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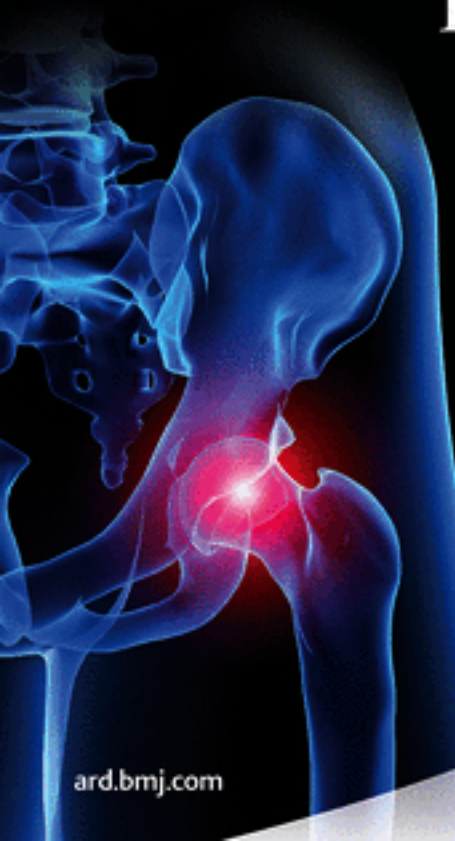

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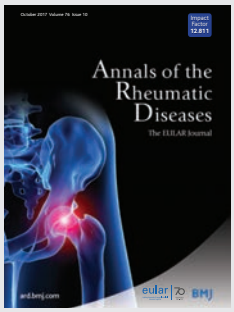

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Thank you and goodbye!

Tore K Kvien

I am grateful for the opportunity to be Editor in Chief for *Annals of the Rheumatic Diseases* (*ARD*) since 1st of April 2008. I was fortunate to succeed Professor Leo van de Putte who had been the successful Editor of this European League Against Rheumatism (EULAR) journal since 1999.[1] Importantly, he was my teacher when I started.[2] Editorial responsibility for a leading scientific journal means that you need to cover a broad area, that is, research work from 'bench to bedside'. My first step was actually to identify weaknesses in my own knowledge and invite experts in these areas as associate editors or editorial board members to fill these gaps of knowledge. These colleagues have all been extremely important for the increasing success of *ARD* during the recent years.

What has then been achieved? First, the impact factor (IF) has been increasing and reached the highest number ever in rheumatology this year (IF 2016 for ARD announced in June 2017 was 12.811). Most rheumatologists have during the recent years considered *ARD* as the world leading journal in rheumatology. We have seen an increasing number of submissions—but the annual number has been stable around 2000 during the most recent years. The acceptance rate for original research articles is now around 12% and the instant reject rate about 70%. Editorial decisions for instantly rejected papers occur in average after 7 days and after 43 days for papers which have been to external review. I feel confident that the quality of published papers has improved—and I am very satisfied to see that the journal publishes a good mixture of high quality science not only in clinical and epidemiological research, but increasingly also in basic and translational science. Most importantly, I think *ARD* is also presenting results from research that will lead to better patient care when new

findings are implemented into clinical practice. The development of lay summaries of selected articles (usually three per issue) has also supported dissemination of research findings that can enhance patient care.

The editorial process is a team work and I am very grateful for the important contributions from many people. The associate editors and the editorial board members have been essential both as advisors, second opinion assessors and reviewers. I am also in particular grateful for the work performed by the editorial assistant, Christine Janssen-Seijkens, based on her knowledge, experience and dedication. The statistical advisor has contributed a lot to the scientific quality of the published papers.[3] More recently, the journal has also benefited from graphic advises. Another recent new important initiative has been to establish a social media group from Emerging EULAR Network.[4] I have enjoyed the support, collaboration and friendship with the editorial team of the BMJ Publishing Group as well as the leadership group of EULAR. Finally, the journal could never have achieved its current standard without the support from many colleagues who submit their high quality research papers to *ARD*—and not least the external reviewers who provide excellent assessments of papers sent out for external review.

I have immensely enjoyed these years as Editor of *ARD*, but it is now time for a replacement. I am very happy about the selection of my good friend and colleague, Professor Josef Smolen (Vienna, Austria), as my successor. He started to handle new submissions as of 1st September (I will continue to handle papers which I have sent out for review until final decisions have been made). I am confident that Josef will maintain and further enhance elements in the editorial process that have been successful, but also that he will bring new and innovative ideas which will further strengthen the quality and standing of the journal. I wish Josef and his editorial team all the best for the coming years!

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The new editor greets you

Josef S Smolen

It is a great privilege and honour to assume the position of editor of the *Annals of the Rheumatic Diseases–The EULAR Journal* and I am very grateful to the selection committee consisting of members from both constituencies for granting me this opportunity.

Even bigger is the challenge to take on this position as a successor of Professor Tore Kvien. During the 9 years of his editorship, Professor Kvien has continued the work of the founding Editor after initiation of the collaboration between BMJ and *EULAR*, Professor Leo van der Putte, and has furthered the impact of *The EULAR Journal* with prudent and far-sighted editorial decisions. These have allowed the *Annals* to become the leading journal in the field of rheumatology. While a success like this is impossible without receiving good manuscript and especially an excellent team of associate editors, editorial board members and reviewers, Tore's visions and leadership were instrumental for this development. Thank you very much, Tore!

The accomplishments of the *Annals* during the 18 years since it became *The EULAR Journal* are so remarkable that much change of the editorial policy is not needed. But there will be a few. Foremost, the team of associate editors as well as the editorial board and the advisory committee will undergo some modification. Many thanks to everyone who has contributed so importantly during the last years—without this support the journal's success would not have been possible. And a wholehearted welcome to the team that will support the efforts during the next term—the editorial team is the major asset of every journal. It will also be a pleasure to collaborate with the team of the BMJ Publishing Group and the EULAR leadership, who have all had important roles in the development of the journal.

Further, plans for some new sections have been made. One of these sections will be called 'Views on News' and is meant to briefly highlight breakthrough research from outside rheumatology that may be pertinent to advance our field. Another

section, 'Heroes and Pillars of Rheumatology', will be devoted to persons or scientific innovations of the past that were pivotal in advancing our discipline—past research tends to be forgotten, younger researchers often do not know enough about the evolution within our specialty and the wheel is often reinvented. This information should not only bring historical aspects into awareness, but also allow to stimulate some novel scientific approaches—much can be learnt from the basics. And thirdly, so that we not only look at the past or at other disciplines, the 'Thinking the Unthinkable' section should solicit and be provided with ideas on accomplishments or developments that may constitute fundamental advancements within our field, but only be realisable within a decade or two—visions, dreams to foster search and most innovative research. I am grateful that Ferdinand Breedveld, Gerd Burmester, Maxime Dougados and David Pisetsky have taken on to lead one or more of these new areas.

Finally, it should be noted that *EULAR*, alone or in collaboration with *ACR*, has developed a number of recommendations and criteria, such as on reporting data from clinical trials in rheumatoid arthritis.[1] Not only is adherence to these (and also other) recommendations, as the smallest denominator for clinical trials reports (more can always be added), scientifically important, but it also facilitates future meta-analyses and systematic reviews. The author information will be amended accordingly.

The *Annals of the Rheumatic Diseases* was founded in 1939 and the joint ownership of the Journal by and collaboration between *BMJ* and *EULAR* started in 1999; thus, the year 2019 will mark a special dual anniversary which to celebrate appropriately will be one of the objectives of the upcoming editorial term. Appropriate celebration materialises primarily through excellent publications, and it is needless to say that Professor Kvien's path and standards constitute a model to be continued during the next years. It is 'value and interest' of manuscripts related to clinical, epidemiological, basic and translational research that will govern editorial decision making in the future as it in the past.

It can hardly be said better today than stated in 1939 in the first issue of the first volume of the *Annals*, when Sir William Willcox, the Chairman of the Empire Rheumatism Council at that time, wrote: 'It is intended that the new journal shall consist entirely of original papers and reports, specially selected for their value and interest'.[2] And he ended his foreword by stating: '…in view of the great activity in so many departments of research into the many manifestations of rheumatic diseases, they may rest assured that they will find no lack of material for the publication …[in] this valuable journal'.

In this light, it is my desire, hope and expectation that the *Annals* will continue to attract the best research work of our field. I also look forward to receiving advice and suggestions for further advancement of the journal's quality from all stakeholders, especially the readership of the *Annals*. Thank you very much in advance for your support!

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European evidence-based recommendations for diagnosis and treatment of paediatric antiphospholipid syndrome: the SHARE initiative

Noortje Groot,[1,2] Nienke de Graeff,[1] Tadej Avcin,[3] Brigitte Bader-Meunier,[4] Pavla Dolezalova,[5] Brian Feldman,[6] Gili Kenet,[7] Isabelle Koné-Paut,[8] Pekka Lahdenne,[9] Stephen D Marks,[10] Liza McCann,[11,12] Clarissa A Pilkington,[10] Angelo Ravelli,[13] Annet van Royen-Kerkhof,[1] Yosef Uziel,[14] Sebastiaan J Vastert,[1] Nico M Wulffraat,[1] Seza Ozen,[15] Paul Brogan,[10] Sylvia Kamphuis,[2] Michael W Beresford[11,12]

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ABSTRACT

Antiphospholipid syndrome (APS) is rare in children, and evidence-based guidelines are sparse. Consequently, management is mostly based on observational studies and physician's experience, and treatment regimens differ widely. The Single Hub and Access point for paediatric Rheumatology in Europe (SHARE) initiative was launched to develop diagnostic and management regimens for children and young adults with rheumatic diseases. Here, we developed evidence-based recommendations for diagnosis and treatment of paediatric APS. Evidence-based recommendations were developed using the European League Against Rheumatism standard operating procedure. Following a detailed systematic review of the literature, a committee of paediatric rheumatologists and representation of paediatric haematology with expertise in paediatric APS developed recommendations. The literature review yielded 1473 articles, of which 15 were valid and relevant. In total, four recommendations for diagnosis and eight for treatment of paediatric APS (including paediatric Catastrophic Antiphospholipid Syndrome) were accepted. Additionally, two recommendations for children born to mothers with APS were accepted. It was agreed that new classification criteria for paediatric APS are necessary, and APS in association with childhood-onset systemic lupus erythematosus should be identified by performing antiphospholipid antibody screening. Treatment recommendations included prevention of thrombotic events, and treatment recommendations for venous and/or arterial thrombotic events. Notably, due to the paucity of studies on paediatric APS, level of evidence and strength of the recommendations is relatively low. The SHARE initiative provides international, evidence-based recommendations for diagnosis and treatment for paediatric APS, facilitating improvement and uniformity of care.

INTRODUCTION

The antiphospholipid syndrome (APS) is defined by vascular thrombosis or pregnancy morbidity (including premature births due to eclampsia, severe pre-eclampsia or unexplained fetal death), combined with persistently positive antiphospholipid antibodies (aPLs).[1] The routine tests to screen for aPL are lupus anticoagulant (LA); anticardiolipin antibodies (aCL) IgG and/or IgM; and/or anti-β2 glycoprotein-I antibodies (anti-β2GPI) IgG and/or IgM.[1] Primary APS is not as well defined in children but comprises the finding of aPL combined with thrombosis.[2] APS may occur in association with other disorders, including particularly childhood-onset systemic lupus erythematosus (cSLE). Patients with cSLE who are positive for aPL can present with aPL-related clinical manifestations.[3–6]

Although APS and SLE can share biological and clinical manifestations, immunogenetic studies in adult patients showed that the two diseases display different combinations of susceptibility genes, suggesting that they are two distinct disease entities.[7–9] In paediatric patients, APS is more commonly associated with SLE than in adults,[3] but no immunogenetic studies were performed in paediatric population.

