6 95% CI: −0.1934 to 0.1137, one-way ANOVA (p=0.02) F (3, 14)=4.361). ANOVA, analysis of variance; ECM,extracellular matrix;GAPDH, glyceraldehyde-3-phosphate dehydrogenase;OA, osteoarthritis.

We found increased sulfation of proteoglyacans using alcian blue/periodic acid–schiff (PAS) staining at pH 1 in the pericellular matrix of the chondrocytes already at 8 weeks in ttw/ttw cartilage, which increased during ageing and OA progression (figure 2B).

To investigate whether canonical Wnt signalling was activated in the cartilage of ttw/ttw mice, we stained for Wnt3a and β-catenin in 8-week-old ttw/ttw cartilage and age-matched wt controls (figure 2C). We found significantly more Wnt3a and β-catenin staining in ttw/ttw cartilage compared with wt controls. The quantification indicates twice as much Wnt3a bound in the pericellular ECM of ttw/ttw cartilage compared with wt for a sixfold increase in β-catenin staining was observed. Alizarin red staining revealed that chondrocyte micro-masses from ttw/ttw mice are more calcified than those from control mice, thereby suggesting that cartilage calcification in ttw/ttw mice is cell-autonomous (figure 2D: wt=12.20±0.29, ttw/ttw=19.95±2.9, n=4, p=0.029).

### Wnt3a binds to BCP crystals thereby increasing the bioavailability for the ligand

As we found BCP crystals and activation of canonical Wnt signalling in murine and human OA cartilage in the same place, we asked the question, whether BCP crystals themselves were able to activate the signalling cascade. We incubated C28/I2 human costal chondrocyte cell line with 0.1 ng/mL BCP crystals and investigated the phosphorylation of LRP6 after 15 and 30 min. We observed an increase in phosphorylation on BCP

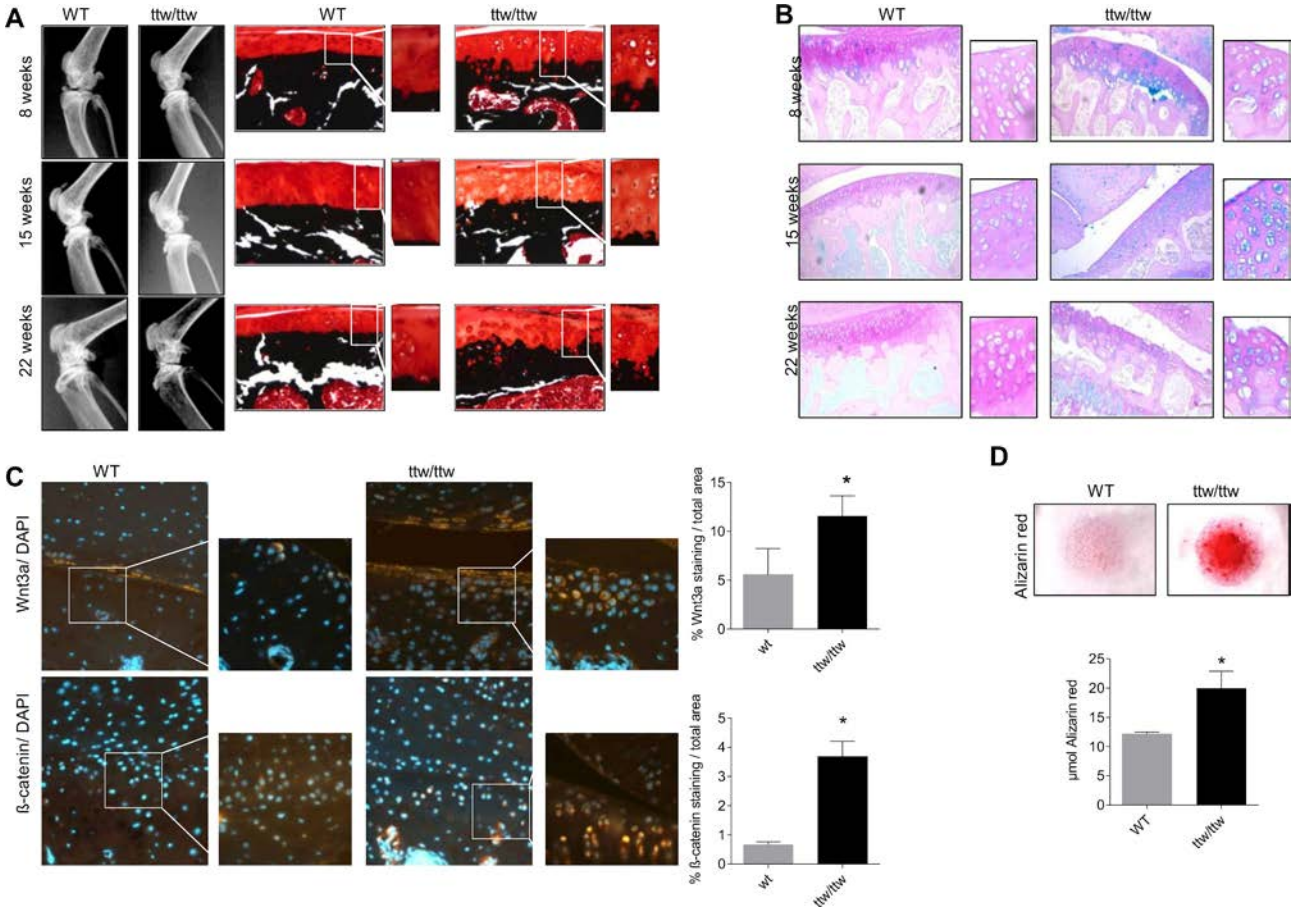


**Figure 2** Canonical Wnt signalling is induced in a calcifying murine model of OA and also accompanied by increased sulfation of ECM: (A) Representative lateral X-Ray of Technovit blocks of wt and ttw/ttw knee joints at different time points. Safranin-Orange/von Kossa staining shows a breakdown of the tidemark and an increase in chondrocyte hypertrophy. (B) Representative alcian/PAS staining pH 1 of wt and ttw/ttw knee joint sections. (C) Representative Wnt3a and β-catenin stainings of murine cartilage of 8-week-old ttw/ttw and wt mice. (Wnt3a: red, DAPI: blue) Quantification of Wnt3a and β-catenin in percentage of total cartilage area is depicted in the corresponding graphs on the right hand side . (Wnt3a: wt=5.55%±2.67% and ttw/ttw=11.58%± 2.04%, p=0.03, n≥9) and β –catenin (wt=0.66%±0.10% and ttw/ttw=3.68%± 0.52%, p<0.0001, n≥9). (D) Alizarin red staining of wt and ttw/ttw micromass cultures. ECM, extracellularmatrix; OA, osteoarthritis; ttw, tiptow walking; wt, wilt type.

stimulation, suggesting an activation of canonical Wnt signalling (figure 3A). In a next step we analysed the stabilisation of β-catenin as readout for canonical Wnt signalling activation. We used two doses of (0.05 and 0.1 ng/mL) BCP crystals, as well as 100 ng/mL Wnt3a as a positive control. We detected a dose-dependent increase in β-catenin, which was less pronounced, as with 100 ng/mL Wnt3a (figure 3B). To analyse whether ttw/ttw chondrocytes, which produce excessive amounts of BCP crystals, exhibit cell-autonomous canonical Wnt activation, we investigated the amount of β-catenin after 24 hours of cultivation. We observed an increase in β-catenin in ttw/ttw chondrocytes compared with wt (figure 3C). To investigate whether the Wnt3a binds directly to BCP crystals, we incubated BCP crystals either with FCS or with FCS and Wnt3a and subsequently stained for Wnt3a. We observed an increased Wnt3a staining on the BCP crystals after in fluorescence microscopy (figure 3D). To further validate this finding, we incubated increasing amounts of BCP crystals with the same concentration of Wnt3a (100 ng). We observed an increase in Wnt3a protein on the western blot with increasing amount of BCP crystals, indicating a direct interaction between the crystals and Wnt3a. To further evaluate the specificity of Wnt3a binding to the BCP crystals we used a fixed dose of BCP crystals with an increasing amount of Wnt3a. Again, we were able to detect Wnt3a at 100 ng/mL. Next, we also incubated BCP crystals with GFP and found no binding to the BCP crystals using recombinant GFP and a native SDS gel (figure 3D). To test the hypothesis that the BCP crystals associate Wnt3a at their surface and thereby increase the local bioavailability of the ligand for the chondrocytes, we used a low concentration of Wnt3a (10 ng/mL) and BCP crystals alone in BSA containing medium. We used BSA to exclude any interfering Wnt-effects from the FCS. BCP crystals alone under serum-free conditions did not increase LRP6 phosphorylation. Wnt3a of 10 ng/mL increased LRP6 phosphorylation after 30 min. The combination of BCP and low-dose Wnt3a however, induced a marked phosphorylation of LRP6. This finding indicates an amplifying effect of BCP crystals on Wnt3a signalling (figure 3E: p=0.01).

### BCP crystals induce hypertrophic differentiation of chondrocytes

To investigate the effect of BCP crystals on the phenotype of chondrocytes we performed a quantitative reverse transcription PCR for chondrocyte marker genes, as well as stainings of micro pellet cultures using wild-type murine chondrocytes for the respective marker gene. As a marker for canonical Wnt signalling activation, Axin2 expression was about 20-fold increased with both doses of BCP crystals. The representative stainings of micro