In 2004, the international Ped-APS registry was initiated to determine the extent and characteristics of paediatric APS. This registry has identified 121 cases of paediatric APS in 14 European countries. Of these patients, 50% had primary APS and 41% had APS in association with cSLE or lupus-like disease.[2 3]

The low prevalence of APS impedes translational research, resulting in a lack of evidence to inform guidelines for disease management. Treatment approaches can differ even within centres and are mostly based on adult-derived studies, anecdotal evidence based on case series in children and clinical expertise. International collaboration is necessary: only by sharing expertise can we optimise and disseminate best practices regarding diagnosis and management of these rare diseases. For this reason, the Single Hub and Access point for paediatric Rheumatology in Europe (SHARE) project was initiated.[10] One of the objectives of this project was to identify best practices for diagnosis and management of paediatric rheumatic diseases (PRDs), including paediatric APS. SHARE-recommendations for juvenile dermatomyositis and autoinflammatory diseases have already been published.[11–13]

METHODS

A European-wide panel of 16 paediatric rheumatologists along with representation of paediatric haematology, with expertise in paediatric APS, was established to develop evidence-based

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recommendations. A project plan for the systematic literature search was written following the European League Against Rheumatism standardised operating procedure of recommendations.[10 14] In short, a systematic literature review based on specific research questions was performed in the MEDLINE, EMBASE and Cochrane databases in July 2013 (see online supplementary table 1). Relevant articles were selected by two authors (NG, NdG) based on predefined inclusion and exclusion criteria (see online supplementary table 2). All selected articles were independently reviewed by two experts (TA, MWB), who were responsible for data extraction, assessment of level of evidence and of methodological quality, according to a predefined proforma (see online supplementary table 3).[15 16] Provisional recommendations for the diagnosis and treatment of paediatric APS were based on the results of the literature review mapped against the initial research questions. The strength of the recommendations was based on the level of evidence relevant for the specific recommendation (see online supplementary table 4). Recommendations were discussed and finalised in two face-to-face consensus meetings (Genoa, March 2014, and Barcelona, March 2015), where the nominal group technique (NGT) was used to reach consensus (defined by at least 80% agreement) among the expert panel.[17]

RESULTS

A total of 1473 articles were found on paediatric primary APS or APS in association with other diseases. After screening on title and abstract and assessing the full text for relevance, 11 articles on primary paediatric APS, 9 articles regarding paediatric APS associated with cSLE and 4 articles on paediatric Catastrophic Antiphospholipid Syndrome (CAPS) fulfilled the inclusion criteria. Sixteen articles were considered to be valid (see online supplementary table 5). The consensus meetings resulted in 14 final recommendations (figure 1). Nine statements were accepted for diagnosis and management of paediatric APS, two statements for children born to mothers with APS being accepted and three statements for treatment of paediatric CAPS (table 1).

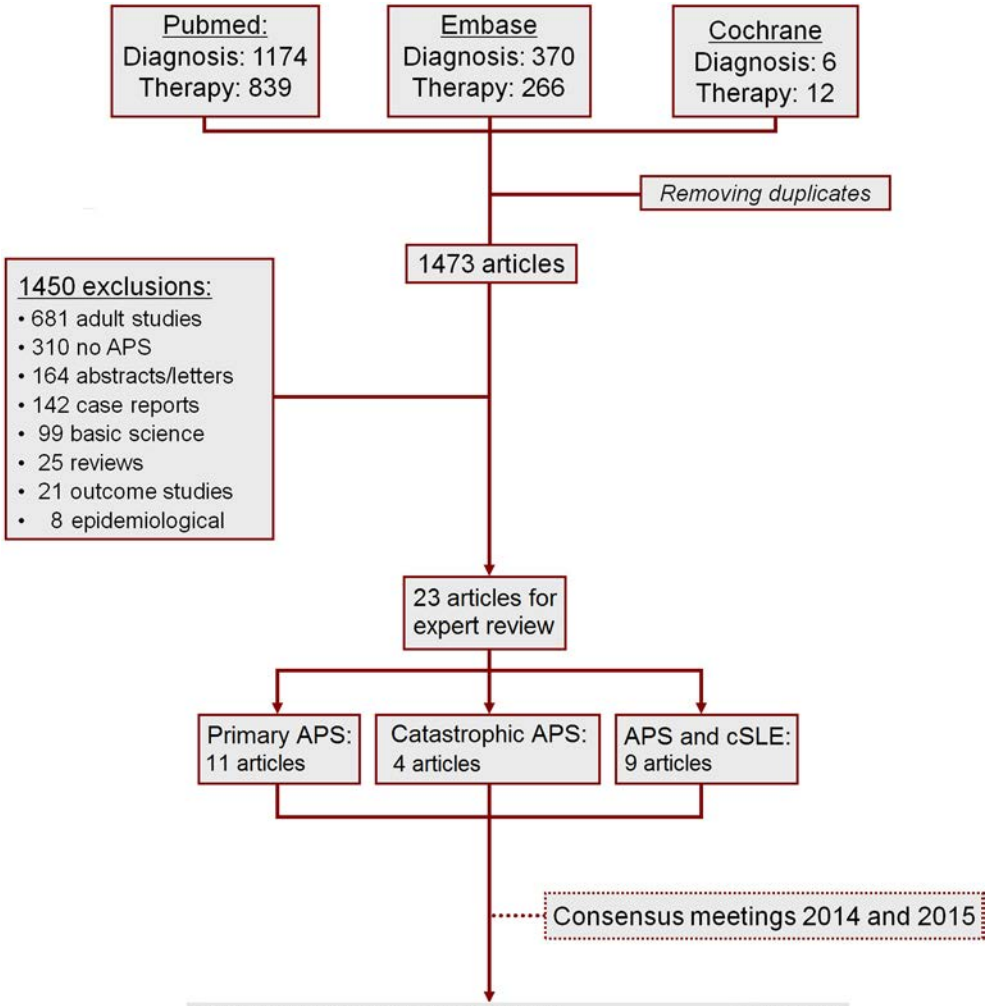

Figure 1 Summary results from the systematic literature review. APS, antiphospholipid syndrome; cSLE, childhood-onset systemic lupus erythematosus.

Table 1 Recommendations for the paediatric antiphospholipid syndrome (APS)

	Level of evidence	Strength	Agreement (%)
Diagnostic recommendations			
1. The adult criteria for APS, while specific, may lack sensitivity for paediatric APS.	3	C	100
2. New classification criteria for paediatric APS are needed that would incorporate non-thrombotic manifestations in children, in addition to thrombosis.	4	D	100
3. The following tests should be performed when suspecting paediatric APS: lupus anticoagulant, anticardiolipin IgG and IgM and anti-β2-glycoprotein-I IgG and IgM.	2A/B	B	100
4. aPL screening should be performed in all patients with cSLE.	3	C	100
Treatment recommendations			
1. In patients with cSLE and aPL, antiplatelet agents could be considered for primary prevention of thrombosis in addition to hydroxychloroquine.	3	C	100
2. When a patient has suffered a venous thrombotic event, anticoagulation therapy is indicated when manifestations are related to aPL.	3	C	100
3. When a patient has suffered a venous thrombotic event associated with persistent aPL positivity, long-term anticoagulation therapy is indicated.	3	C	100
4. When a patient has suffered an arterial thrombotic event associated with persistent aPL positivity, adequate long-term anticoagulation therapy or combined anticoagulation and antiaggregation therapy is indicated.	3	C	100
5. When a patient has suffered a recurrent thrombotic event associated with persistent aPL positivity despite oral anticoagulation with a target INR 2.0–3.0, long-term anticoagulation therapy to a target INR 3.0–4.0 or alternative therapies such as extended therapeutic dose of low-molecular- weight heparin yielding a target anti-Xa is indicated.	3	C	100
Recommendations for children born to mothers with APS			
1. Perinatal thrombosis associated with aPL is a very rare complication in infants born to mothers with positive aPL. Recurrence rates in infants with perinatal thrombosis are extremely low and there are no uniform guidelines for the therapeutic approach. In general, infants with perinatal arterial ischaemic stroke associated with aPL should not usually receive anticoagulation.	3	C	100
2. Children born to mothers with APS may exhibit a higher frequency of neurodevelopmental abnormalities; regular neurodevelopmental assessments during their long-term follow-up may be considered.	3	C	87
Recommendations for treatment of paediatric CAPS			
1. In a patient with paediatric CAPS, immediate combination treatment with anticoagulants, corticosteroids, plasma exchange with or without intravenous immunoglobulins should be considered.	3	C	100
2. In a patient with paediatric CAPS, rituximab or other immunosuppressive therapy may also be considered as a treatment option.	3	C/D	100
3. In CAPS, there are too few data to support the routine use of antiaggregation therapy.	4	D	100

Level of evidence; for diagnostic and observational studies: 1A, meta-analysis of cohort studies; 1B, meta-analysis of case–control studies; 2A, cohort studies; 2B, case–control studies; 3, non-comparative descriptive studies; 4, expert opinion; and for treatment studies: 1A, meta-analysis of randomised controlled trial; 1B, randomised controlled study; 2A, controlled study without randomisation; 2B, quasi-experimental study; 3, descriptive study; 4, expert opinion; Strength, strength of recommendation: A, based on level 1 evidence; B, based on level 2 or extrapolated from level 1; C, based on level 3 or extrapolated from level 1 or 2; D, based on level 4 or extrapolated from level 3 or 4 expert opinion,[8–10] Agreement indicates % of experts agreeing on the recommendation during the final voting round of the consensus meeting.
aPL, antiphospholipid antibodies; CAPS, Catastrophic Antiphospholipid Syndrome; cSLE, childhood-onset systemic lupus erythematosus; INR, international normalised ratio.

Recommendations on the diagnosis of paediatric primary APS and APS associated with other diseases

The current classification criteria used for APS in adults include two clinical criteria: vascular thrombosis and pregnancy morbidity.[1] Although the former is also an important feature in paediatric APS, the latter was deemed less relevant in children. However, in addition to thrombosis, other non-thrombotic features such as haematological or neurological manifestations are also frequently present in children.[3 18–25] Incorporation of these clinical features in a specific set of classification criteria for paediatric APS is therefore important.

The prognosis of APS is dependent on the number, type and titre of specific aPL (LA, aCL, anti-β2GPI) present. When APS is suspected, screening for all three aPL types should be performed. As the prevalence of aPL in patients with cSLE can range from 11% to 87% (depending on aPL subtypes present), it is important to screen for aPL in all patients with cSLE at baseline.[5 26–34] Timely diagnosis and appropriate management of paediatric APS can then be facilitated. When the presence of aPL is detected, but no thrombotic events have occurred, a diagnosis of APS cannot be made.

There is increasing evidence of differences in the cut-off threshold for detection of positive aPL between paediatric and adult population.[35 36] The majority of data pertaining to paediatric APS literature however refer to the adult cut-off values.[31 37]

Recommendations on the treatment of paediatric primary APS and APS associated with other diseases

A meta-analysis examining the preventive effect of aspirin in patients positive for aPL demonstrated a significant decrease in the risk of first thrombotic event in those taking aspirin.[38 39] Furthermore, a study of seven children with acute cerebral infarction associated with the presence of aPL indicated that aspirin may be effective in the prevention of recurrent thrombotic events.[40] Extrapolating from adult evidence, while recognising paucity of specific evidence available in children, it was concluded that addition of an antiplatelet agent (such as aspirin at an antiplatelet dose) to the therapy of patients with cSLE who are positive for aPL should be considered, in addition to the use of hydroxychloroquine.

After a thrombotic event, long-term anticoagulation therapy is indicated when manifestations are related to persistent aPL

positivity. If the thrombotic event initially seemed to be related to aPL, but persistence of aPL was not found, long-term anticoagulation therapy is not indicated. Although no specific evidence is available supporting this directly in paediatric APS, it was considered reasonable to continue anticoagulation therapy in the case of persistent aPL positivity 0.1as the patient is prone to develop a second thrombotic event when aPLs remain positive.[3] If a patient has suffered an arterial thrombotic event associated with persistent aPL positivity, adequate long-term anticoagulation therapy or combined anticoagulation and antiaggregation (such as aspirin) therapy is indicated. If recurrent thrombotic events associated with persistent aPL positivity occur despite oral anticoagulation, a higher target international normalised ratio (INR) or alternative therapies should be considered. In all instances of paediatric APS associated with other diseases, the primary disease (including cSLE and other PRDs including systemic vasculitis) should be treated appropriately.

Recommendations for children born to mothers with APS

There are few data on the outcomes of children born to mothers with APS. A European registry was set up to follow these children prospectively.[41] None of the 134 children that were included developed perinatal thrombosis, illustrating the rarity of the event. Evaluation of the management of infants with recurring perinatal thrombosis should be done on a case-by-case basis. It was agreed that in general infants with perinatal arterial ischaemic stroke associated with aPL should not usually receive anticoagulation.

From the above registry experience during the 5-year follow-up, three children had impaired neuropsychological development (axial hypotony, autism, hyperactive behaviour and a combination of feeding disorders, language delay and growth failure).[41] It was recommended that neurodevelopmental assessment should be considered to detect these problems early on.

Recommendations for treatment of paediatric CAPS

CAPS is the most severe, acutely life-threatening form of APS, characterised by multiple organ involvement and extensive small vessel thrombosis.[42] By definition, manifestations develop simultaneously or in less than a week. Histopathology of small vessel occlusion is found in at least one organ or tissue, along with the presence of aPL, according to the validated classification criteria for CAPS.[43 44]

A recent subanalysis of 45 paediatric patients included in the CAPS registry showed minimal differences in clinical and laboratory features between adult and paediatric patients with CAPS . Similar to adults, mortality in this group was high (27%). None of the patients who received only partial treatment with anticoagulants, corticosteroids, plasma exchange, with or without intravenous immunoglobulins (IVIG), survived the catastrophic event.[45] Three paediatric CAPS patients reported in a case series received combination treatment (heparinisation with high-dose corticosteroids and IVIG, in one patient additional rituximab). This led to resolution of symptoms in all three patients.[46] These results underline the importance of immediate combination treatment with anticoagulants, corticosteroids, plasma exchange with or without IVIG in paediatric CAPS, as is advised in adults with CAPS.[47]

Evidence regarding the use of biologics or other immunosuppressive drugs is very sparse in paediatric CAPS.[46] Some evidence is available in adults with CAPS. Based on an analysis of 20 adult CAPS patients who were treated with rituximab, there may be a role for rituximab in patients with haematological and/or microthrombotic manifestations.[48] Adults with CAPS associated with SLE could benefit from treatment with cyclophosphamide.[49] The use of eculizumab, a complement pathway inhibitor, has been described in several case reports and may be promising in the prevention of recurrence of CAPS.[50–54] As evidence in the paediatric population is lacking for these medications, their use should be considered carefully and with caution.

DISCUSSION

Following systematic review of the literature and international NGT consensus methodology, a total of 14 recommendations regarding paediatric APS were accepted with at least 80% agreement. These recommendations should help specialists with the diagnosis and treatment of children with APS. There is very little evidence on paediatric primary APS and APS associated with other diseases; consequently, the recommendations have a low level of evidence and strength. The collaboration already initiated with the Ped-APS registry, combined with generalised treatment for these patients and with improved classification criteria for APS in children, should result in a good documentation of treatment outcomes of this patient population. The SHARE recommendations should be updated with the evidence of future publications from this registry to keep improving diagnosis and therapeutic strategies for these patients.

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EXTENDED REPORT

Acute coronary syndrome in new-onset rheumatoid arthritis: a population-based nationwide cohort study of time trends in risks and excess risks

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ABSTRACT

Background Acute coronary syndrome (ACS) and other cardiovascular diseases are the main drivers of the increased morbidity and preterm mortality in rheumatoid arthritis (RA). ACS in RA has been linked to inflammation and RA severity. During recent years and with new therapeutic options and treat-to-target strategies, increasing efforts have been made to reach RA remission as soon as possible after diagnosis, and the average level of RA disease activity has declined. Whether this has resulted in declining excess risks for RA comorbidities remains unclear.

Methods We performed a nationwide population-based cohort study of patients with new-onset RA from 1997 to 2014, and matched general population comparators. In the Swedish healthcare system, all residents have equal access to healthcare services. Healthcare is monitored using high-quality population-based registers that can be linked together. 15 744 patients with new-onset RA, identified from the Swedish Rheumatology Quality Register, and 70 899 general population comparator subjects were included.

Results Seven hundred and seventy two patients with RA developed an ACS during 103 835 person-years of follow-up (crude incidence, 7.4 per 1000), corresponding to an overall HR versus the general population of 1.41 (95% CI 1.29 to 1.54). Whereas the ACS incidence declined over calendar time in both the RA and the general population cohort, the excess and the relative risks of ACS remained the same.

Conclusions Despite improved disease control in new-onset RA, the elevated risk of ACS in RA remains a concern.

INTRODUCTION

Cardiovascular disease (CVD) is the main driver of the excess morbidity and preterm mortality in patients with rheumatoid arthritis (RA).[1–3] We and others have previously demonstrated an increased risk of acute coronary syndrome (ACS) in patients with RA compared with the general population, already within a few years of the RA diagnosis.[4] The mechanisms behind this risk increase are not completely elucidated, although the risk of ACS in RA has repeatedly been linked to RA disease activity and severity.[5–8] Because of this, and in addition to close monitoring of traditional cardiovascular risk factors, clinical cardioprotective recommendations in RA include active treatment aiming for control of the RA inflammatory activity.[9]

During the last two decades, more intense treatment strategies and new therapeutic options have been introduced in the management of new-onset RA, and the average level of RA disease control has improved. Taken together, 'modern rheumatology' might thus have impacted the risk of RA-associated comorbidities, including the elevated risk of CVD in RA.[10–13] Few studies have, however, addressed the risk of ACS in RA over calendar time periods and disease duration with focus on patients diagnosed with RA during the most recent decade. The aim of this study was therefore to investigate whether improved management in terms of treatment options, treatment algorithms and cardiovascular vigilance in new-onset RA collectively have led to a reduction in the excess risk of ACS.

METHODS

Design

We performed a nationwide population-based cohort study of patients with newly diagnosed RA (defined as RA diagnosis within 12 months of patient-reported symptom onset, to be able to assess the temporal development of ACS in relation to disease onset, not just diagnosis) with matched general population comparator subjects, based on prospectively recorded register data.

Setting

The publicly funded Swedish healthcare system enables access to all healthcare services, including specialised care for chronic diseases such as RA, for all residents. Patients with RA are diagnosed and cared for by rheumatologists or internists, typically in hospital outpatient settings. Prescribed drugs are subsidised and, beyond an upper annual limit of SEK2200 (approximately US$260), provided free of charge. All residents are assigned a unique personal identity number that can be used for linkage of different data resources, including several national health registers of high quality.[14]

Study population

The new-onset RA cohort

The Swedish Rheumatology Quality (SRQ) Register was initiated in 1995 and includes individuals aged 16 years or older fulfilling the 1987 American College of Rheumatology criteria for RA.[15] The current coverage (on a national basis) is estimated to above 80% of all new-onset RA.[16] The register collects information on age, sex,

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rheumatoid factor (RF) status, date of first symptom of RA, date of inclusion into the register and the personal identification number. The register also contains information on drug treatment and disease activity, for example, number of swollen and tender joints, erythrocyte sedimentation rate, serum concentration of C reactive protein, patient's and physician's assessment of global disease activity, and Disease Activity Score 28-joint count (DAS28), from the inclusion visit and onwards. For this study, we identified all individuals diagnosed with RA between 1 January 1997 and 31 December 2014 who were included in the register within 12 months of first symptoms of RA (n=16 214). Validations against other data sources suggest that less than 0.8% of all new-onset RA in SRQ represents prevalent RA misclassified as new-onset (D di Giuseppe, personal communication, SRQ 2016). For this study, the date of inclusion into the register (typically the date of RA diagnosis) was used as index date.

General population comparators cohort

For each unique patient with RA, we randomly selected up to five individuals from the Swedish Population Register (which includes all Swedish residents), matched on sex, year of birth and residential area (n=72 939). Each subject was assigned the same index date as the corresponding patient with RA.

Data sources used for follow-up and to detect ACS

Using the personal identification number, we linked the cohort of patients with RA and the matched general population comparator cohort with the following data sources, for which data were available through 31 December 2014: The National Patient Register, the Population Register, the Cause of Death register, and the Integrated Database for Labor and Education. The National Patient Register contains information on inpatient care since 1964, with nationwide full coverage since 1987.[17] The register lists date of admission, date of discharge and the discharge diagnosis (primary and secondary diagnoses) as set by the discharging physician and classified according to the calendar year-specific version of the International Classification of Diseases (ICD, since 1997: ICD-10). The Population Register includes information on deaths, emigration and immigration for the entire Swedish population. The Cause of Death register holds information on cause of death coded according to ICD. The Integrated Database for Labor and Education holds information on educational level. Through these linkages, we identified all hospitalisations and non-primary care outpatient visits, before or after index date, and all deaths and emigrations during follow-up. The nationwide and near complete coverage of the National Patient Register ensured very low (but formally not assessable) missingness.

Follow-up and occurrence of ACS

ACS was defined as hospitalisation listing a primary ICD-10 diagnosis of I21 (acute myocardial infarction) or I20.0 (unstable angina pectoris), or an acute myocardial infarction listed as the underlying cause of death. This definition has a positive predictive value of 95%.[18] In both cohorts, all individuals with a diagnosis of ACS prior to the start of follow-up were excluded. The RA cohort and the comparison cohort were followed from the index date until the first ACS, death, emigration or 31 December 2014, whichever came first.

Statistical analyses

Descriptive baseline data were summarised and presented as proportions, means and medians as appropriate. The incidence of ACS was described and assessed by dividing the number of ACS (overall and in subgroups based on sex, age and calendar year of diagnosis) with the corresponding person-years of follow-up. The excess incidence of ACS was defined as the difference between the incidence in the RA cohort and the corresponding incidence in the general population cohort. The HR (used as measure of relative risk) of ACS in RA compared with the general population was calculated using Cox' regression models adjusted for residential area, sex, year of diagnosis, age at diagnosis and educational level. Analyses were stratified by RF status, sex, age at index date, calendar period of index date, time since start of follow-up and DAS28 (≤3.2 and >3.2) at RA diagnosis. All analyses were carried out with SAS V.9.4 software package. This study was approved by the Stockholm Ethics Review Board.

RESULTS

After excluding individuals with a history of ACS at start of follow-up (470 (2.9%) patients with RA and 2040 (2.8%) general population comparator subjects), 15 744 patients with RA and 70 899 comparator subjects remained for analysis. Sixty-nine per cent of all individuals were women and the mean age at index date was 57 years (table 1).

On average, patients with RA had a somewhat lower level of education than the general population comparators (25% vs 29% with >12 years of education). When our study period was split into four groups as defined by calendar period of RA diagnosis, the gender distribution was stable, but the distributions of age (increasingly higher), RF status (increasingly more seronegative RA) and educational level (increasingly higher) varied over time (table 1).