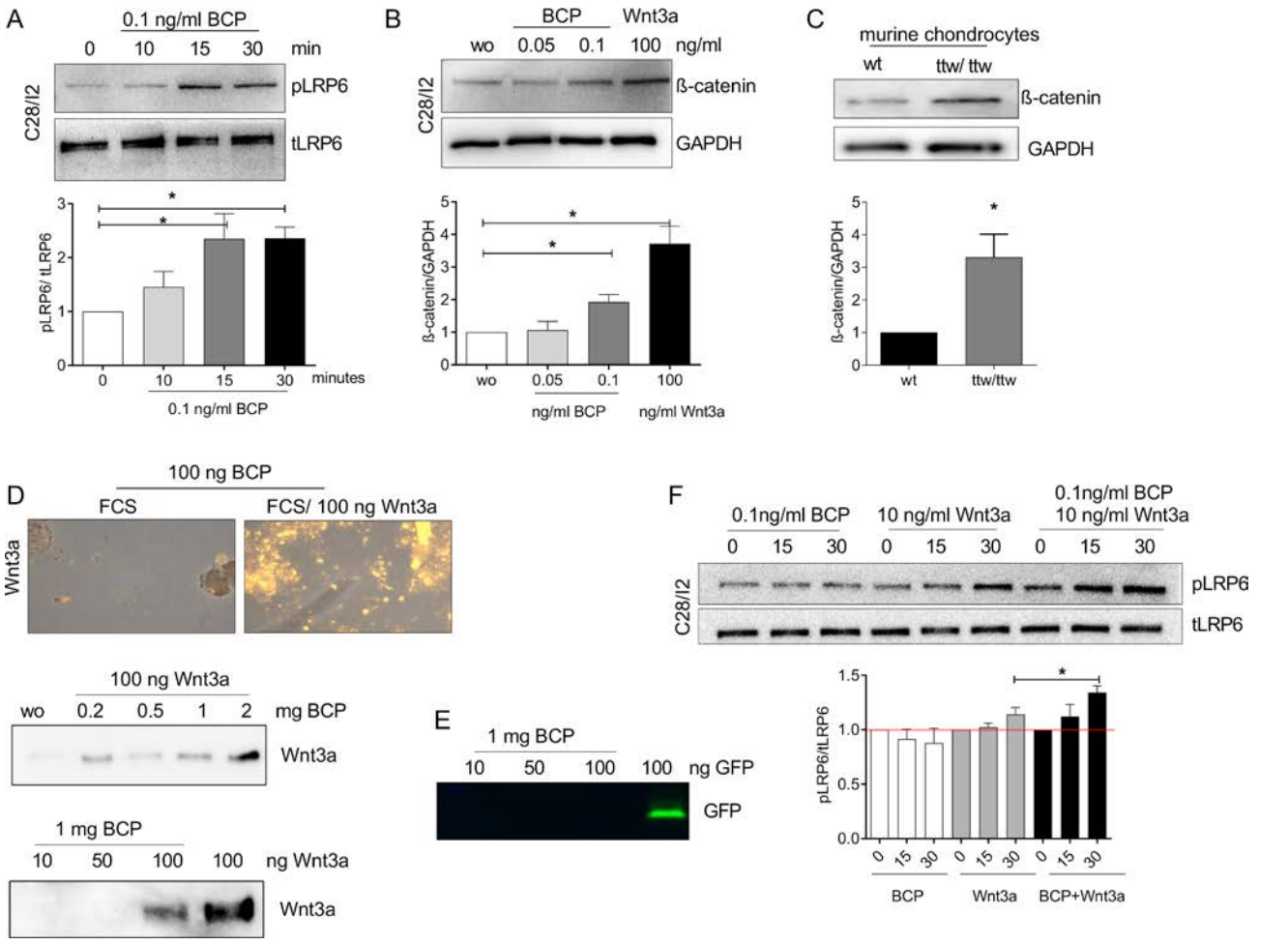


**Figure 3** Wnt3a binds to BCP crystals thereby increasing the bioavailability for the ligand: (A) Representative western blot for LRP6 phosphorylation over a time course of 30 min after stimulation with 0.1 ng/mL BCP crystals. Total LRP6 served as loading control. Quantification of LRP6 western blots is given in the graph (0 vs 10 min 95% CI: −1.566 to 0.6514, 0 vs 15 min 95% CI: −2.458 to −0.2400 (p=0.017) and 0 vs 30 min 95% CI: −2.471 to −0.2532 (p=0.016), one-way ANOVA (p=0.014) F (3, 12)=5.364). The values are normalised to the untreated control. (B) Representative western blot for β- catenin after stimulation with BCP crystals. GAPDH served as loading control. Quantification of β-catenin western blots is given in the graph (wo vs 0.05 ng BCP 95% CI: −0.9690 to 0.8315, wo vs 0.1 ng BCP 95% CI: −1.826 to −0.02596 (p=0.04) and wo vs 100 ng Wnt3a 95% CI: −3.618 to −1.818 (p<0.0001), one-way ANOVA (p<0.0001) F (3, 16)=17.76). The values are normalised to the untreated control. (C) Total β-catenin was detected in whole cell lysates of wt and ttw/ttw chondrocytes without external stimulation. GAPDH served as loading control. (p=0.001, n=4). The values are normalised to the wt. (D) Immunohistological staining of BCP crystals incubated with FCS and Wnt3a (Wnt3a: yellow). Representative western blot of increasing amounts of BCP crystals incubated with 100 ng Wnt3a and increasing amounts of Wnt3a (0, 50, 100 ng) with 1 mg BCP crystals. (E) Representative western blot analyses of increasing amounts of GFP (0, 50, 100 ng) with 1 mg BCP crystals.(F) Representative western blot for LRP6 phosphorylation over a time course of 30 min after stimulation with 0.1 ng/mL BCP crystals, 10 ng/mL Wnt3a or the combination of both. Total LRP6 served as loading control. (0 vs 30 BCP +Wnt3 a 95% CI: −0.6349 to −0.05008 (p=0.0163), one-way ANOVA (p=0.0052) F (8, 27)=3.659). The values are normalised to the untreated control. ANOVA, analysis of variance; BCP,basic calciumphosphate; GAPDH, glyceraldehyde-3-phosphatedehydrogenase.

pellets incubated with both dosages of BCP crystals indicated the similar effect of Axin2 reduction on protein level (figure 4A). The main transcription factor involved in keeping the chondrocytic phenotype is Sox9. We found a fivefold decrease in Sox9 after incubation with BCP crystals for both concentrations. A similar trend was observed in the Sox9 immunostainings of micro pellet cultures (figure 4B). There was no detectable change in collagen II expression after BCP crystal incubation. The immunostaining in micro pellets of wild-type chondrocytes confirmed that there were no differences in collagen II expression on treatment with BCP crystals. However, Aggrecan was significantly downregulated by BCP crystal in a dose-dependent manner. Again, we performed an immunostaining for Aggrecan in the micro pellet culture. We observed the same trend of decreased Aggrecan expression with increasing BCP content. The hypertrophic differentiation markers collagen X and MMP13 were dose-dependently upregulated by BCP crystal incubation. These results were confirmed by immunohistological stainings on micro pellet cultures indicating the same upregulation of MMP13 and collagen X on protein level. The expression data indicate a clear shift of the chondrocyte phenotype induced by BCP crystals incubation towards hypertrophy.

## DISCUSSION

We have shown that accumulation of Wnt3a binding to HSPGs and the increased production of crystals in OA cartilage cooperate leading to increased activation of canonical Wnt signalling