Clinical RA characteristics

During the study period, the duration of RA symptoms at index date (diagnosis) decreased somewhat from 1997 to 2002 and from 2011 to 2014 (table 2). The disease activity at index date and at the return visit at 3–6 months also declined modestly. The use of any disease-modifying antirheumatic drug within the first year after RA diagnosis was high; the proportion prescribed glucocorticoids, methotrexate or biological drugs during the first year after RA diagnosis increased during the study period.

Incidence of ACS during follow-up

During 103 835 person-years of follow-up (median/interquartile follow-up=5.7/7.1 years) in the RA cohort, 772 individuals developed a first-ever ACS. In the comparator cohort (median/interquartile follow-up=5.7/7.3), 2418 individuals developed a first-ever ACS during 466 930 person-years of follow-up (table 3). In both the RA and the general population comparator cohort, the incidence of ACS was higher among men, increased with age and decreased markedly over successive calendar periods of start of follow-up (figure 1). During follow-up, 1685 (10.7%) of the patients with RA and 7336 (10.4%) of the general population subjects died. Seventy seven (0.5%) of patients with RA and 824 (1.2%) of the general population emigrated from Sweden during follow-up and where therefore censored at these time-points.

Table 1 Demographics at year of diagnosis among Swedish patients with rheumatoid arthritis (RA) overall and by calendar period of RA diagnosis, and in matched general population comparator subjects

	Patients with RA					General population
	All	1997–2002	2003–2006	2007–2010	2011–2014	All
	n=15 744	n=3422	n=3181	n=4179	n=4962	n=70 899
Women	69	70	69	69	70	69
Age, mean (SD)	15	15	15	15	16	15
Age at diagnosis						
<53 years	33	35	33	33	32	34
53–62 years	26	27	30	26	22	26
63–71 years	22	20	19	23	26	22
≥72 years	19	19	19	18	21	19
Educational level						
<9 years	30	38	32	28	26	28
10–12 years	44	40	43	46	46	42
>12 years	25	21	25	26	27	29
Seropositive RA	65	67	68	64	62	NA

Expressed in % if not stated otherwise.

Excess incidence of ACS by calendar period and time since RA diagnosis

Overall, the excess incidence of ACS increased from around 1/1000 person-years during the first year after RA diagnosis to between 2/1000 and 3/1000 person-years during the next 10 years. There was no evidence of any clear secular trend in the point estimates for excess risk, but the excess risk remained similar in all calendar periods (around 1/1000 person-years during the first year to between 2/1000 and 3/1000 person-years during the next 10 years).

Relative risk of ACS by calendar period and time since RA diagnosis

Overall, RA was associated with approximately 40% higher risk of ACS, HR 1.41 (95% CI 1.29 to 1.54, table 3). We noted statistically significantly increased risks, and similar relative risks, in all subsets defined by age and sex, but also that the excess risk was confined to patients with seropositive RA and to patients with DAS28 above 3.2 at diagnosis (table 3).

Further, within each calendar period of RA diagnosis, the overall relative risks remained similar: 1997–2002, HR=1.41 (95% CI 1.24 to 1.60); 2003–2006, HR=1.47 (95% CI 1.24 to 1.74); 2007–2010, HR=1.38 (95% CI 1.13 to 1.68); and 2011–2014, HR=1.19 (95% CI 0.85 to 1.67) (p=0.9). When the calendar years of RA diagnosis and RA disease duration were cross-tabulated, we noted similar HRs per follow-up interval for each calendar period of RA diagnosis (table 4).

The same pattern of lack of distinct calendar trend emerged when the same analyses were performed separately for seropositive RA and seronegative RA (for which the relative risks were typically not increased), and by DAS28 at diagnosis (online supplementary tables 1 and 2).

DISCUSSION

In this nationwide population-based study, we observed a close to 40% decline in the incidence of ACS in the general Swedish population in 1997–2012. In our inception cohort of patients with RA, the level of decline in incidence was similar, but the overall risk of ACS was approximately 40% higher than in the general population. The increase was restricted to patients with DAS28 above 3.2 at RA diagnosis and to patients who were RF-positive. Because the decline in incidence was equally pronounced in the RA cohort as in the general population, there was no evidence of any decline in the excess risk for patients diagnosed with RA in more recent years.

Studies evaluating time trends of mortality and morbidity in CVD in RA based on data from 1950 to 2000 have shown conflicting results. A declining trend of standardised mortality ratio (SMR) from myocardial infarction was observed in an earlier study in patients with debut of RA symptoms before

Table 2 Clinical characteristics of Swedish patients with new-onset rheumatoid arthritis (RA) at inclusion in the Swedish Rheumatology Quality register, including data on treatments initiated within 1 year of inclusion, by calendar period of RA diagnosis

Year of RA diagnosis	All	1997–2002	2003–2006	2007–2010	2011–2014
DAS28 at inclusion*	4.9 (1.4)	5.1 (1.3)	5.3 (1.3)	4.9 (1.5)	4.6 (1.5)
DAS28 at 3–6 months*†	3.2 (1.4)	3.5 (1.4)	3.4 (1.4)	3.1 (1.3)	3.1 (1.4)
Symptom duration at index date, weeks,* mean (SD)	25 (17)	27 (18)	26 (18)	25 (16)	24 (14)
Oral glucocorticoids within first year of diagnosis‡	59	46	48	69	66
DMARD, any within first year of diagnosis‡	93	91	96	94	92
DMARD, Mtx within first year of diagnosis‡	82	66	86	87	86
Biological drugs within first year of diagnosis‡	13	7	14	14	16

*Expressed as mean (SD).
†DAS28 at first revisit to rheumatologist within 3–6 months of diagnosis.
‡Expressed as % of all individuals.
DMARD, disease-modifying antirheumatic drug; Mtx, methotrexate.

Table 3 Number of events (N), person-years at risk (PY) and incidence rates (N/1000 PY) of acute coronary syndrome in new-onset rheumatoid arthritis (RA) identified between 1997 and 2014 and in an individually matched general population comparator

	Patients with new-onset RA			General population comparator subjects			
	N	PY	Incidence rate	N	PY	Incidence rate	HR (95% CI)
Overall	772	103 835	7.4 (6.1 to 8.8)	2418	466 930	5.2 (4.6 to 5.7)	1.41 (1.29 to 1.54)
Sex							
Women	395	73 749	5.4 (4.0 to 6.7)	1125	329 087	3.4 (2.9 to 3.9)	1.53 (1.35 to 1.73)
Men	377	30 085	12.5 (9.4 to 15.7)	1293	137 842	9.4 (8.1 to 10.7)	1.30 (1.15 to 1.47)
Rheumatoid factor							
Positive	513	69 012	7.4 (5.8 to 9.1)	2418	466 930	5.2 (4.6 to 5.7)	1.64 (1.48 to 1.83)
Negative	259	34 823	7.4 (5.2 to 9.7)	2418	466 930	5.2 (4.6 to 5.7)	1.09 (0.94 to 1.26)
DAS28 at diagnosis							
≤3.2	45	9588	4.7 (2.7 to 6.7)	2418	466 930	5.2 (4.6 to 5.7)	0.81 (0.58 to 1.14)
>3.2	652	85 360	7.6 (6.7 to 8.6)	2418	466 930	5.2 (4.6 to 5.7)	1.45 (1.32 to 1.60)
Age at diagnosis							
<53	58	38 454	1.5 (0.5 to 2.6)	150	176 302	0.9 (0.5 to 1.2)	1.76 (1.28 to 2.42)
53	186	28 929	6.4 (4.0 to 8.9)	483	130 874	3.7 (2.8 to 4.6)	1.73 (1.45 to 2.07)
63≤72	224	20 879	10.7 (7.3 to 14.1)	718	92 796	7.7 (6.4 to 9.1)	1.37 (1.17 to 1.61)
≥72	304	15 571	19.5 (14.5 to 24.5)	1067	66 956	15.9 (13.8 to 18.1)	1.22 (1.06 to 1.40)
Calendar period							
1997–2002	375	42 363	8.9 (5.7 to 12.0)	1209	191 849	6.3 (5.0 to 7.6)	1.41 (1.24 to 1.60)
2003–2006	208	28 575	7.3 (4.3 to 10.2)	603	127 185	4.7 (3.6 to 5.9)	1.47 (1.24 to 1.74)
2007–2010	144	23 413	6.2 (3.8 to 8.5)	441	105 264	4.2 (3.3 to 5.1)	1.38 (1.13 to 1.68)
2011–2014	45	9483	4.7 (2.8 to 6.7)	165	42 630	3.9 (3.1 to 4.7)	1.19 (0.85 to 1.67)

HR and 95% CI with the matching factors taken into account and adjusted for age and educational level. By sex, serostatus, DAS28 at diagnosis, age at diagnosis and calendar period of diagnosis.
DAS28, Disease Activity Score 28.

1970 compared with RA diagnosed from 1980 through 1997.[19] A previous Swedish study observed a decrease in the overall mortality (incidence), but similar standardised ratios (relative risks) of morbidity and mortality from CVD comparing patients with established RA assessed in 1978 with patients assessed in 1995.[20] This finding is in line with a meta-analysis that presented unchanged excess risks of death (here: SMRs, relative risks) from CVD in RA studies between approximately 1945 and 1995.[3] Published studies evaluating time trends in CVD incidence in RA cohorts comprising patients with disease debut after 2000 are scarce. Our present study thus contributes new knowledge regarding contemporary patients, but otherwise corroborates the results from RA cohorts from previous decades and treatment paradigms.

On the one hand, our results suggest that patients with RA may have benefited from the CVD prevention and risk factors intervention that have been implemented in society at large, at least to the same extent as the rest of the population. On the other hand, our results suggest that despite improved RA disease control and increasing recognition of cardiovascular (CV) risks in RA over time, the gap in risk between patients with RA and the general population remains, at least among patients with seropositive RA. Potential explanations include that the increasing RA treatment intensity and efficiency have not affected the excess risk of CV comorbidity, or that such true gains have been offset by risks inherent with these treatment strategies.

A distinct decrease in incidence and mortality from coronary artery disease in the general population has been observed in developed countries since half a century. In part this has been attributed to population-level changes in CV risk factors (eg, smoking, dyslipidaemia, hypertension, inactivity), in part to improved medical treatment.[21] It is probable that the positive changes in the general population have also affected individuals with present or forthcoming RA disease. However, as several CV risk factors are also associated with the development of RA and with active RA disease (eg, smoking, dyslipidaemia, physical inactivity, obesity), seropositive RA in particular, it could be argued that the improvement in risk factors might have been less pronounced in the subpopulation that later will develop RA. Under such a scenario, an excess risk of CVD would be observed already prior to RA symptom onset, which was not the case in one of our earlier studies but has been reported from elsewhere (and in our current study, the proportion with a history of ACS at entry was not higher in the RA cohort).[22 23] Even if traditional CV risk factors seem to have less impact on the risk of CVD in RA than in the general population, some level of risk reduction due to improved CV risk factor profile is to be expected,[24–26] although it is not possible to tell whether the remaining gap in morbidity from ACS is attributable to factors related to the RA disease and its treatment rather than to any increased background risk in the subpopulation with RA.

Our observation of a rapid increase in the risk of ACS after RA diagnosis is in line with our previous results from this cohort and the results from other studies.[4 5 20] Patients with recent RA onset have typically experienced a period with active RA disease before the effect of treatment, in the present study with a mean of DAS28 4.6–5.3 at diagnosis, and disease activity at RA diagnosis was clearly linked to excess ACS risk. This inflammatory activity could theoretically have negative effects on plaque stability as well as the formation of thrombus.[27 28] Use of prescribed or over-the-counter COX inhibitors or oral glucocorticoids during the first months of RA disease duration might be more frequent than during periods with well-controlled disease activity. It is thus possible that these factors may contribute to the increased morbidity during the first

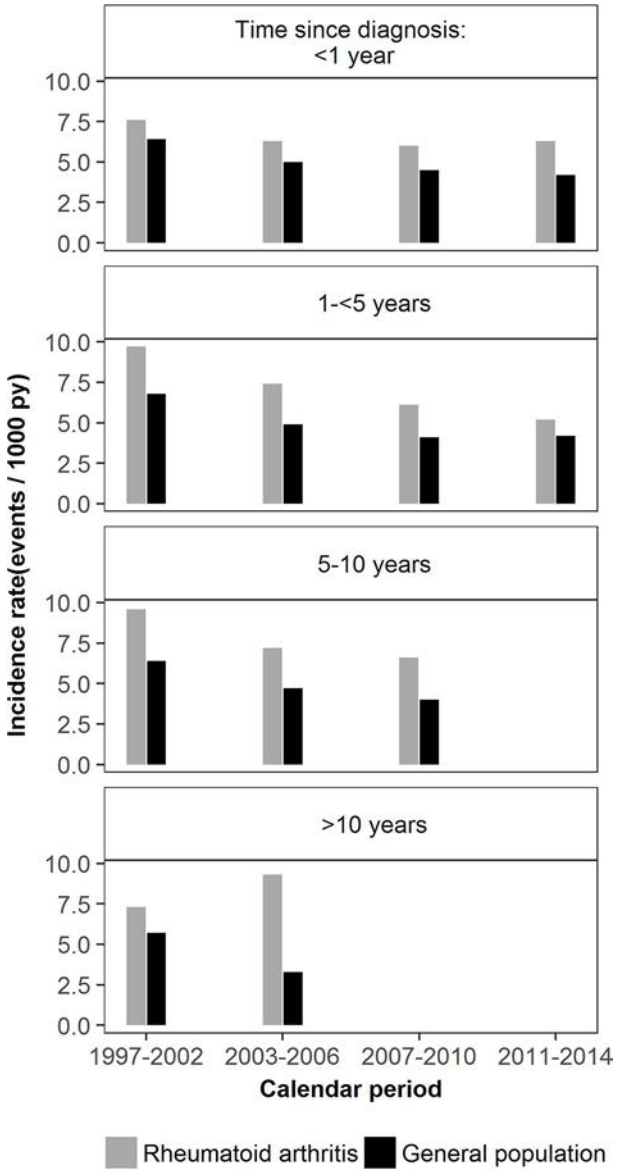

Figure 1 Panels refer to the number of events/1000 person-years by calendar period and time since diagnosis in RA and general population.

year. The average RA disease activity in our study was well controlled 3–6 months after diagnosis, with the lowest disease activity estimate in the most recent calendar period. The use of methotrexate in early RA increased, and the use of biological treatment doubled during the study period, which, if anything, is likely to exert beneficial rather than detrimental effects on CV risks.[29] Interestingly, however, there was also an increasing use of glucocorticoids, for which adverse CV effects are documented.[30] The main strength of this study is the large, nationwide, population-based RA inception cohort, with the possibility to add prospectively collected and linked data on comorbidity and covariates from mandatory public registers. In terms of patients with RA, we believe that the present study has a good generalisability as our cohort represents patients from daily routine clinical care rather than patients recruited from, for example, tertiary referral centres or into a research cohort. Similarly, the publicly funded healthcare in Sweden might limit the impact of socioeconomic factors. Also, the Swedish RA treatment guidelines are in good agreement with most other RA guidelines, at least in the western world. In terms of ACS, the trend towards declining incidences in the general population is observed in many countries. Therefore we believe that our results may also be applicable to other settings other than Sweden. The risk of misclassification of the outcome is low; a previous validation found a positive predictive value of 95% for this definition of ACS in the context of early RA.[18] Misclassification of prevalent as new-onset RA in SRQ is, as indicated, very low, and thus unlikely to have influenced our results. Although the coverage of the SRQ is high, some degree of selection is possible as individuals with an expected short survival due to age or concomitant morbidity might stand a lower chance of being included in any longitudinal clinical monitoring system. Such selection would, however, not explain the increases in overall HRs in our study, and can only explain the absence of time trends in HRs if the coverage of patients with poor prognosis (and elevated CV risks) has increased over time. Irrespective, our results suggest that RA is still associated with an increased risk of new-onset coronary artery disease. Similarly, despite an increasing proportion of seronegative RA and an increasing proportion of patients with DAS28 <3.2 at entry (both of which would have inflated any declining calendar trend), we did not observe any marked reduction in relative risk during the more recent calendar periods of RA diagnosis. Although this is a large cohort, the numbers of events in some strata were low. We did not have data on traditional risk factors for patients or referents, which is a limitation, although our primary aim was to assess rather than to attribute clinical excess risks. The frequencies of unidentified but true ACS (eg, silent myocardial infarction) in the RA and comparator cohorts are

Table 4 HR and 95% CI adjusted for sex, residential area, year of diagnosis age and educational level

Calendar period of RA diagnosis	RA duration categories HR (95% CI)					p Value for heterogeneity in HRs by disease duration*
	Total follow-up	<1 year	1-<5 years	5–10 years	>10 years	
Total study period	1.41 (1.29 to 1.54)	1.12 (0.88 to 1.43)	1.43 (1.26 to 1.63)	1.57 (1.34 to 1.83)	1.30 (1.01 to 1.68)	0.13
1997–2002	1.41 (1.24 to 1.60)	1.14 (0.73 to 1.78)	1.42 (1.15 to 1.76)	1.60 (1.29 to 1.97)	1.26 (0.96 to 1.65)	0.42
2003–2006	1.47 (1.24 to 1.74)	1.18 (0.71 to 1.97)	1.47 (1.14 to 1.90)	1.59 (1.22 to 2.07)	1.77 (0.75 to 4.18)	0.76
2007–2010	1.38 (1.13 to 1.68)	1.20 (0.76 to 1.90)	1.38 (1.07 to 1.76)	1.56 (0.95 to 2.54)	. (.-.)	0.60
2011–2014	1.19 (0.85 to 1.67)	0.96 (0.57 to 1.6)	1.48 (0.94 to 2.32)	. (.-.)	. (.-.)	0.47
p Value for heterogeneity in HRs by calendar period*	0.67	0.80	0.91	0.94	0.81	

Overall and stratified by calendar period of RA diagnosis and time since RA diagnosis.
*Wald χ^2 tests of heterogeneity with 2–3 df, depending on the number of variables tested.
RA, rheumatoid arthritis.

unknown, but based on previous studies unlikely to be higher among the general population comparator subjects.[22]

In conclusion, in spite of a decline in the absolute risks, the excess and the relative risks of ACS have not declined over calendar time, such that patients with early RA are at an approximately 40% increased risk of ACS compared with the general population. Whether RA disease-related or treatment-related or not, coronary artery disease in RA thus remains a concern and calls for continuous vigilance and implementation of preventive measures early in the RA disease course.

Contributors MH had full access to all of the data used for analyses in this study and takes full responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: JA, MH, LL. Acquisition of data: MH, JA. Statistical analysis: MH, LL. Analysis and interpretation of data: MH, LL, JA. Drafting of manuscript: MH, LL. Critical revision of manuscript and final approval given: MH, LL, JA. Obtained funding: JA. Study supervision: JA.

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EXTENDED REPORT

ADA2 deficiency (DADA2) as an unrecognised cause of early onset polyarteritis nodosa and stroke: a multicentre national study

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ABSTRACT

Objectives To analyse the prevalence of *CECR1* mutations in patients diagnosed with early onset livedo reticularis and/or haemorrhagic/ischaemic strokes in the context of inflammation or polyarteritis nodosa (PAN). Forty-eight patients from 43 families were included in the study.

Methods Direct sequencing of *CECR1* was performed by Sanger analysis. Adenosine deaminase 2 (ADA2) enzymatic activity was analysed in monocyte isolated from patients and healthy controls incubated with adenosine and with or without an ADA1 inhibitor.

Results Biallelic homozygous or compound heterozygous *CECR1* mutations were detected in 15/48 patients. A heterozygous disease-associated mutation (p.G47V) was observed in two affected brothers. The mean age of onset of the genetically positive patients was 24 months (6 months to 7 years). Ten patients displayed one or more cerebral strokes during their disease course. Low immunoglobulin levels were detected in six patients. Thalidomide and anti-TNF (tumour necrosis factor) blockers were the most effective drugs. Patients without *CECR1* mutations had a later age at disease onset, a lower prevalence of neurological and skin manifestations; one of these patients displayed all the clinical features of adenosine deaminase 2deficiency (DADA2) and a defective enzymatic activity suggesting the presence of a missed mutation or a synthesis defect.

Conclusions DADA2 accounts for paediatric patients diagnosed with PAN-like disease and strokes and might explain an unrecognised condition in patients followed by adult rheumatologist. Timely diagnosis and treatment with anti-TNF agents are crucial for the prevention of severe complications of the disease. Functional assay to measure ADA2 activity should complement genetic testing in patients with non-confirming genotypes.

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INTRODUCTION

The deficiency of adenosine deaminase 2 (DADA2) is a recently described autoinflammatory disease caused by loss-of-function homozygous or compound heterozygous mutations in *CECR1* (Cat Eye Syndrome Chromosome Region 1) gene.[1 2] DADA2 is characterised by an early onset vasculopathy with clinical and histopathological features of polyarteritis nodosa (PAN), associated with haemorrhagic and ischaemic strokes.[1–3] Hypogammaglobulinaemia with reduction of memory and terminally differentiated B cells and plasma cells may be present.[1 4] A severe clinical picture dominated by cytopenia and lymphoproliferation has been also described.[5 6] Even if the disease's onset is commonly in the paediatric age, some patients with adulthood onset have been described as well.[2]

ADA2 has a homology to the ADA1 protein, which is associated with a form of severe combined immunodeficiency. ADA1 and ADA2 have a key role in the regulation of the purinergic signalling pathway by converting adenosine to inosine and 2′-deoxyadenosine to 2′-deoxyinosine, respectively.[7] While ADA1 is ubiquitously expressed in all cell types, ADA2 is mostly expressed in monocytes and cells of myeloid lineage.[7]

ADA2 acts as a growth factor, playing a pivotal role in the development of endothelial and haematopoietic cells.[7 8] ADA2 also displays an autocrine activity and is able to induce monocyte proliferation and macrophage differentiation.[9] Monocytes of ADA2-deficient patients display a defect in the differentiation of M2 (anti-inflammatory) macrophages, which leads to the prevalence of M1 (proinflammatory) cells.[1]

Eighteen mutations of *CECR1* have been detected so far.[3] The p.G47R mutation has been detected in homozygous state in most patients of Georgian Jewish and Turkish ancestries.[1 2] The estimated carrier frequency of this mutation in the Georgian Jewish population is 10%.[2] The p.R169Q mutation is most frequently found in the Caucasian populations living in the Northern Europe where carriers might be up to two in 1000 individuals.[5 6 10]

In this study, we have analysed the prevalence of *CECR1* mutations among patients with a clinical picture characterised by early onset PAN, livedo

reticularis or stroke referred to the Italian Pediatric Rheumatology centres.

PATIENTS AND METHODS

Since February 2014, a national survey among the Italian centres of Pediatric Rheumatology was performed. Criteria of inclusion in the study were (1) early onset livedo reticularis associated with chronic or recurrent signs of systemic inflammation, (2) haemorrhagic/ischaemic stroke or signs of peripheral nervous system involvement associated with systemic inflammation, and/or (3) previous diagnosis with childhood onset PAN.[11] The study was approved by the Ethical Review Board of G. Gaslini Institute.