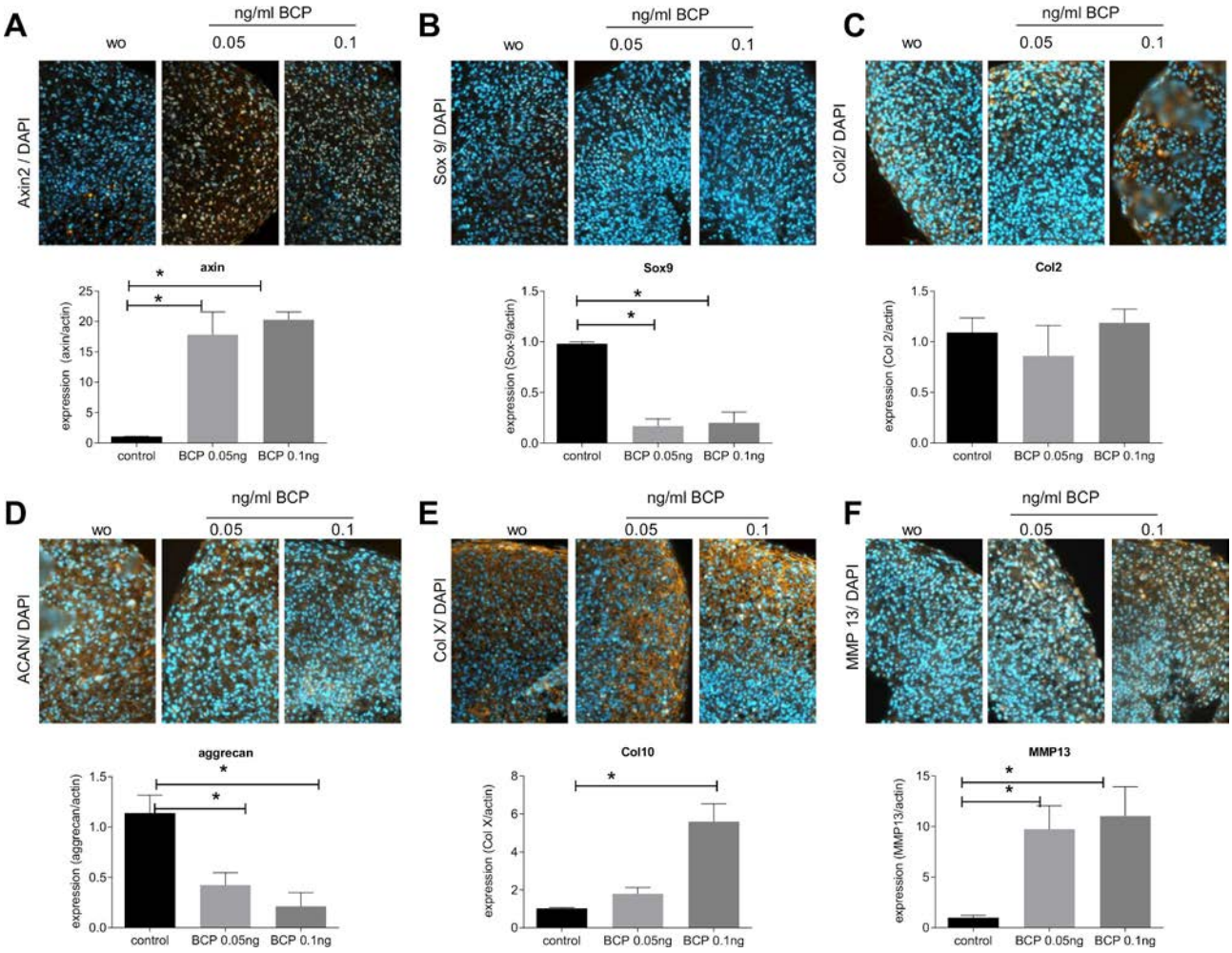


**Figure 4** BCP crystals induce hypertrophic differentiation of chondrocytes: Quantitative RT-PCR of chondrocyte marker gene expression in wt murine chondrocytes after 24 hours of incubation with BCP crystals (0.05 and 0.1 ng/mL). Actin was used as housekeeping gene. The values are normalised to the untreated control and analysed using a one-way ANOVA with Dunnett's post hoc test for statistical significance. Corresponding representative stainings of micro pellet cultures for the respective marker are given above the graph. (A) Representative pictures of Axin2 staining in micro pellet cultures incubated with 0.05 or 0.1 ng/ mL BCP crystals. Graph depicts Axin2 expression of murine chondrocytes in monolayer culture after incubation with 0.05 and 0.1 ng/mL BCP crystals in qRT-PCR (wo vs 0.05 ng/mL 95% CI: −24.66 to −8.933 (p=0.0002) and wo vs 0.1 ng/mL BCP 95% CI: −27.09 to −11.36 (p<0.0001), one-way ANOVA (p<0.0001) F (2, 15)=21.09). (B) Representative pictures of Sox9 staining in micro pellet cultures incubated with 0.05 or 0.1 ng/ mL BCP crystals. Graph shows Sox9 expression of murine chondrocytes in monolayer culture after incubation with 0.05 and 0.1 ng/mL BCP crystals in qRT-PCR (wo vs 0.05 ng/mL 95% CI: 0.5581 to 1.071 (p=0.0001) and wo vs 0.1 ng/mL BCP 95% CI: 0.5246 to 1.037 (p=0.0003), one-way ANOVA (p<0.0001) F (2, 15)=38.46. (C) Representative pictures of collagen II staining in micro pellet cultures incubated with 0.05 or 0.1 ng/ mL BCP crystals. No significant difference was found for collagen II expression of murine chondrocytes in monolayer culture after incubation with 0.05 and 0.1 ng/mL BCP crystals in qRT-PCR. (D) Representative pictures of Aggrecan staining in micro pellet cultures incubated with 0.05 or 0.1 ng/ mL BCP crystals. Graph shows Aggrecan expression of murine chondrocytes in monolayer culture after incubation with 0.05 and 0.1 ng/ mL BCP crystals in qRT-PCR (wo vs 0.05 ng/mL: 95% CI: 0.2181 to 1.218 (p=0.0055) and wo vs 0.1 ng/mL BCP 95% CI: 0.4291 to 1.429 (p=0.0006), one-way ANOVA (p=0.0008) F (2, 18)=10.92. (E) Representative pictures of collagen X staining in micro pellet cultures incubated with 0.05 or 0.1 ng/ mL BCP crystals. Graph shows collagen X expression of murine chondrocytes in monolayer culture after incubation with 0.05 and 0.1 ng/mL BCP crystals in qRT-PCR (wo vs 0.05 ng/mL 95% CI: −2.740 to 1.220 and wo vs 0.1 ng/mL BCP 95% CI: −6.555 to −2.596 (p<0.0001), one-way ANOVA (p<0.0001) F (2, 15)=18.26. (F) Representative pictures of MMP13 staining in micro pellet cultures incubated with 0.05 or 0.1 ng/mL BCP crystals. Graph shows MMP13 expression of murine chondrocytes in monolayer culture after incubation with 0.05 and 0.1 ng/mL BCP crystals in qRT-PCR (wo vs 0.05 ng/mL 95% CI: −16.03 to −1.454, (p=0.0187) and wo vs 0.1 ng/mL BCP 95% CI: −17.33 to −2.752 (p=0.0074), one-way ANOVA (p=0.0077) F (2, 18)=6.459. ANOVA, analysis of variance; BCP,basic calciumphosphate; quantitative reverse transcription PCR (qRT-PCR);wt, wilt type.

and ultimately to hypertrophic differentiation of chondrocytes. Increased 6-O-sulfation sensitises chondrocytes to Wnt3a induced canonical Wnt signalling. BCP crystals bind Wnt3a directly on their surface and facilitate their bioavailability, thereby driving hypertrophic differentiation of chondrocytes.

Healthy cartilage chondrocytes are protected from hypertrophic differentiation during and after embryogenesis.[35] Calcification and hypertrophic differentiation of chondrocytes in the articular cartilage are hallmarks of OA.[24–26] It has been suggested that various triggers are involved in the loss of the chondrocyte phenotype during early OA. One important trigger is the activation of canonical Wnt signalling.[36 37] Wnt3a is one of the best studied canonical Wnts in articular cartilage. Wnt3a is known to mediate the inhibition of chondrogenesis, by the

β-catenin and the canonical pathway. However, Wnt3a might also use non-canonical pathways to induce other effects in articular cartilage.[2 38 39] An enhanced activation of canonical Wnt signalling has already been reported in OA cartilage in humans and mouse, as well as following cartilage injury in mice.[3 4 40 41] The inhibition of canonical Wnt signalling has been shown to inhibit hypertrophic differentiation of chondrocytes and thereby prevent or delay OA onset.[42 43] Results of functional studies using mouse genetics, however, gave conflicting results, as both repression and forced activation of canonical Wnt signalling, resulted in OA like cartilage degeneration.[6 7] These data indicate that the manipulation of canonical Wnt signalling via knockout and overexpression is not sufficient to maintain cartilage homoeostasis.[37]

Our data confirm the activation of canonical Wnt signalling in human and murine OA cartilage. We show a clear correlation between OA severity and accumulation of Wnt3a around chondrocytes. Although Wnt3a has been extensively studied as model Wnt for cartilage degeneration during OA, the exact location of Wnt3a in the osteoarthritic cartilage has not been shown previously. We show an accumulation of Wnt3a around the chondrocyte clusters in osteoarthritic cartilage. Wnt3a is has been described to induce ECM degradation and to amplify interleukin-1β signalling in articular cartilage,[5] thereby contributing also to the inflammatory mediated cartilage degeneration.[44–46]

It is known that Wnts avidly bind to HSPGs.[47] HSPGs not only mediate the binding of Wnts at the cell surface, but also contribute to the activation and regulation of Wnt signalling.[8 48 49] We showed in our experiments that treatment with heparitinase effectively removed the Wnt3a from the chondrocyte clusters in OA cartilage, highlighting the important role of HSPGs in Wnt3a-mediated signalling during OA. Interestingly, it has been described that the response of cells to Wnt3a is regulated by the sulfation pattern of HSPGs.[50] An increase in 6-O-sulfation has been attributed to an increased Wnt3a-induced canonical Wnt signalling.[12 13 50] 6-O-sulfation has been show to activate canonical Wnt signalling.[14 50 51] 2-O-sulfation on the other hand seems to favour deactivation of Wnt signalling.[51] This effect is thought to be mediated by increased Wnt binding capacity to the higher sulfated HS side chain.[51] We found a marked increase in ECM sulfation in OA cartilage around the chondrocyte clusters in murine and human OA cartilage, as well as an increase in the expression of HS6ST1, which mediates 6-O-sulfation. These data indicate that OA cartilage might be primed to activate canonical Wnt signalling, due to the increased presence of 6-O-sulfate HSPGs.

As the Wnt3a accumulation was mainly found around the calcified chondrocyte clusters, we examined whether the BCP crystals themselves can interact with Wnt3a. It has been shown before that monosodium urate crystals have a high affinity for binding proteins at their surface and that the coating regulates the cellular response.[52 53] A correlation of this MSU crystal coating in gout with disease activity has been described before.[54] For this reason, we investigated the binding of Wnt3a to the surface of BCP crystals. We show that Wnt3a associates to the surface of BCP crystals in a dose-dependent manner. This finding indicates that BCP crystals in the ECM might not be a mere epiphenomenon, resulting from chondrocyte hypertrophy, but contribute actively to chondrocyte differentiation by keeping Wnt3a at the cell surface.

The mechanism of how proteins associate to the crystal surface is not well described. For mono sodium urate (MSU) crystals it has been described, that the crystal surface is predominantly negatively charged due to the negatively charged oxygen atoms prominent at the crystal surface.[55] Interestingly, most secreted proteins, including morphogens and cytokines, are lipidated during the secretion process.[56] Also Wnt proteins are lipidated during intracellular processing before secretion into the extracellular space.[57] Wnt3a is known to be lipidated with a palmitoleic acid moiety at Ser209.[58] Lipidation induces a positive charge at the specific site of the protein, which might interact electrostatically with the negative charge of BCP crystals. This phenomenon might explain how BCP crystals can associate Wnt3a at their surface and thereby increase the local availability of the ligand, thereby lowering the threshold for canonical Wnt signalling activation. This also explains how BCP crystals can induce canonical Wnt signalling without addition of a Wnt ligand, as the serum in cell culture contains various morphogens, which will associate at the surface of the crystal. That the BCP crystal itself does not mediate any effect has been proven by the use of BSA in the LRP6 phosphorylation experiment, where the BCP crystals were not sufficient to induce a response, as well as 10 ng/mL Wnt3a.

Figure 5 depicts the proposed mechanism of BCP-induced hypertrophic differentiation of chondrocytes. The sulfation pattern of HSPGs in OA cartilage is changed. An increase in

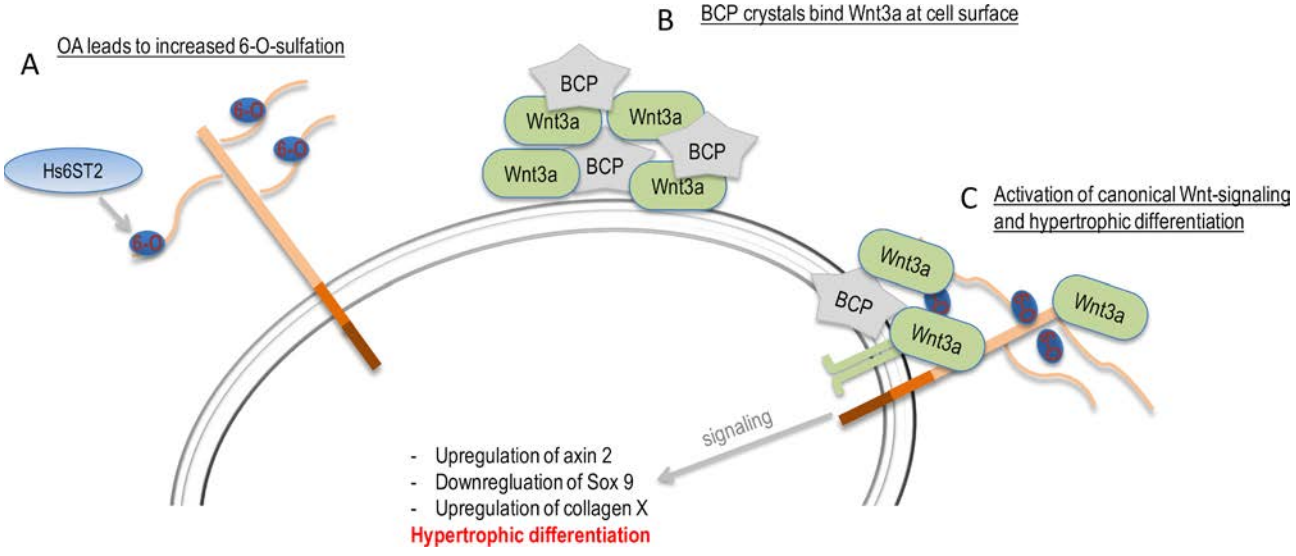


**Figure 5** Cartoon of proposed mechanism of BCP induced changes in the chondrocyte phenotype. BCP, basiccalcium phosphate; OA, osteoarthritis.

total sulfation and especially 6-O-sulfation can be observed. The change in 6-O-sulfation primes the chondrocytes to activate canonical Wnt signalling. At the same time BCP crystals around the chondrocytes accumulate Wnt3a at their surface, thereby increasing the local availability of the ligand. Chondrocytes therefore, activate canonical Wnt signalling inducing hypertrophic differentiation and loss of cartilage homoeostasis.