Genetic analysis

Molecular testing was performed on DNA samples extracted from peripheral blood lymphocytes by standard methods. All nine coding exons (from 2 to 10) of the *CECR1* gene (NM_001282228) were analysed by means of amplification followed by direct sequencing; the intronic regions were not analysed. Primers for PCR amplifications, amplicon lengths and PCR conditions are listed in the online supplementary material table S1. All the PCRs were performed as previously described.[12] Patients 1, 2 and 3 were screened by QZ and IA, as previously described.[1]

Cytokine profile assessment in monocytes

Following purification by peripheral blood mononuclear cells (PBMCs) adherence,[13] fresh 10% fetal calf serum (FCS) RPMI medium was added and monocytes were incubated at 37°C, 5% CO_2 for 6–18 hours in the presence/absence of zymosan (20 μg/mL) and Lipopolysaccharide (LPS) (1 μg/mL). Supernatants were collected at experimental time points (ie, 6 and 18 hours) and samples stored at −80°C. Six hours released tumour necrosis factor (TNF)-α and 18 hours secreted interleukin (IL)-1β and IL-6 were then quantified by ELISA assay.

ADA2 enzymatic activity

ADA2 plasma levels were detected with ELISA kit (eBioscience). ADA2 activity was assessed in primary monocytes. PBMCs were isolated through Ficoll-Paque and monocytes isolated by adherence, incubated for 1 hour in 24-well plate with RPMI 1% penicillin–streptomycin (Sigma Aldrich), 1% L-glutamine (EuroClone) in 5% CO_2 at 37°C. Monocytes were then cultured in PBS in the presence of exogenous adenosine (Sigma Aldrich), with or without the ADA1 inhibitor (erythro-9-(2-hydroxy-3-nonyl)adenine, EHNA, Sigma Aldrich). After 4 hours of incubation at 37°C and 5% of CO_2, supernatants were collected and the activity evaluated through the measurement of the adenosine-derived products (inosine, hypoxanthine) in high-performance liquid chromatography.

Statistical analysis

Comparison among genetically confirmed patients with DADA2 and mutation-negative patients was performed with χ^2 test. Non-parametric Mann-Whithney U test was used for the biological assays.

RESULTS

Molecular characterisation

From March 2014 to June 2016, we enrolled 48 patients, who fulfil the study inclusion criteria, from 43 families that were identified in eight Italian centres: 14 patients with early onset livedo reticularis associated with chronic or recurrent signs of systemic inflammation, 13 patients with haemorrhagic/ischaemic stroke or signs of peripheral nervous system involvement associated with systemic inflammation, 20 patients with a previous diagnosis with childhood onset PAN.

Homozygous or compound heterozygous *CECR1* mutations were found in 15 patients coming from 11 families. Four variants (G47A, G47R, P251L, Y435C) had already been associated with DADA2;[1 2] six (c.138_144delG, L249P, R312X, E328D, P344L, T360A) were novel mutations predicted to have deleterious effects on the protein function (table 1 and supplementary figures 1 and 2).

Consanguinity (third cousins) was reported in the parents of patient 3. Patients 13 and 14 were born from apparently non-consanguineous parents living in geographic isolation. The remaining 12 patients were born from unrelated parents. The living parents of five families were also analysed: all of them were heterozygous for one *CECR1* mutation (online supplementary figure 3).

A single disease-associated mutation (G47V) was observed as the sole genetic defect in two affected brothers and in their unaffected father and brother (table 1 and online supplementary figure 3).

A number of common single-nucleotide polymorphisms (L46L, N53N, H335R, Y453Y) were found both in patients carrying deleterious *CECR1* mutations and in the remaining 31 'genetically negative' patients.

Clinical presentation and disease course of genetically confirmed patients with DADA2

The main demographic features and the clinical manifestations at disease onset and during follow-up of the 15 genetically confirmed patients with DADA2 are summarised in table 1 (see also online supplementary material): two patients were in the group of early onset livedo reticularis associated with chronic or recurrent signs of systemic inflammation, 10 patients in the group of haemorrhagic/ischaemic stroke/peripheral nervous system involvement associated with systemic inflammation (seven of them received a histological diagnosis of PAN), two patients in the group of a previous diagnosis with childhood onset PAN. Patient 12 did not fulfil the inclusion criteria presenting a milder phenotype but was included in the study since his brother (patient 11) presented a typical phenotype.

The mean age at molecular analysis was 16.5 years (range 1–35 years); nine patients were children, six adults.

The clinical features observed during the disease course are reported in table 2 and figure 1, and discussed in detail in the online supplementary materials. The disease course was chronic or recurrent in nine and six patients, respectively (table 1).

Acute phase reactants were elevated in all but one patient. No patient displayed severe haematological manifestations, such as cytopenia. Low immunoglobulin levels, requiring substitutive treatment (IgG <500 mg/dL), were observed in three patients only; patient 12 displayed a reduced level of IgM. None of the patients displayed a clear history consistent with recurrent infections. Of note, patient 7's sister, who had the same clinical manifestations as the brother (early onset livedo reticularis, ischaemic stroke and inflammation) died at the age of 18 due to a septic shock secondary to an episode of pyelonephritis.

Clinical features of patients with an incomplete genotype and heterozygous family members

Patients 16 and 17 were heterozygous for the p.G47V variant and presented with a full picture of a DADA2-like disease.

Table 1 Clinical and demographic data of genetically confirmed and heterozygous patients with DADA2

Pt./sex	Age at onset/at diagnosis	Disease course	Fever	Skin manifestations	Stroke (number)	Peripheral nervous system involvement	Hypertension	Immunohaemato-logical symptoms	Other symptoms	Biopsy	*CECR1* mutations
Genetically confirmed patients											
1 M	2 years/14 years	Chronic	Yes	Livedo reticularis Subcutaneous nodules	Yes (2)	Peripheral neuropathy	Yes	HGG	Growth hormone deficiency, arthralgia	Polyarteritis nodosa (skin)	R312X E328D
2 M	9 months/7 years	Chronic	Yes	Livedo reticularis	Yes (1)	Peripheral neuropathy	Yes	HGG	Small bowel invagination	Polyarteritis nodosa (bowel)	R312X E328D
3 M	6 months/8 years	Recurrent → chronic	Yes	Livedo reticularis	Yes (2)	Peripheral paresis of the VII cranial nerve Neurosensory hearing loss	Yes	No	Myocarditis	Not done	T360A T360A
4 M	1 year/10 years	Recurrent → chronic	Yes	Livedo reticularis Subcutaneous nodules Ecchymotic lesions	No	No	No	Recurrent infection of upper airways	Diarrhoea	Polyarteritis nodosa (skin)	G47A P251L
5 F	3 years/12 years	Chronic	Yes	Livedo reticularis Urticarial rash	No	No	No	No	–	Leucocytoclastic vasculitis (skin)	c.138/144delG T360A
6 M	5 years/19 years	Chronic	No	Livedo reticularis Necrotic ulcers of extremities	No	No	Yes (with PRES)	No	Small bowel invagination	Polyarteritis nodosa (skin)	L249P T360A
7 M	8 months/24 years	Recurrent → Chronic	Yes	Livedo reticularis Purpuric lesions	Yes (1)	Peripheral paresis of the III cranial nerve Optic neuritis	Yes	No	Hepatomegaly, splenomegaly	Polyarteritis nodosa (skin)	G47R T360A
8 F	7 years/7.5 years	Chronic	Yes	Livedo reticularis	No	No	No	Recurrent infections of upper airways	Hepatomegaly	Not done	Y453C Y453C
9 F	12 months/17 years	Chronic	Yes	Livedo reticularis Erythematous skin rash Necrotic ulcers of extremities	Yes (3)	Peripheral neuropathy	Yes	No	–	Polyarteritis nodosa (skin)	T360A T360A
10 F	5 years/5 years	Chronic	Yes	Livedo reticularis Necrotic ulcers Subcutaneous nodules	Yes (1)	Peripheral neuropathy	Yes	No	Hepatomegaly, splenomegaly, arthralgia, arthritis	Polyarteritis nodosa (skin)	T360A T360A
11 M	12 months/12 months	Chronic	Yes	Livedo reticularis	Yes (1)	Neurosensory hearing loss	Yes (with PRES)	No	Generalised adenopathy, diarrhoea, oral aphthosis, arthralgia, arthritis	Not done	L249P P344L
12 M	5.5 years/6 years	Chronic → Recurrent	Yes	No	No	No	Yes	HGG (IgM)	Hepatomegaly, splenomegaly, arthralgia	Not done	L249P P344L
13 F	3 months/24 years	Recurrent	Yes	Livedo reticularis	Yes (2)	Peripheral paresis of cranial nerves	No	No	oral aphthosis	Polyarteritis nodosa (skin)	T360A T360A
14 M	6 years/22 years	Recurrent	No	No	Intracranial haemorrhage	No	No	No	No	Not done	T360A T360A
15 M	7 years/7 years	Chronic	Yes	Livedo reticularis psoriasis	Yes (1)	Peripheral neuropathy	Yes	HGG	Arthralgia	Leucocytoclastic vasculitis (skin)	Y453C Y453C

Continued

Table 1 Continued

Pt./sex	Age at onset/at diagnosis	Disease course	Fever	Skin manifestations	Stroke (number)	Peripheral nervous system involvement	Hypertension	Immunohaematological symptoms	Other symptoms	Biopsy	*CECR1* mutations
Heterozygous patients											
16 F	1.5 years/10 years	Chronic	No	Livedo reticularis Subcutaneous nodules	Yes (3)	No	Yes	HGG recurrent infections of upper airways	Diarrhoea, abdominal pain, colic ulcerations	Leucocytoclastic vasculitis (skin), panarteritis nodosa (bowel)	G47V/WT
17 M	2 years/9 years	Chronic	No	Livedo reticularis Subcutaneous nodules Necrotic ulcers of extremities	No	Peripheral neuropathy	Yes	HGG	Diarrhoea, abdominal pain, arthralgia	–	G47V/WT

F, female; HGG, hypogammaglobulinaemia; M, male; PRES, posterior reversible encephalopathy syndrome; Pt., patient.

Both of them had an early disease onset (20 and 24 months) manifesting with livedo reticularis, subcutaneous nodules and necrotic ulcers of extremities in one of them (figure 1). Patient 16 suffered from multiple episodes of symptomatic ischaemic stroke. Hypogammaglobulinaemia was present in both siblings. Their father and the younger brother were heterozygous for the same mutation and asymptomatic. No mutation was detected in the asymptomatic mother (online supplementary figure 3). The second disease-causing mutation, a possible null allele inherited from the mother, could not be identified despite the analysis of the *CECR1* transcript in all the five members of the family (data not shown).

The study of the families trees of some genetically confirmed patients with DADA2 allowed the identification of other members presenting some DADA2-related clinical manifestations. The DNA from some of these individual was available for the analysis of *CECR1* (online supplementary figure 3). Several individuals from the family of patients 9 and 10, displayed manifestations consistent with an inflammatory vasculopathy (online supplementary figure 3). The mother presented at the age of 46 with systemic vasculitis (skin rash, low-grade fever) with good response to treatment with steroids. A few months later she presented with an episode of stroke. The father died of a cardiovascular attack. A third sister presented with severe systemic hypertension at the age of 20 years, associated with persistent arthralgia, responding to steroids. Both the son of this sister and patient 10's son presented Kawasaki disease at the age of 1 year, responding to treatment with immunoglobulins. With the exception of the father, whose DNA was not available, all these paucisymptomatic individuals were heterozygous for the T360A mutation (online supplementary figure 3). Patient 4's father, heterozygous for the P251L mutation, presented a myocardial infarction at the age of 40 years. Unfortunately, none of the above heterozygous patients were analysed from a functional point of view, so far.