Wnt3a has been shown before to drive chondrocytes down the hypertrophic differentiation pathway.[37] Wnt3a is known to induce hypertrophic changes in the chondrocyte phenotype.[39] BCP crystals bind Wnt3a at the cell surface, keeping the ligand at the cell surface.

In conclusion our data show that BCP crystals are not only an epiphenomenon during OA development, but an active driver of chondrocyte hypertrophic differentiation by perpetuating canonical Wnt signalling, by associating Wnt3a at their surface, in the already 6-O-sulfation primed cartilage.

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**Contributors** JB performed most experiments and wrote the manuscript, TK performed the mouse histology and stainings and the Western Blots for activation of Wnt signalling, TG performed the human Alcian/PAS stainings, JS helped writing the manuscript and discussing the data, FR provided the ttw/ttw mice, FL provided the basic calcium phosphate crystals and helped discussing the data, FDL helped discussing the data and writing of the manuscript, CHL provided the human samples and discussed the data, MB performed the safranin-orange stainings of the human samples, AH performed the Wnt3a and ß-catenin stainings of the human samples, TP helped writing the manuscript and discussed the data.

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**Competing interests** None declared.

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

**Patient consent for publication** Not required.

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**Data availability statement** Data are available upon reasonable request. Protocols and statistical analyses can be made available upon request. Please contact the corresponding author (jesscia.bertrand@med.ovgu.de).

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# Hope

I am a rheumatologist. I am NOT on the front lines of the pandemic, yet, but I AM scared.

I am scared for myself. I am scared when my husband and I discuss our advanced directives as he awaits a 'deployment' to the Intensive Care Unit. I am scared for my family, my friends, my colleagues and my patients. I am scared for the new normal.

I cope. I cope by maintaining a level of normalcy by conducting tele health visits. I cope by attending virtual yoga classes, making fresh pizza, doing silly dances with my nephew and listening to music. I am fortunate to be able to cope with all the above.

I break. I break when I learn my patient's wife passed away from COVID-19 after visiting Disneyland. I break when I hear about my friends and family members working on the front lines without adequate personal protective equipment. I break because patient's family members are not able to say goodbye to their loved ones. I break because there is a story in each death which has instead become a statistic.

I try. I try to examine for synovitis on a video visit. I try to calm my friends down when they call in the middle of the night, concerned they have contracted COVID-19. I try to stay optimistic.

I rage. I rage when my lupus patients cannot get hydroxychloroquine. I rage when people do not follow social distancing guidelines. Sometimes, I rage without a reason.

I grieve. I grieve each time I hear the news. I grieve at the loss of the warmth of a hug.

I am thankful. I am thankful for everyone who puts their life at risk to save us. There are way too many people to be thankful for. I am thankful for the altruism and love that surround me.

I cry. I cry because the enormity of the situation is too difficult to absorb. I cry at the surge of cases around the world and the surge of emotions inside me.

I contemplate. I contemplate about life, death, the uncertainties and the future. I contemplate about the collective experience we are all going through, courtesy of an invisible virus.

I contemplate about my identity as a rheumatologist and my role as a physician during a pandemic. My conscience pulls me to be on the front lines and help my colleagues. Dr Louis Lasagna mentioned in the modern Hippocratic oath, 'I will remember that there is art to medicine as well as science, and that warmth, sympathy and understanding may outweigh the surgeon's knife or the chemist's drug'.[1] I do my part by being there for my patients and by commiserating; I heal myself in the process.

I hope. I hope that once on the other side of the pandemic, undoubtedly damaged and scarred, to be more appreciative, humble, grateful and thankful.

I adapt. I persevere. I trudge onward.

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## Susceptibility and severity of COVID-19 in patients treated with bDMARDS and tsDMARDs: a population-based study

Patients with autoimmune conditions treated with biological agents have an increased risk of severe infections.[1 2] Very few studies have evaluated the susceptibility and severity of coronavirus disease 2019 (COVID-19) in patients treated with biological disease-modifying antirheumatic drugs (bDMARDs) or targeted synthetic DMARDs (tsDMARDs).[3 4] Some of these studies suggest a protective role of these drugs for COVID-19; however, they consist of small series, and the results are unclear.

Therefore, we decided to evaluate in a population-based study the risk of COVID-19 infection and its severity in the patients treated with bDMARDs or tsDMARDs in a geographic area (Emilia Romagna) at high diffusion of COVID-19.

We identified 1195 patients treated with the bDMARDs or tsDMARDs listed in table 1 in Reggio Emilia area on 31 December 2019. Biological agents were classified according to the mechanism of action. The patients were registered in the database of the Hospital Pharmaceutical Service of the Reggio Emilia area, which delivers the drug directly to the patients. The database is updated every 3 months. All residents of Reggio Emilia area who have had rhinopharyngeal swabs, positive swabs and were hospitalised or died from COVID-19 from the beginning of the outbreak (27 February 2020) are registered in a centralised index. Swabs were performed in symptomatic patients at risk of having COVID-19. The fiscal code was used to identify and match patients treated with biological agents and with COVID-19 infection. We used data updated at 24 April. Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

**Table 1** Residents of the Reggio Emilia area treated with bDMARDS or tsDMARDS versus all residents: comparison among residents tested and residents positive for COVID-19 stratified by gender and classes of age

| Residents of the Reggio Emilia area treated with bDMARDs or tsDMARDs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total, n | | | Tested, n | | | Positive, n | | |
| | Male | Female | Total | Male | Female | Total | Male | Female | Total |
| Overall | 523 | 672 | 1195 | 8 | 17 | 25 | 3 | 6 | 9 |
| Hospitalised | | | | | | | | | 4 |
| Death | | | | | | | | | 1 |
| Age | | | | | | | | | |
| ≤45 | 143 | 167 | 310 | 2 | 4 | 6 | 0 | 0 | 0 |
| 45–65 | 267 | 302 | 569 | 3 | 7 | 10 | 3 | 3 | 6 |
| >66 | 113 | 203 | 316 | 3 | 6 | 9 | 0 | 3 | 3 |
| Drug mechanism of action | | | | | | | | | |
| Anti-TNF-alpha* | 334 | 436 | 770 | 4 | 13 | 17 | 2 | 3 | 5 |
| Anti-IL-1† | 12 | 7 | 19 | 0 | 1 | 1 | 0 | 1 | 1 |
| Anti-IL-6R‡ | 14 | 56 | 70 | 0 | 0 | 0 | 0 | 0 | 0 |
| Anti-IL-12/IL-23 anti-IL-23§ | 41 | 29 | 70 | 1 | 0 | 1 | 0 | 0 | 0 |
| Anti-IL-17°¶ | 103 | 85 | 188 | 2 | 2 | 4 | 0 | 2 | 2 |
| Anti-JAK** | 14 | 39 | 53 | 1 | 1 | 2 | 1 | 0 | 1 |
| Abatacept | 5 | 20 | 25 | 0 | 0 | 0 | 0 | 0 | 0 |
| **All residents of the Reggio Emilia area** | | | | | | | | | |
| | Total, n | | | Tested, n | | | Positive, n | | |
| | Male | Female | Total | Male | Female | Total | Male | Female | Total |
| Overall | 261 563 | 270 328 | 531 891 | 3405 | 4542 | 7947 | 1697 | 2049 | 3746 |
| Hospitalised | | | | | | | | | 1342 |
| Death | | | | | | | | | 383 |
| Age | | | | | | | | | |
| ≤45 | 133 037 | 126 701 | 259 738 | 835 | 1377 | 2212 | 294 | 453 | 747 |
| 45–65 | 81 057 | 82 183 | 163 240 | 1177 | 1514 | 2691 | 655 | 691 | 1346 |
| >66 | 47 469 | 61 444 | 108 913 | 1393 | 1651 | 3044 | 748 | 905 | 1653 |

*Etanercept, infliximab, adalimumab and their biosimilars, certolizumab pegol and golimumab.
†Anakirna and canakinumab.
‡Tocilizumab and sarilumab.
§ustekinumab and guselkumab.
¶Secukinumab, brodalumab and ixekizumab.
**Tofacitinib and baricitinib.
bDMARDs, biological disease-modifying antirheumatic drugs; tsDMARDs, targeted synthetic disease-modifying antirheumatic drugs.

Table 1 compares the residents of the Reggio Emilia area treated with bDMARDs or tsDMARDs versus all residents. The difference regarding the frequencies of patients with swabs was significant (1.7% vs 1.4%, p=0.001), not that of positive swabs (36.0% vs 47.1%, p=0.318), nor that of hospitalised or dying patients (44.4% vs 35.8%, p=0.730; 11.1% vs 10.2%, p=1.000, respectively). table 1 also shows the different bDMARDs and tsDMARDs grouped by the mechanism of action. None of the 70 patients treated with IL-6 blockers and only 1 of the 70 patients treated with anti-IL-12/IL-23 and anti IL-23 were tested. The one tested resulted negative. At multivariate logistic and Cox proportional hazards analyses adjusted by sex and age, patients treated with bDMARDs or tsDMARDs had a tendency of being more frequently tested (OR 1.19, 95% CI 0.80 to 1.77) and hospitalised (HR 1.28, 95% CI 0.32 to 5.11) and to be less frequently positive when tested (OR 0.62, 95% CI 0.27 to 1.42); however, the differences were not significant.

In our study, which had an accurate case ascertainment from two reliable sources and a sufficiently long follow-up to observe deaths, we did not find any statistically significant difference regarding the probability of being tested, having a positive swab when tested, being hospitalised and dying in our patients treated with bDMARDs or tsDMARDs . The observed tendency towards a reduced probability of being positive at swabs is probably related to the higher proportion of patients tested compared with general population. Our data confirm some preliminary data from Lombardia, the Italian area with the highest incidence of COVID-19, which seem to indicate that patients treated with traditional immunosuppressive drugs or bDMARDs or tsDMARDs are not at increased risk of severe COVID-19, but we did not observe a protective role.[3 4] We cannot exclude that patients with immune-mediated disorders taking IL-6 inhibitors or compounds suppressing IL-12/IL-23 axis might be somewhat protected against COVID-19 infection. In conclusion, our study did not show a different susceptibility and severity of COVID-19 in patients treated with bDMARDs or tsDMARDs. The number of patients is too small to provide definitive conclusions; further larger prospective studies need to be done to confirm our results.

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# Clinical features and outcomes of COVID-19 in patients with rheumatic diseases treated with biological and synthetic targeted therapies

From the beginning of the COVID-19 pandemic, more than 4.7 million cases have been detected in the world, Spain being one of the countries hardest hit by the SARS-CoV-2.[1] The role of the immune system and immunomodulatory therapies in the evolution of this infection is still controversial.[2] The study of patients with rheumatic and musculoskeletal diseases (RMDs) such as rheumatoid arthritis (RA), spondyloarthropathies (SpA) or systemic lupus erythematosus, treated with immunomodulatory therapies is essential to understand the prognosis of COVID-19 in this specific population and to the management of these patients.

BIOBADASER is a multicentre prospective observational registry promoted by the Spanish Society of Rheumatology (SER) and supported by the Spanish Agency of Drugs and Medical Devices. It is aimed at assessing safety in patients with RMDs starting treatment with any biological (bDMARD) or targeted synthetic disease-modifying antirheumatic drug (tsDMARD). More than 6600 patients are prospectively followed up in BIOBADASER 3.0.

This report describes the clinical characteristics and outcomes of patients with COVID-19 in BIOBADASER. We have identified 41 patients with RMDs treated with bDMARD and tsDMARD diagnosed of COVID-19 at 15 hospitals in the registry. Thirty-one patients were diagnosed because positive PCR test for SARS-CoV-2, and 10 patients because a highly compatible clinical picture and close contact with confirmed positive cases. Table 1 shows baseline characteristics of the patients. Twenty-five (61.0%) patients were female and 16 (39,0%) male, with a mean age of 59.4 years. They had long-standing (12.8 years) refractory (three previous bDMARD/tsDMARDs) diseases with 5.7 years of bDMARD/tsDMARD therapy duration. Twenty-one patients (51.2%) had RA. Comorbidities included hypertension (36.6%), past or current smoker (36.8%), diabetes (9.8%) and high body mass index (BMI) (27.7 (5.6) kg/m$^2$—mean (SD)). Eighteen patients (43.9%) were using TNF inhibitors, seven JAK inhibitors (17.1%, 9.8% baricitinib and 7.3% tofacitinib) and five (12.2%) IL-6 inhibitors. Seventeen (41.5%) patients were using methotrexate and four (9,8%) hydroxychloroquine.

Three patients died (7.3%); a 63-year-old RA male on anakinra—plus prednisone 5 mg/day—(comorbidities: smoker, BMI 34.6); a 56-year-old SpA female on secukinumab—no glucocorticoids—(past smoker, BMI 28.4) and a 91-year-old vasculitis female on rituximab—plus prednisone 5 mg/day—(hypertension). Hospitalisation was required in 28 patients (68.3%) and intensive care unit (ICU) admission in 6. Thirty-five (85.4%) patients are fully recovered at the moment of this analysis, and three patients are still hospitalised, none in ICU.

Data on COVID-19 in patients with RMDs is still scarce.[3–6] Because of the rapid evolution of the pandemic, it is important to accrue information on the clinical course of rheumatic patients on bDMARD/tsDMARDs developing COVID-19. The reduced number of patients in our study limits the possibility of drawing solid conclusions. However, these findings point in the direction that COVID-19 course and mortality in patients

**Table 1** Clinical features and treatments in patients with rheumatic diseases on targeted therapies with the diagnosis of COVID-19

| Variable | RA | SpA | Other rheumatic diseases | Total |
|---|---|---|---|---|
| N | 21 | 12 | 8 | 41 |
| Age at COVID-19 onset, years (SD) | 61.3 (13.9) | 57.1 (11.5) | 57.1 (23.9) | 59.4 (15.6) |
| Sex, female, n (%) | 14 (66.6) | 5 (41.7) | 6 (75.0) | 25 (61.0) |
| Disease duration (time since rheumatic diagnosis to COVID-19), years (SD) | 12.0 (8.3) | 15.0 (14.2) | 11.4 (7.9) | 12.8 (9.8) |
| Time with bDMARDs/tsDMARDs (time since beginning of treatment to COVID-19), years (SD) | 5.8 (5.2) | 5.3 (5.8) | 5.7 (9.6) | 5.7 (5.7) |
| *Comorbidities and risk factors* | | | | |
| Charlson index, mean (SD) | 2.5 (1.6) | 2.3 (1.7) | 3.4 (3.1) | 2.6 (2.0) |
| BMI, mean (SD) | 27.9 (5.1) | 29.4 (4.7) | 25.2 (7.5) | 27.7 (5.6) |
| Hypertension, n (%) | 6 (28.6) | 5 (41.7) | 4 (50.0) | 15 (36.6) |
| Smoking status, n (%) | | | | |
| Never smoker | 14 (66.7) | 8 (66.7) | 5 (62.5) | 27 (65.8) |
| Current smoker | 2 (9.5) | 0 (0.0) | 2 (25.0) | 4 (9.8) |
| Former smoker | 5 (23.8) | 4 (33.3) | 1 (12.5) | 10 (24.4) |
| *COVID-19 diagnosis, evolution and outcome* | | | | |
| COVID-19 diagnosis, n (%) | | | | |
| Confirmed cases (positive PCR test) | 16 (76.2) | 8 (66.7) | 7 (87.5) | 31 (75.6) |
| Suspicious cases (highly compatible clinical picture) | 5 (23.8) | 4 (33.3) | 1 (12.5) | 10 (24.4) |
| COVID-19 outcome | | | | |
| Recovered without sequelae | 18 (85.7) | 11 (91.7) | 6 (75.0) | 35 (85.4) |
| Not yet recovered | 2 (9.5) | 0 (0.0) | 1 (12.5) | 3 (7.3) |
| Death | 1 (4.8) | 1 (8.3) | 1 (12.5) | 3 (7.3) |
| Hospitalisation, n (%) | 16 (76.2) | 8 (66.7) | 4 (50.0) | 28 (68.3) |
| Intensive care unit, n (%) | 4 (19.0) | 2 (16.7) | 0 (0.0) | 6 (14.6) |
| *Rheumatic disease: treatment and clinical features* | | | | |
| Last DAS-28 available (previous to COVID-19), mean (SD) | 3.9 (1.4) | 3.3 (1.3) | – | 3.6 (1.4) |
| bDMARD/tsDMARDs previous to COVID-19), n (%) | | | | |
| TNF inhibitors | 7 (33.3) | 7 (58.3) | 4 (50.0) | 18 (43.9) |
| Anti-IL6 monoclonal antibodies | 3 (14.3) | 0 (0.0) | 2 (25.0) | 5 (12.2) |
| Anti-CD20 monoclonal antibodies | 2 (9.5) | 0 (0.0) | 1 (12.5) | 3 (7.3) |
| Anti-IL1 monoclonal antibodies | 1 (4.8) | 0 (0.0) | 0 (0.0) | 1 (2.4) |
| Anti-IL17A monoclonal antibodies | 0 (0.0) | 5 (41.7) | 0 (0.0) | 5 (12.2) |
| Abatacept | 1 (4.8) | 0 (0.0) | 1 (12.5) | 2 (4.9) |
| JAK inhibitors | 7 (33.3) | 0 (0.0) | 0 (0.0) | 7 (17.1) |
| Baricitinib | 4 (9.0) | 0 (0.0) | 0 (0.0) | 4 (9.8) |
| Tofacitinib | 3 (14.3) | 0 (0.0) | 0 (0.0) | 3 (7.3) |
| Number of previous bDMARD/tsDMARDs, mean (SD) | 4.2 (2.9) | 2.0 (1.0) | 1.6 (0.7) | 3.0 (2.4) |
| Use of concomitant csDMARDS | | | | |
| Methotrexate | 11 (52.4) | 4 (33.3) | 2 (25.0) | 17 (41.5) |
| Hydroxychloroquine | 2 (9.5) | 0 (0.0) | 2 (25.0) | 4 (9.8) |
| Others | 0 (0.0) | 2 (16.7) | 0 (0.0) | 2 (4.9) |
| Monotherapy | 12 (57.1) | 6 (50.0) | 4 (50.0) | 22 (53.7) |
| Use of glucocorticoids, n (%) | 13 (61.9) | 2 (16.7) | 5 (62.5) | 20 (83.3) |
| Dose of glucocorticoids (before COVID-19), mg, mean (SD) | 5.5 (3.3) | 7.5 (2.1) | 6 (2.2) | 5.8 (2.9) |
| Concomitant use of NSAIDs, n (%) | 7 (33.3) | 3 (25.0) | 0 (0.0) | 10 (24.4) |

bDMARDs, biological disease-modifying antirheumatic drugs; BMI, body mass index; n, number of patients; NSAIDs, non-steroidal anti-inflammatory drugs; RA, rheumatoid arthritis; SpA, spondyloarthropathies; tsDMARDs, targeted synthetic disease-modifying antirheumatic drugs.

with RMDs treated with b/tsDMARD do not differ from the general population (12.0% mortality rate and hospitalisation rate 53.6% by COVID-19 in Spain[1]). Of interest, these high mortality and hospitalisation rates are likely due to a diagnostic bias with PCR testing reserved for the most symptomatic patients, as suggested by a recent (unpublished) report by the Spanish Ministry of Health showing a prevalence of IgG seroconversion to SARS-Cov-2 of 5% in Spain. That is 10 times greater than the PCR confirmed cases (1). The present data, in addition to previous publications, is crucial to clarify the risks of patients with rheumatic diseases and their immunosuppressive medications. Doubtlessness, additional studies are still needed. To this end, the SER is prospectively collecting information on COVID-19 in three registries (BIOBADASER, RELESSER and CARMA) in more than 9000 patients with rheumatic diseases.

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# TARA study: a new perspective on tapering drugs in RA

We read with great interest the article on 'Gradual tapering TNF inhibitors vs conventional synthetic DMARDs after achieving controlled disease in patients with rheumatoid arthritis: first-year results of the randomised controlled TARA study'[1]by van Mulligen *et al*. This was the first head-to-head comparison between two tapering strategies—biological versus conventional in rheumatoid arthritis. The final results favour tapering tumour necrosis factor inhibitors (TNFis) before conventional synthetic disease-modifying antirheumatic drugs (csDMARDs). However, certain points need clarifications.

First, many patients in the study used combination csDMARDs, but no data have been provided on their number and specific combinations used. Furthermore, no clarity has been given on how the tapering was done in these patients who were on combination csDMARDs—was methotrexate the only drug reduced and stopped or were all the drugs in the combination reduced and stopped. In their trial registration (NTR2754), the authors have only mentioned methotrexate tapering, whereas the study title mentions csDMARD tapering.

Second, in their statistical analysis, they mention an 'intention-to-treat analysis'. However, in their results, the authors have presented the clinical response after 12 months for both tapering groups, for only 85 and 89 patients in table 2 (final number of patients at 12 months) rather than the 94 and 95 patients who were initially randomised to the two tapering arms.

Third, the number of patients in clinical remission (disease activity score (DAS) <1.6) in TNFi tapering arm has been reported in table 2 as 58. However, the number of patients at risk at 12 months has been mentioned as only 54 (figure 2B). Thus, it is unclear that how the number of patients in clinical remission at 12 months more than the number at risk—ideally, all patients who dropped out or required to restart biologics or csDMARDs because of flare should be not considered in remission.

Finally, in the study protocol, the use of intra-articular glucocorticoids (GCs) and one intramuscular (IM) injection of GCs during a flare (as bridging therapy) was permitted. However, four and five patients, respectively, in csDMARDs and TNFi tapering arms received oral GCs and three patients in each arm got more than one IM injections. What was the effect of excluding these patients on the analysis would be interesting to know?