The family history of patients 13 and 14 was significant for several family members who presented with cerebrovascular or cardiovascular events, in some cases associated with inflammatory manifestations (online supplementary figure 3); however, genetic testing for the other members is not available.

Clinical characteristics of patients with negative genetic test

Thirty-one patients fulfilling the inclusion criteria of the study tested negative for mutations in *CECR1*. Complete clinical data were available for 21 of these subjects and compared with 15 genetically confirmed patients with DADA2 (table 2). Genetically negative patients had a later disease onset, a lower prevalence of livedo reticularis and a higher prevalence of subcutaneous nodules (table 2). They also displayed a less frequent central nervous system (CNS) involvement: only three patients reported CNS manifestations (one with two ischaemic strokes, one with a single haemorrhagic stroke, one with encephalitis). Peripheral neuropathy (in three cases secondary to the use of thalidomide) was more common in genetically confirmed patients. No difference was observed in the incidence of hypogammaglobulinaemia (table 2).

Among *CECR1*-negative patients, a 6-year-old girl from Sardinia presented the whole clinical spectrum associated with DADA2. At the age of 3 months, she presented livedo reticularis, fever, elevation of acute phase reactants associated with severe myocarditis and hypertension. The skin biopsy was consistent with a PAN. Persistent hypogammaglobulinaemia was also detected. High doses of steroid with subsequent slow tapering were able to control the

Table 2 Comparison between *CECR1* genetically confirmed and *CECR1*-negative patients

	Genetically confirmed patients with DADA2 (n=15)	*CECR1* gene negative patients (n=21)	p Value
Mean age at onset (range)	2.9 years (3 months–7.5 years)	7 years (2 months–16 years)	0.001
Fever (%)	13 (86)	18 (85)	NS
Skin manifestations (%)	13 (86)	19 (90)	NS
Livedo reticularis	13 (86)	8 (38)	0.008
Subcutaneous nodules	3 (20)	15 (71)	0.004
Ulcerations of extremities	3 (20)	2 (9)	NS
Other	5 (33)	11 (52)	NS
Biopsy (%)	10 (66)	10 (47)	NS
Polyarteritis nodosa	8 (53)	8 (38)	NS
Leucocytoclastic vasculitis	2 (13)	1 (4)	NS
Other	0	2 (9)	NS
CNS involvement (%)	10 (66)	3 (14)	0.003
Stroke	9 (60)	2 (9)	0.004
Intracranial haemorrhage	1 (6)	0	NS
Other	0	1 (4)	NS
PNS involvement (%)	9 (60)	2 (16)	0.004
Peripheral neuropathy	5 (33)	2 (16)	NS
Cranial nerve paralysis	3 (20)	0	NS
Hearing loss	3 (20)	0	NS
Other	1 (6)	0	NS
Hypertension (%)	10 (66)	8 (38)	NS
Gastrointestinal manifestations (%)	5 (33)	9 (42)	NS
Diarrhoea	2 (13)	2 (9)	NS
Abdominal pain	0	8 (38)	NS
Bowel ischaemia	2 (13)	0	NS
Other	2 (13)	2 (9)	NS
Immunologic manifestations (%)	6 (40)	4 (21)	NS
Hypogammaglobulinaemia	4 (26)	2 (9)	NS
Recurrent infections	2 (13)	3 (14)	NS
Hepatomegaly (%)	4 (26)	8 (38)	NS
Splenomegaly (%)	3 (20)	6 (28)	NS
Generalised adenopathy (%)	1 (6)	1 (4)	NS
Articular manifestations (%)	5 (33)	13 (61)	NS
Arthralgia	5 (33)	13 (61)	NS
Arthritis	2 (13)	6 (28)	NS

CNS, central nervous system; PNS, peripheral nervous system.

inflammatory manifestations; however, two episodes of stroke were observed when steroid was withdrawn.

ADA2 enzymatic activity

ADA2 activity in plasma samples was significantly lower in patients with DADA2 compared with age-matched healthy controls (online supplementary figure 4).

We specifically analysed the ADA2 activity of circulating monocytes through the evaluation of inosine and hypoxanthine concentrations in the supernatant of adenosine stimulated cells in the presence or in the absence of an ADA1 inhibitor. Patients with DADA2[1 2 4] showed a lower enzymatic activity, as demonstrated by the absence of inosine in patient cells supernatant, compared with seven healthy subjects. Notably, patient 4, presenting with a mild phenotype, displayed a minimal residual enzymatic activity (figure 2A). As expected, ADA1 activity was normal in all the subgroups analysed (data not shown). Two genetically negative patients with a history of cutaneous PAN and the absence of strokes displayed a normal ADA2 activity (Ctr1 and Ctr2, figure 2). Conversely, the Sardinian patient (Ctr3), presenting with a full-blown DADA2 phenotype but negative for *CECR1* mutations, displayed a complete lack of ADA2 activity (figure 2A), despite plasmatic levels of ADA2 comparable to those of the healthy donors (data not shown), suggesting that this patient likely carries atypical mutation(s) in *CECR1* that was not detected by standard sequencing.

Both clinically affected heterozygous patients (16 and 17) displayed a complete absence of enzymatic activity, similar to patients with biallelic mutations. Conversely, the two heterozygous asymptomatic family members (the father and the brother), as well as the mother, displayed a preserved enzymatic activity (figure 2B).

Long-term response to treatment in patients with DADA2

The treatments applied to patients with DADA2 are reported in table 3 (see also supplementary material). The response to treatment was considered (i) complete, in case of persistent control of inflammatory parameters with no disease flares or complications in the absence of any steroid treatment; (ii) partial, in case of a good control of disease activity with sporadic relapses and need of steroid on demand or increased steroid dosage and (iii) poor, in case of little or absent response with persistence of systemic flares and/or complications (table 3). All patients showed a partial response to NSAID and complete response to high dose of steroids. However, all patients with a chronic disease course relapsed following steroid tapering. The most severe manifestations of disease (ie, cerebral strokes and intestinal invagination) occurred at the time of steroid tapering or steroid withdrawal.

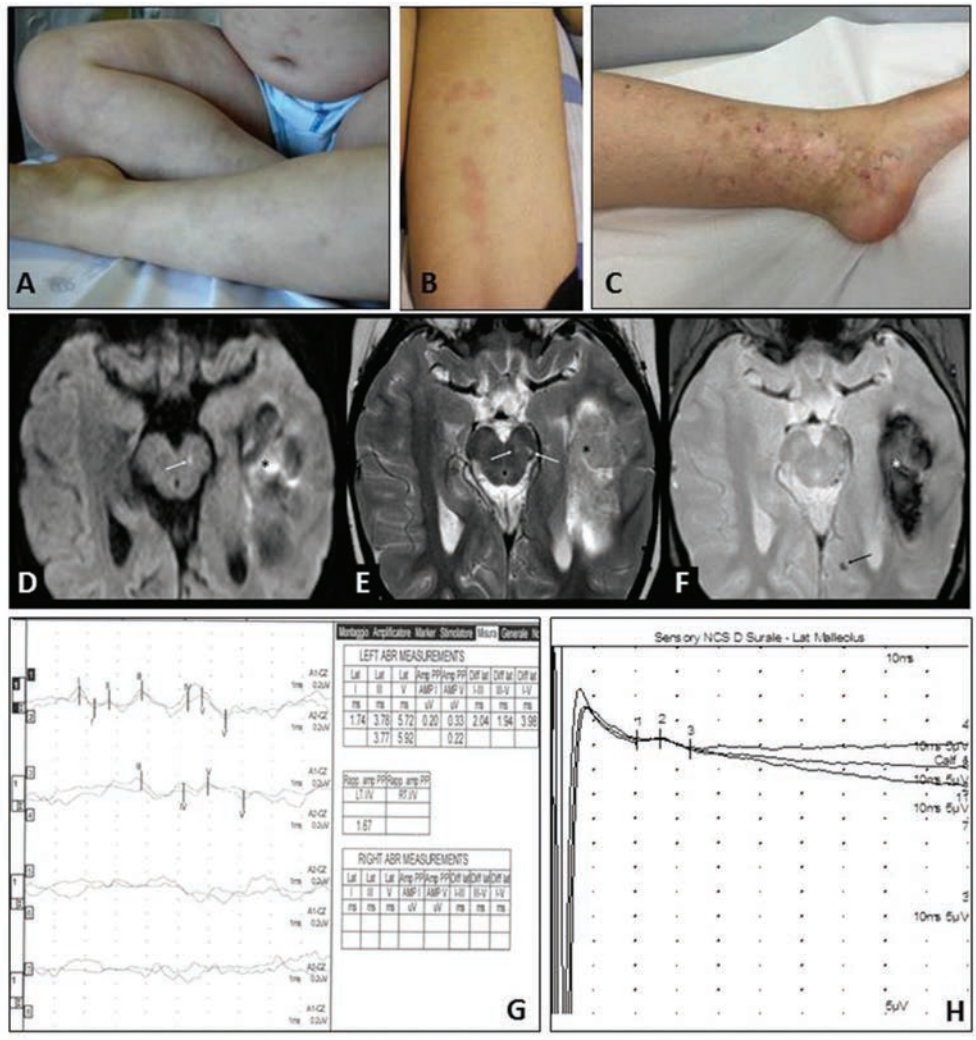

Figure 1 Clinical features in patients with adenosine deaminase 2 deficiency. (A) Livedo reticularis in patient 1. (B) Painful subcutaneous nodules in patient 4. (C) Scars after a deep necrotic ulcer at right lower limb in one heterozygous patient (16). (D–F) Brain MRI performed in patient 1 at 6 years of age before anti-TNF treatment. Axial T2* (D), diffusion (DWI) (E), T2-weighted (F) images show a large acute haemorrhagic infarct in the left temporal lobe (asterisks) associated with an acute small asymptomatic ischaemic infarct of the midbrain (E, arrows) and multiple small chronic lacunar infarcts at the level of the left cerebral peduncle (arrow, F). (G) Identification of complete acute hearing loss in patient 3 with auditory brainstem response (ABR). (H) Reduced amplitude of the sensitive action potential of the sural nerve following antidromic stimulation (mild absonal injury).

Immunosuppressive therapies (azathioprine, ciclosporin, cyclophosphamide, methotrexate, mycophenolate mofetil) were generally associated with a poor or partial response (table 3 and online supplementary material). Patient 5 had a poor response to treatment with an IL-1 receptor antagonist.

Interestingly, treatment with thalidomide (mean dose: 2 mg/kg/daily, maximum 50 mg/day) gave better results in seven patients (online supplementary material) with a complete response achieved in six patients. In three patients, the drug was withdrawn after 20 months, 25 months and 5 years due to neurological toxicity.

Ten patients received anti-TNF treatment with a complete remission in nine of them (table 3); only one patient was still steroid dependent. The median duration of treatment with etanercept in the 10 treated patients is now 3.9 years (range 0.9–13 years). No severe infections or other complications have been reported. The same good response was also observed in the heterozygous patients 16 and 17 (table 3).

We analysed the pattern of cytokine production from isolated monocytes in a patient with DADA2 with active disease (Patient 3) and three patients in remission (6, 7 and 9). LPS-stimulated monocytes from the patients with DADA2 with inactive disease displayed higher secretion of TNF-α compared with age-matched healthy donors (supplementary figure 5A). The TNF secretion was substantially higher in monocytes from patient 3 (supplementary figure 5B), with a clear downmodulation 1 month after the beginning of anti-TNF treatment (supplementary figure 4A).

DISCUSSION

The present paper describes the largest series of European Caucasian patients affected with DADA2. In this cohort, we identified six novel disease-causing mutations. We observed a variability of the clinical phenotypes associated with DADA2, and we describe a small number of patients presenting with typical clinical manifestations of DADA2 (vasculopathy, stroke, hypogammaglobulinaemia) and non-confirming or negative genetic analysis. We developed a novel enzymatic assay to analyse the ADA2 activity of circulating monocytes that was able to distinguish affected patients from healthy controls and healthy heterozygous carriers.