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# Response to: 'TARA Study: a new perspective on tapering drugs in RA' by Mishra *et al*

We are pleased about the interest in our article by Mishra *et al* and we would like to respond to their questions so that there can be no ambiguity.[1 2]

First of all, there is some clarification needed on the conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) that were used in combination with the TNF-inhibitors at baseline in the TApering strategies in Rheumatoid Arthritis (TARA) study. In table 1, we elaborate on the different combinations of csDMARDs that were used for each intervention arm separately. In the csDMARD tapering group, the methotrexate (MTX) was tapered, except for the three patients who did not use MTX. These patients gradually tapered leflunomide (n=1) and sulfasalazine (n=2).

Mishra *et al* also had a question about our intention-to-treat (ITT) analysis. In an ITT analysis, patients are analysed in the groups to which they were randomised, regardless of whether they received or adhered to the allocated intervention. Therefore, in the clinical response table of the original article, we should have given the total numbers instead of the patients who were still participating in the TARA trial at 12 months.[2] If we had given the total numbers, the results would be similar.

Third question was about explaining the difference between the number of patients who are in remission after 12 months of follow-up and the number of patients below the Kaplan-Meier (KM) curve at 12 months. In a KM curve, only the patients at risk are given. Patients are censored if they experience a flare or drop-out, which results in a decreasing number of patients at risk over time. In the original TARA article, on the other hand, the number of patients in clinical remission (defined as a disease activity score (DAS) <1.6) at 12 months of follow-up is given. Thus, the interpretation of the numbers given in the KM curve and the number of patients in clinical remission is different and, therefore, the numbers are non-identical.

Finally, it would be interesting to know if the primary outcome would change if we use a modified per-protocol approach as brought up by Mishra *et al*. For this reason, we excluded the patients who used oral glucocorticoids, n=4 and n=5, respectively, in the csDMARD and TNF-inhibitor tapering group, or had more than one intramuscular injection, n=3 in each tapering group. With aforementioned approach a 30% (95% CI, 21% to 41%) flare rate was seen in the csDMARD tapering group, and a 39% (95% CI, 31% to 52%) flare rate in the TNF-inhibitor group (p=0.15). The difference in flare rates between the two tapering arms is similar to the one found in the original article.[2]

**Table 1** Use of csDMARDs at baseline in the TARA study specified for two groups: tapering csDMARDs and tapering TNF-inhibitors

| Use of csDMARDs at baseline | Tapering csDMARD (n=93) | Tapering TNF-inhibitor (n=95) |
|---|---|---|
| MTX monotherapy, n (%) | 64 (69) | 49 (52) |
| MTX+hydroxychlorquine, n (%) | 17 (18) | 27 (29) |
| MTX+sulfasalazine+hydroxychloroquine, n (%) | 5 (5) | 6 (6) |
| MTX+sulfasalazine, n (%) | 3 (3) | 2 (2) |
| MTX+leflunomide, n (%) | 1 (1) | 0 (0) |
| Sulfasalazine monotherapy, n (%) | 0 (0) | 3 (3) |
| Sulfasalazine+hydroxychloroquine, n (%) | 2 (2) | 0 (0) |
| Sulfasalazine+leflunomide, n (%) | 0 (0) | 1 (1) |
| Leflunomide monotherapy, n (%) | 1 (1) | 3 (3) |
| Leflunomide+hydroxychloroquine, n (%) | 0 (0) | 1 (1) |
| Hydroxychloroquine monotherapy, n (%) | 0 (0) | 3 (3) |

MTX, methotrexate; TARA, TApering strategies in Rheumatoid Arthritis; csDMARD, conventional synthetic disease-modifying antirheumatic drug.

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# Tapering without relapse in rheumatoid arthritis patients with high TNF blocker concentrations: data from STRASS study

We read with interest the paper by l'Ami *et al*[1] reporting the safety of a single step-down strategy without flare-up of disease in rheumatoid arthritis (RA) patients treated with adalimumab associated with high trough concentrations. At the time of personalised medicine, prediction of the absence of relapse during tapering strategy is a huge challenge to improve this approach. Furthermore, EULAR recommendations proposed in RA patients in remission without glucocorticoids to first step down the bDMARDs.[2] So, we investigated the interest of TNF blocker blood concentration assessment in order to predict the absence of relapse during tapering in the STRASS study.[3] The STRASS study demonstrated the feasibility of step-down therapeutic strategy compared with maintenance strategy in RA patients in clinical remission treated with adalimumab or etanercept. In contrast to l'Ami study, which performed a single tapering, successive tapering step every 3 months in RA patient still in remission was performed. Among the 137 patients included in STRASS study, 132 serum samples were collected solely at baseline without other blood collections and assessed by ELISA with Lisa Tracker (adalimumab or etanercept kit by Theradiag, Marne-La-Vallee, France). We defined high level of TNF blocker, by concentration higher or equal to upper detection limits in serums (8 µg/mL for adalimumab and 5 µg/mL for etanercept). For adalimumab, this definition was similar to the definition of high trough concentrations defined by l'Ami.

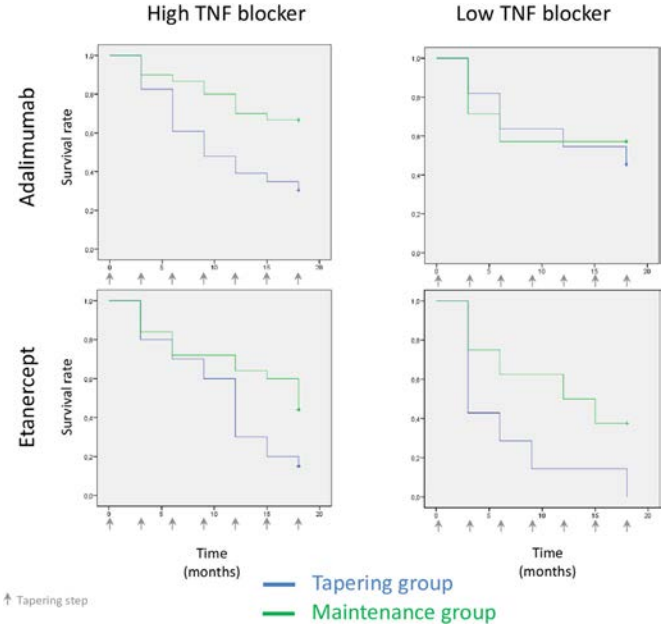


**Figure 1** Survival without relapse according to TNF blockers trough concentration. Survival without relapse was the same between tapering or maintenance group in case of high TNF blocker trough concentration at baseline during the first 3 months and almost the same over 9 months for etanercept. However, the survival rate in the tapering group strongly dropped after 6 months for adalimumab. This was in accordance to l'Ami study since adalimumab trough concentration decreased under the high concentration threefold at 12 and 24 weeks after only one tapering.

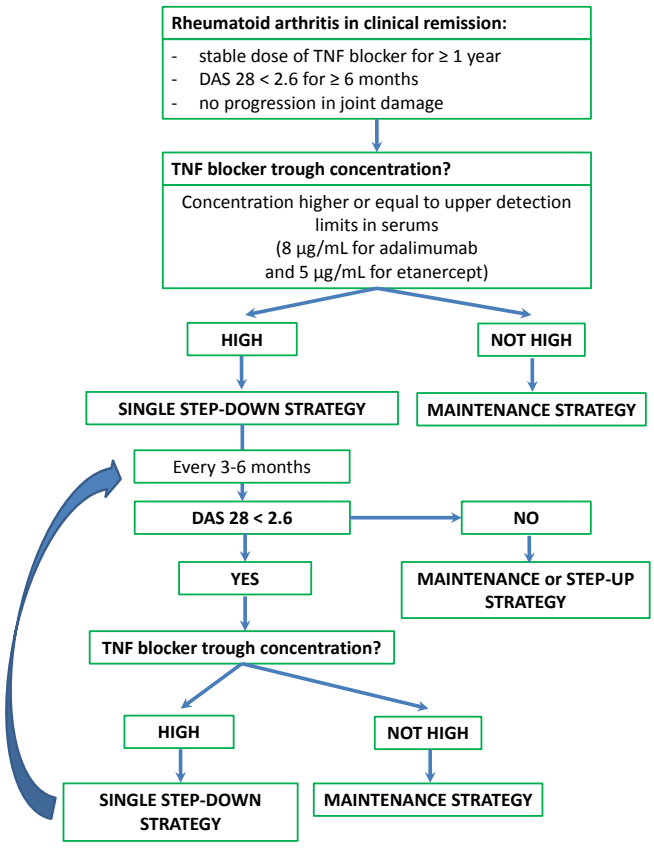


**Figure 2** Proposed algorithm based on therapeutic drug monitoring to improve tapering strategy.

Overall, in STRASS study, no clear effect was observed between high blood levels of TNF blockers at baseline and persistence of remission over 24 months. However, when focusing at 6 months (that means two first steps-down in the spacing arm), the proportion of patients without relapse was higher in case of high TNF blockers concentration at baseline ($\chi^2$=6.22; p=0.01; figure 1). In the l'Ami study, adalimumab trough concentration decreased under the high concentration level at 12 and 24 weeks after only one tapering. This could explain the increased rate of relapse after the third tapering in STRASS (figure 1). Furthermore, no data with etanercept on tapering are available to date. Difference pattern of flares between RA patients treated by adalimumab or etanercept could be due to the absence of cut-off previously reported for etanercept.

Our data suggest to perform a drug monitoring before each tapering, in order to avoid the situation with low TNF blockers blood trough concentration leading to clinical relapse. Furthermore, to reduce the high TNF blockers trough concentration could be also benefit for the RA patients in remission since high TNF blockers trough concentration was reported to be associated with a strong risk of infection.[4]

The clinical utility of TNF blockers monitoring and determination of specific cut-offs in predicting clinical remission had already been explored especially in inflammatory bowel diseases.[5] Here, we claim the monitoring of trough concentrations in order to improve successful tapering strategy (figure 2).

In conclusion, we confirmed that tapering is feasible without an increased rate of relapse in RA patients with

clinical remission and high TNF blocker blood concentration. Furthermore, since the initial concentration of STRASS study will be predictive of RA relapse in case of TNF blocker injection spacing, we propose to assess trough TNF blocker concentration before each tapering step in order to maintain remission and avoid a relapse in RA patients with clinical remission. Finally, we proposed an algorithm to manage step-down strategy (figure 2), which should be confirmed in a prospective study.

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# Response to: 'Tapering without relapse in rheumatoid arthritis patients with high TNF blocker concentrations: data from the STRASS study' by Marotte *et al*

We thank *Marotte et al* for their interest in our study and for presenting their own recent study. The Spacing of TNF-blocker injections in Rheumatoid ArthritiS Study (STRASS) results on drug concentrations are highly valuable, as this is a large, pragmatic, randomised trial. *Marotte et al* found that high drug concentrations were related to lower relapse rates after 6 months of tapering.[1] This is in line with our study, where we found that patients with high adalimumab concentrations could safely reduce the dose.[2]

In the STRASS study, 39% of the patients discontinued the biologic disease-modifying antirheumatic drugs (bDMARDs) successfully.[3] These patients, apparently, did not need the drug anymore, suggesting that any drug concentration was too high. This might explain why *Marotte et al* did not find an association between drug concentrations and relapse rates at long-term follow-up. Other patients in the study did require the drug, but could achieve the same result with less of it. For this latter group, in particular, therapeutic drug monitoring (TDM) can be helpful to reduce the dose safely. However, it is currently impossible to differentiate between these two groups of patients before tapering starts.

Moreover, as *Marotte et al* emphasised as well, the critical tumour necrosis factor (TNF) inhibitor concentration necessary to control the disease is incompletely defined. Especially, for etanercept, data are lacking. On the basis of our recent study, an adalimumab trough concentration of 1 µg/mL might be adequate to control TNF blockade.[4] It is, however, challenging to derive such results from observational studies with fixed-dosed treatment. A considerable number of patients with low concentrations is required to define the threshold adequate concentration, which are unavailable in the clinical setting.

Furthermore, individual bDMARDs should be studied separately, because pharmacokinetic aspects differ substantially. For instance, the half-life time for an antibody (eg, adalimumab) is about 21 days, assuming no antidrug antibodies, versus 3 days for the TNF receptor antagonist (etanercept). With pharmacokinetic modelling, an algorithm can be developed to determine individualised dose reduction based on drug concentrations.

In conclusion, we welcome the study of *Marotte et al* in the field of TDM. We expect that determination of critical concentrations and development of algorithms based on pharmacokinetic modelling will further contribute to the use of TDM in the treatment of rheumatic diseases.

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# Interosseous tendon inflammation of rheumatoid arthritis: what's the real meaning?

We read with deep interest the article by Mankia *et al*[1] related to interosseous tendon inflammation (ITI) of rheumatoid arthritis (RA). This retrospective analysis suggested that ITI occurs in anticyclic citrullinated peptide positive at-risk (CCP+at-risk) individuals and could precede the onset of clinical synovitis. The ITs may be important non-synovial extracapsular targets in the development and progression of RA. Their finding suggested a new extra-articular involvement of RA. We really appreciate the work that has been done by the authors. However, there are some worthwhile issues that need to be explored.

The authors found that no IT tenosynovial sheath was identified and no communication between the IT and the joint in cadavers on dissection or histological studies. Thus, they concluded that MRI findings represented 'peritendonitis'. The result was different with Rowbotham *et al*,[2] who reported that tenosynovitis of the hand ITs was found in 47.7% of patients with RA, and in the majority of cases, this was adjacent to metacarpophalangeal joint synovitis. We agree with the authors' point of view. We can tell from the MRI that the signal change is around interosseous muscles, other than in the tendon and muscle fibres of themselves. In fact, there is still a thin layer of fascia wrapping the interosseous muscle with many connective tissue cells, as peritendon showing in figure 2C,D.[1] In our opinion, this should be fasciitis around the interosseous muscles. Essentially, it is an extra-articular manifestation of RA, similar to rheumatoid vasculitis, rheumatoid heart disease, rheumatoid lung disease and so on.[3 4] It provides a new perspective for the research of RA: rheumatoid fasciitis. This might be the intrinsic value of this study. Previous studies on isolated peritendinous inflammation of the digital extensor tendons have also proved this point.[5]

Generally speaking, this study is very important and interesting. However, some aspects still need to be further improved. First, the sample size of this study was relatively small. Only 93 CCP+at-risk, 47 early RA (ERA), 28 late RA (LRA) and 20 health controls (HC) were included. On the basis of such a small sample size, the positive rates of CCP+ and ITI might not accurate enough. The sample size needs to be expanded. Second, women account for the vast majority in each group: 69% in CCP+at-risk group, 74% in ERA group, 93% in LRA group and 75% in HC group, respectively. The results may be gender-biassed. If gender-related data were improved, the results would be more objective and reliable. In addition, the related factors of RA were not analysed and excluded. For example, the age of the sample was not sufficiently representative. If we can analyse several age subgroups, the results will be more convincing. Furthermore, if fasciitis is found in other parts of the patient, the findings will be more meaningful.

Aside from the factors related to RA, risk factors of ITI also need to be excluded. Yet, there is no report on risk factors associated with ITI. Many general factors may relate to tendinitis or fasciitis of hand, such as trauma, diabetes, inflammatory arthritis, renal disease, gout and so on.[6 7] Unfortunately, these factors are not statistically analysed in this study. The activity, labour and exercise load of the hand could also affect the occurrence of tendinitis and fasciitis.[6 7] Were the HC and other groups in the same conditions? However, this information was not provided in the text. It is difficult to tell whether inflammation around the interosseous muscles was caused by RA or mechanical factors.

In addition to all the above, there are some other issues that puzzled us. The word 'epitendon' was used in the illustration of figure 2 and some sections. It was incorrect and lead to a misunderstanding. According to our understanding, it should be changed to 'peritendon'. Besides, 'EPM', 'MF' and 'EM' were marked in figure 2, but there was no explanation for these abbreviations.

We respect the great contributions of the authors and would also be very much interested in the authors' response to these issues.

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# Response to: 'Interosseous tendon inflammation of rheumatoid arthritis: what's the real meaning?' by Deng *et al*

We thank Deng for their interest in our study,[1] in which we identified MRI interosseous tendon inflammation (ITI) in anti-cyclic citrullinated peptide positive at-risk individuals (CCP+ at risk) without clinical synovitis.[2] Given the MRI appearances and absence of a tendon sheath on histological examination, we suggested ITI is a peritendonitis rather than a tenosynovitis. ITI was originally described as a tenosynovitis by Rowbotham *et al*.[3] However, in the discussion, it was acknowledged that this may not be the correct terminology as the MRI features were not typical of tenosynovitis and the microstructure of the tendons had not been well described.[3] Indeed, it was conceded that ITI may be better described as peritendinous inflammation or 'paratenonitis' rather than a true tenosynovitis. The lack of a tendon sheath demonstrated in the current study certainly supports this assertion.

We were interested in the view that ITI represents a fasciitis, which may be considered an extra-articular manifestation of rheumatoid arthritis (RA), similar to rheumatoid lung or rheumatoid vasculitis.[1] We agree that ITI, like these other features, may be viewed as an extra-articular consequence of RA autoimmunity. However, other extra-articular manifestations in RA are not periarticular and have different associations, generally seen in the setting of longstanding joint disease with increased prevalence in males and smokers.[4] They are also very unusual to find in at-risk individuals prior to the development of arthritis. Instead, ITI appears to frequently precede arthritis and occurs adjacent to the metacarpophalangeal joints, raising important questions about its role in the development of RA.

Although, to the best of our knowledge, the current study is the largest MRI study in CCP+ at-risk individuals, we agree larger studies should be done to confirm our findings. We also agree that ITI may not be specific to RA, and may be associated with mechanical factors or other conditions; we could not assess these factors in our study and acknowledged this in the discussion of the manuscript. Deng questioned whether the results could be gender-biassed as our subjects were predominantly female.[1] However, RA is more frequent in females, with a sex ratio of around 3:1. As such, we aimed to describe ITI and its associations in a population representative of the condition of interest rather than the general population. Similarly, the mean age of the RA patients included in our study was between 50 and 60 years, which is representative of the RA demographic.[5] Whether ITI is as prevalent in different age groups is an interesting question, which could be addressed in future work.

In describing histological findings, we followed the histological terminology of the Federative International Committee on Anatomical Terminology (2008).[6] We regret that some of the abbreviations were accidentally omitted from the figure legend (EM, endomysium; EPM, epimysium; MF, muscle fascicles).

Finally, we agree that it would be interesting to know if peritendinous inflammation or fasciitis is a generalised phenomenon found at other sites in symptomatic at-risk individuals. For example, CCP+ at-risk individuals often present with foot pain without synovitis[7] and it is possible that extracapsular inflammation may be responsible. Further imaging studies would certainly be useful in this regard.

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# Comment on: 'Idiopathic inflammatory myopathies and antisynthetase syndrome: contribution of antisynthetase antibodies to improve current classification criteria' by Greco *et al*

With great interest, we read the letter titled 'Idiopathic inflammatory myopathies and antisynthetase syndrome: contribution of antisynthetase antibodies to improve current classification criteria' by Greco *et al*[1] published in the *Annals of the Rheumatic Diseases*.

The authors analysed if the detection of anti-aminoacyl transfer RNA synthetase (ARS) autoantibodies other than anti-Jo1 could improve the European League Against Rheumatism/Amercian College of Rheumatology (EULAR/ACR) classification criteria[2] for adult and juvenile idiopathic inflammatory myopathies (IIM) and classification of antisynthetase syndromes (ASSD). These analyses were performed retrospectively assessing a cohort of 37 patients with clinical suspicion of IIM or ASSD and positive ARS using myositis immunoblots. The authors observed that all patients with clinically objectified muscle weakness and positivity for non-anti-Jo1 ARS did not fulfil EULAR/ACR IIM criteria but could be re-classified as IIM, if assigning non-anti-Jo-1 ARS the same weight as anti-Jo1 ARS.

We appreciate the effort of Greco *et al* to highlight the importance of ARS autoantibodies. We believe, however, that careful interpretation of ARS autoantibody status is necessary as various autoantibody assays are currently used, often resulting in misleading results. Furthermore, in a recent analysis[3] at our centre, we could show that only 27/160 (17%) individuals with ARS autoantibodies (using immunoblot technique) had clinical evidence for ASSD presenting with at least one of the triad findings: arthritis, myositis and interstitial lung disease. It would, therefore, be interesting to know if ARS autoantibody status was validated. In an effort to improve and harmonise the classification of ASSD, the CLASS (classification criteria of ASSD) project has recently been funded by the American College of Rheumatology and the European League Against Rheumatism. It will be interesting to see if similar results can be repeated using a large and carefully selected cohort.

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# Response to: 'Comment on: 'Idiopathic inflammatory myopathies and antisynthetase syndrome: contribution of antisynthetase antibodies to improve current classification criteria' by Greco *et al*' by Knitza *et al*

We have with great interest read the letter entitled 'Response to: 'Idiopathic inflammatory myopathies and antisynthetase syndrome: contribution of antisynthetase antibodies to improve current classification criteria' by Greco *et al*' by Knitza *et al*, to be published in the *Annals of the Rheumatic Diseases*.[1]

We appreciate the thoughtful comments made by the authors and agree on the importance of aminoacyl transfer RNA synthetase (ARS) autoantibodies, as well as other autoantibodies for correct classification of the associated diseases. The discussion stems from the European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification of idiopathic inflammatory myopathies (IIM) where anti-Jo-1 autoantibody positivity is included.[2] The authors emphasise the importance of careful interpretation of assays used to detect anti-ARS autoantibodies. In the centre of the authors, they found a low specificity for positive anti-ARS autoantibodies in relation to clinical manifestations of anti-synthetase syndrome (ASSD) where only 17% of their cases with positive anti-ARS antibodies had one of the clinical manifestations including arthritis, myositis or interstitial lung disease (ILD). We completely agree with the concern of the authors. This concern applies to the use of tests for myositis-specific autoantibodies in general, and not only for the anti-ARS autoantibodies, in clinical settings as a tool for diagnosis and classification. We agree with the authors that validation of commercially available immune blot techniques is important. We also want to emphasise that the positive predictive value depends on the context in which the anti-ARS antibodies are tested. In a recent study from our group, we found a high agreement between anti-Jo-1 positivity by a line blot assay and clinical manifestation of ASSD with ILD present in 15/18 (83%) anti-Jo-1+ patients with IIM. This was similar to the presence of ILD in 11/12 anti-Jo-1+ patients by immunoprecipitation.[3] The results presented by Knitza *el al* emphasise the importance to limit the testing of anti-ARS antibodies to patient populations with a high suspicion of ASSD or IIM. As anti-Jo-1 autoantibodies are one of the variables in the 2017 EULAR/ACR classification criteria for IIM, we want to underline careful interpretation of commercially available assays and welcome more validation studies that support the value of different assays.

Within the Euromyositis register collaboration, our continued efforts to standardise and harmonise methods for systematic collection and handling of samples, as well as analysing and interpreting autoantibody data have been implemented.[4] These data will provide a basis for future revision of classification criteria and evaluation of other autoantibodies in this context.[5]

We appreciate and commend all initiatives and comments aimed at improving the accuracy of the EULAR/ACR classification criteria for IIM and encourage the use of the criteria to evaluate their precision as well as clinical relevance.

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# Antisynthetase antibodies in clinical laboratories: the importance of clinical correlation and indirect immunofluorescence. Response to: Comment on: 'Idiopathic inflammatory myopathies and antisynthetase syndrome: contribution of antisynthetase antibodies to improve current classification criteria' by Greco *et al*' by Knitza *et al*

We have recently published a retrospective two centres study of 37 patients with clinical suspicion of idiopathic inflammatory myopathies (IIM) or antisynthetase syndrome (ASSD), and antiaminoacyl-transfer RNA synthetase (ARS) autoantibodies in the myositis immunoblot. In it, we discussed the role of the ARS in the IIM and the ASSD classification criteria, and a possible overlapping between both of them.[1]

Regarding the detection of ARS, previous studies have shown differences in the specificity between different commercial assays; thus, in agreement with the highly appropriate commentaries raised by Knitza *et al* on our report, we consider that a careful interpretation of them is mandatory.[2 3] In this way, in a recent review Damoiseaux *et al* proposed that to safeguard a high specificity of myositis-specific autoantibodies in multispecific-assays, it could be useful[1]: to establish adequate cut-off values in the immunoblot[2]; to correlate the results with another monospecific-assay (ie, ELISA or immunoprecipitation test) or[3] with the HEp-2 indirect immunofluorescence assay (IIFA); and[4] to correlate them also with the clinical information.[2] Additionally, it is interesting to mention that recent recommendations of the International Consensus on Antinuclear antibodies Patterns (ICAP), describes that not all ARS produce an IIFA pattern and that they are more likely to present AC-19 (dense fine speckled) and AC-20 (fine speckled) patterns.[4]

As for the daily practice application of these methodological aspects in our laboratories. (1) The blot assay used was the Euroline myositis profile three by Euroimmun, and the manufacturer's reference ranges were respected.[1] (2) All cases with anti-Jo1 positivity were validated by ELISA; nevertheless, as in most clinical laboratories, we do not have ELISA for other than anti-Jo1 ARS or immunoprecipitation test availability. (3) All cases were correlated with IIFA; however, due that it is not sufficiently sensitive for the spectrum of ARS, cases with clinical suspicion of IIM or ASSD were included in our series even without presenting a cytoplasmic pattern.[2] (4) In our laboratories, the myositis immunoblots are performed only under an adequate clinical suspicion; thus, 33 of 37 cases (89.2%) presented at least one of the clinical manifestations included in the classic triad of the ASSD (table 1).

Concerning the IIFA's, it is interesting to mention that we observed differences in how our laboratories reported them. In one centre they were reported as positive (title ≥1/160) or negative, without discriminating its pattern; thus, only six of 10 analysed cases were reported as positive, including those that fulfilled Solomon's ASSD criteria (n=2). In the other centre, whose Autoimmunity Laboratory has been externally certified by the UK National External Quality Assessment Service for the last 15 years, the IIFA reports were more rigorous. In it, all cases (n=27) presented positive IIFA (title ≥1/80): 19 cases (70.4%) with a cytoplasmic speckled pattern or AC-19/AC-20 pattern if were performed after the first ICAP publication in 2015 (10 of them presented an associated nuclear pattern), and eight cases (29.6%) presented only a nuclear pattern. Evaluating the ASSD diagnosis criteria, 25 cases (92.6%) fulfilled those of Connors', and 15 of them (55.5%) also met Solomon's criteria. Correlating these, 13 of 27 cases with clinical suspicion of IIM or ASSD and positive ARS (48.1%) presented a cytoplasmic speckled IIFA pattern and also fulfilled Solomon's criteria; representing the 68.4% of the cases with these IIFA patterns and the 86.6% of those that met Solomon's criteria. The other two cases that fulfilled Solomon's criteria (13.3%) presented only a nuclear pattern.

To conclude, in line with previous studies, our results suggest that an adequate ASSD clinical suspicion and the presence of cytoplasmic speckled patterns in the IIFA, can safeguard the specificity of the ARS detected by myositis immunoblots, and also increases the probability of fulfilling Solomon's ASSD criteria. Additionally, the differences observed in the IIFA reports between our centres highlights the importance of the International Autoantibody Standardization and the ICAP initiatives[4–6];

**Table 1** Clinical manifestations and ASSD diagnosis criteria fulfilment in patients with positive antisynthetase antibodies*

| | Anti-JO1 (n=17) | Anti-PL12 (n=8) | Anti-PL7 (n=4) | Anti-EJ (n=4) | Anti-OJ (n=4) | Total (n=37) |
|---|---|---|---|---|---|---|
| Clinical manifestations included in ASSD diagnosis criteria | | | | | | |
| Arthritis† | 13 (76.5) | 3 (37.5) | 1 (25) | 1 (25) | 3 (75) | 21 (56.7) |
| Interstitial lung disease† | 10 (58.8) | 3 (37.5) | 3 (75) | 1 (25) | 3 (75) | 20 (54.1) |
| Muscle weakness† | 8 (47.1) | 5 (62.5) | 2 (50) | 1 (25) | 2 (50) | 18 (48.6) |
| Raynaud phenomenon | 7 (41.2) | 2 (25) | 1 (25) | 2 (50) | 2 (50) | 14 (37.8) |
| Mechanic hands | 7 (41.2) | 2 (25) | 0 (0) | 0 (0) | 2 (50) | 11 (29.7) |
| Fever without known cause | 5 (29.4) | 1 (12.5) | 1 (25) | 0 (0) | 1 (25) | 8 (21.6) |
| Sum of classic triad components | | | | | | |
| 3/3 | 2 (11.8) | 1 (12.5) | 0 (0) | 0 (0) | 1 (25) | 4 (10.8) |
| 2/3 | 11 (64.7) | 3 (37.5) | 2 (50) | 0 (0) | 2 (50) | 18 (48.6) |
| 1/3 | 3 (17.6) | 2 (25) | 2 (50) | 3 (75) | 1 (25) | 11 (29.7) |
| 0/3 | 1 (5.9) | 2 (25) | 0 (0) | 1 (25) | 0 (0) | 4 (10.8) |
| ASSD diagnosis criteria | | | | | | |
| SOLOMON | 11 (64.7) | 2 (25) | 1 (25) | 0 (0) | 3 (75) | 17 (45.9) |
| CONNORS | 16 (94.1) | 7 (87.5) | 3 (75) | 4 (100) | 4 (100) | 34 (91.9) |

*Results are expressed as number (percentage).
†Clinical classic triad components.
ASSD, antisynthetase syndrome.

whose implementation in clinical laboratories could facilitate the development of multicentre studies, and consequently the evaluation of low-frequency antibodies and also of different IIFA patterns to be considered in future IIM and ASSD classification criteria.

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# Response to: 'Can IL-1 be used as a target for osteoarthritis?' by Cheng *et al*

We thank Cheng, Tian and Zhang[1] for their interest in our article[2] that showed that targeting interleukin (IL)-1α and IL-1β with lutikizumab did not significantly improve clinical and imaging outcomes in patients with inflammatory erosive hand osteoarthritis (HOA). The results of the study were indeed disappointing and, appropriately, should stimulate discussion about the role of IL-1 in osteoarthritis.

In our trial, levels of IL-1 were significantly reduced in subjects with erosive HOA and moderate to severe inflammation indicated by joint swelling, joint pain and synovitis.[1] Although levels of IL-1 were not measured after week 4 of treatment, other biomarkers (eg, neutrophils, high-sensitivity C-reactive protein and matrix metalloproteinase–degraded collagen type 1) were monitored for 26 weeks and also exhibited significant reductions with lutikizumab compared with placebo (figure 3 in the original article). Modest differences between lutikizumab and placebo were observed for several other biomarkers through week 26 (online supplementary table 6 in the original article), although additional biomarkers showed no such effects. This suggests that the pharmacodynamic effects of lutikizumab were not limited to short-term changes in concentrations of IL-1 but rather were consistent with the long-term neutralisation of IL-1α and IL-1β.

We agree that assessing patient-rated, quality-of-life (QoL) and functional outcomes in clinical trials is important to understand fully the potential impact of the therapy under consideration. Regarding patient global assessment, health-related QoL and functional outcomes in our study, we refer Chen *et al* and other readers to supplementary table 4, which is available online at the journal's website and which is called out in the original article as follows: "Other efficacy outcomes (pain, stiffness, grip strength and patient-reported outcomes) were also not different between the placebo and lutikizumab groups (online supplementary table 4)". In line with the main results of the study, there were no significant differences between lutikizumab and placebo in changes from baseline to week 26 for any of the endpoints described in online supplementary table 4, including Australian/Canadian Osteoarthritis Hand Index Stiffness scale, subject assessment of hand pain intensity, patient global assessment, grip strength of the index hand, 36-item short-form health survey, Patient Reported Outcomes Measurement Information System Physical Function Questionnaire, Michigan Hand Outcomes Questionnaire and Functional Index for Hand Osteoarthritis.

Finally, similar to our results, a recently published article about a randomised, placebo-controlled, double-blind phase II study of lutikizumab in subjects with knee osteoarthritis demonstrated no benefit of active treatment compared with placebo.[3] Taken together, these two studies of lutikizumab call into question the strategy of targeting IL-1 in patients with osteoarthritis.

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