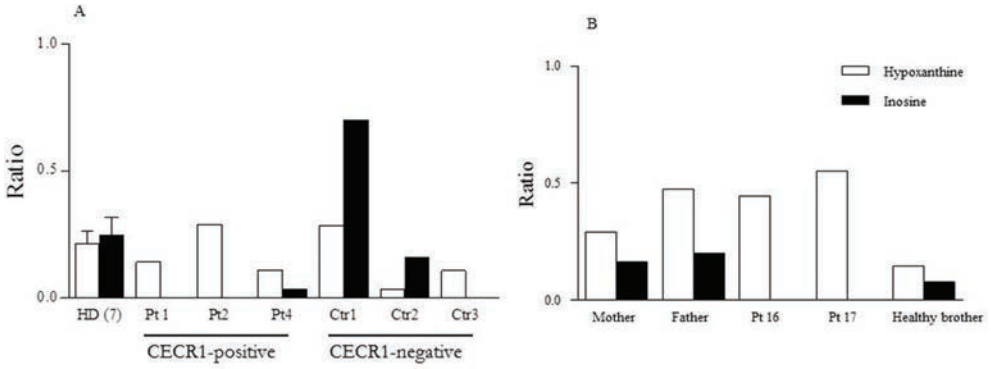

Figure 2 Adenosine deaminase 2 (ADA2) enzymatic activity was assessed in monocytes of patients with different ADA2 mutations compared with age-matched healthy controls. Inosine (black column) and hypoxanthine (white column), derivative products of the metabolism of adenosine mediated by ADA2, were measured in the monocyte supernatant after 4 hours of incubation with adenosine 15 µM and ADA1 inhibitor 30 µM. Values refer to the amount of adenosine present in the supernatants. (A) ADA2 enzymatic activity in patients 1, 2 and 4 in age-matched healthy donors (HD), in genetically negative patients with PAN (Ctr1 and Ctr2) and in the genetically negative Sardinian patient carrying a complete DADA2-like phenotype (Ctr3, see also text). (B) The complete loss of ADA2 enzymatic activity in the two heterozygous patients (16 and 17) with a typical DADA2-like phenotype and the normal activity in the two healthy family members carrying the same mutation and in the wild-type mother (see also text).

Our study confirms that treatment with anti-TNF therapies has a sustained beneficial effect in patients with DADA2, and we showed the first time that the treatment with thalidomide maybe an effective and less expensive alternative therapeutic strategy.

Our study shows a rather high prevalence of *CECR1* mutations in the Italian paediatric population diagnosed with a PAN-like vasculopathy and/or presenting with a history of strokes associated and inflammatory disease. The screening for *CECR1* mutations should be performed in adult patients with a similar phenotype. An early onset symptom, livedoid rash and a history of cerebral stroke were associated with a higher likelihood to identify *CECR1* mutations. Nevertheless, a milder disease course characterised by sole manifestations of a cutaneous PAN disease can be also present in *CECR1* mutation-positive patients (patient 4).

Despite this study covers most of the Italian paediatric patients with PAN vasculitis or stroke associated to inflammatory vasculopathy, it is conceivable that patients with a prevalence of haematological abnormalities may have been missed. The same could also be for patients with an adult onset or with a milder phenotype. Moreover, no data are so far available on the prevalence of *CECR1* mutations in the general Italian population.

Among the *CECR1* mutation-positive patients, a number of unreported mutations, mainly located in the catalytic domain of the protein, thus likely to disrupt the enzymatic activity, have been identified. Some of them, such as the T360A variant, appear to be rather specific for the Italian population, suggesting a possible founder effect. In three patients with the clinical and enzymatic phenotype consistent with DADA2, we identified either one (heterozygous) or none mutations in *CECR1*. Although our preliminary search for the putative second mutation did not produce results, it suggests that mutations affecting regulatory non-coding sequences or genomic deletions likely account for atypical mutations in this gene. This search requires a different set of analysis.

We observed the presence of clinical manifestations possibly associated with DADA2 in a number of family members who are heterozygous carriers. This finding could support the hypothesis of a possible digenic or polygenic inheritance, as recently observed in other autoinflammatory conditions.[14] Whole exome sequencing approach is currently ongoing in the Sardinian patient presenting with a complete clinical and functional DADA2 phenotype, with the aim to identify other genes that may affect the ADA2 enzymatic activity. Another possibility is that this patient carries either genomic deletion(s), intronic mutation(s) and/or regulatory mutation(s).

Our study describes a wide phenotypic variability and diseases expressivity in patients with DADA2.[1 2 5 6 15–18] The clinical course can be chronic or characterised by recurrent flares of systemic inflammation. In patients with a recurrent disease course, the most severe clinical manifestations (ie, strokes or other vascular accidents) were mainly observed during the inflammatory flares, thus suggesting the need for a continuous treatment in all patients. As previously reported, neurological manifestations of central and peripheral nervous system represent the most severe clinical features leading to permanent damage and/or functional dysfunction.[1 2] Acute and permanent unilateral hearing loss has been experienced during disease inflammatory flares in two patients of the present series and represents an additional complication of the disease. Myocarditis was the clinical manifestation at disease onset of patient 3 and was also reported in the Sardinian patient negative for *CECR1* mutations. This finding has anecdotally been associated with polyarteritis in children.[19]

Though severe cytopenia has been described in the spectrum of DADA2 associated phenotypes,[20] none of our patients displayed any haematological features. A minority of our patients displayed a hypogammaglobulinaemia with a history of recurrent infections, resembling clinical features of patients followed for common variable immunodeficiency (CVID).[21] This finding is likely due to the selection of our patients from the paediatric rheumatology community and should not underestimate the multifaceted phenotype of patients with DADA2, some of whom may display major haematological and/or immunological features of the disease.

In the present study, we confirm the dramatic and persistent efficacy of treatment with anti-TNF therapies that completely controlled the inflammatory manifestations preventing the occurrence of vascular events in all treated patients without severe complications.[2] Circulating TNF-α levels in patients with DADA2 are rather variable.[2 4] However, a clear expression of TNF was found in the inflammatory infiltrate of affected tissues.[2] In the present study, we had the opportunity to analyse the pattern of TNF production in LPS-stimulated monocytes

Table 3 Response to treatment in genetically confirmed and heterozygous patients with DADA2.

Pt	NSAIDs	Steroids*	Thalidomide	AZT	CTX	MMP	CyA	MTX	Anakinra	Adalimumab	Infliximab	Etanercept
1	Poor	Complete*	Complete	–	Poor	–	–		–	–	–	Complete
2	Poor	Complete*	Complete	–	–	–	–		–	–	–	Complete
3	Poor	Complete	–	–	–	–	–		–	–	–	Complete
4	–	Complete	–	–	–	–	–		–	–	–	Complete
5	Poor	Complete*	Partial	–	–	–	Poor	Complete (+adalimumab)	Poor	Complete (+MTX)	–	Complete
6	–	Partial	–	–	Poor	Complete	–		–	–	Complete	Complete
7	–	Complete*		Poor	–	Partial	–		–	–	–	–
8	–	Complete	–	–	–	–	–		–	–	–	Complete
9	–	Complete*	Complete	Poor	Complete (with low doses steroid)	poor	–		–	–	–	–
10	–	Complete*	Complete	Poor	–	–	–	Poor	–	–	–	–
11	–	Partial	–	–	–	–	–	–	–	–	–	Complete
12	–	Complete	–	–	–	–	–	–	–	–	–	Complete
13	–	Complete	Complete	–	–	–	–	Poor	–	–	–	–
14	–	–	–	–	–	–	–	–	–	–	–	–
15	Poor	Complete*	Complete	Poor	Poor	–	Poor	–	–	–	–	Partial
16	–	Complete*	–	–	Poor	Partial	–	–	–	–	–	Complete
17	–	Complete*	–	Poor	–	Partial	–	–	–	–	–	Complete

*Only for doses higher than 1 mg/kg daily, with several relapses during tapering.

Complete, Persistent control of inflammatory parameters with no disease's flares or complications in the absence of any steroid treatment; *partial*, good control of disease activity with sporadic relapses and need of steroid on demand or increased steroid dosage; *poor*, little or absent response with persistence of systemic flares and/or complication.

NSAID, Non steroidal anti.inflammatory drugs; AZT, azathioprine; CTX, cyclophosphamide; CyA, ciclosporin A; MMP, mycophenolate mofetil; MTX, methotrexate.

of a patient with DADA2 with active disease and following the anti-TNF treatment.

Interestingly enough, the same persistent therapeutic effect obtained with anti-TNF agents has been observed in majority of the patients treated with thalidomide, which exerts a potent and specific anti-TNF effect.[22 23] Despite the possible toxic effects on peripheral nervous system, this drug has the advantage of a lower cost and could be considered in developing countries in which the use of biological treatments is economically limited.

Our experience suggests that the anti-TNF treatment should be initiated at the moment of the diagnosis, even in patients who do not have two identified mutations in *CECR1*. In these patients, functional assay should be used to confirm the lack of ADA2 activity. Although several reports showed that haematopoietic stem cell transplantation is a potential curative therapeutic strategy for patients with DADA2,[5] our study supports the use of anti-TNF treatment as a first choice intervention, at least in patients with a prevalent inflammatory phenotype. In our study, only one patient (patient 9) withdrew the effective treatment due to persistent complete well-being with a subsequent severe disease flare (see online supplementary material). We therefore suggest not to discontinue the effective treatment in patients with a clear enzymatic defect.

In conclusion, DADA2 may account for a number of patients with PAN-like disease, neurological manifestations and systemic inflammation in children and might represent an unrecognised condition in adult patients followed by adult rheumatologists. A timely diagnosis and a prompt treatment with anti-TNF agents are crucial for the prevention of severe complications of the disease, even in patients with non-conclusive *CECR1* genotype.

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Contributors RC, MG: coordination of the study, interpretation of the results, elaboration of the manuscript. FP, AO, FS: functional immunological studies. AG, QZ: genetic analysis. MS, CG: neuroimaging. AS, GD: biochemical studies. AI, MA, GC, FM, PP, AT, SM, CM, RG, RAP, FF, FG, AMo, MP: clinical characterisation of patients, interpretation of the results, critical reading of the manuscript. AMa, AR, IA, IC: interpretation of the results and critical reading of the manuscript.

Competing interests None declared.

Patient consent Patient or guardian according to the age.

Ethics approval G. Gaslini IRCCS Ethics Board.

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EXTENDED REPORT

Knee extensor strength and body weight in adolescent men and the risk of knee osteoarthritis by middle age

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ABSTRACT

Objectives To assess the extent to which knee extensor strength and weight in adolescence are associated with knee osteoarthritis (OA) by middle age.

Methods We studied a cohort of 40 121 men who at age 18 years in 1969/1970 underwent mandatory conscription in Sweden. We retrieved data on isometric knee extensor strength, weight, height, smoking, alcohol consumption, parental education and adult occupation from Swedish registries. We identified participants diagnosed with knee OA or knee injury from 1987 to 2010 through the National Patient Register. We estimated the HR of knee OA using multivariable-adjusted Cox proportional regression model. To assess the influence of adult knee injury and occupation, we performed a formal mediation analysis.

Results The mean (SD) knee extensor strength was 234 (47) Nm, the mean (SD) weight was 66 (9.3) kg. During 24 years (median) of follow-up starting at the age of 35 years, 2049 persons were diagnosed with knee OA. The adjusted HR (95% CI) of incident knee OA was 1.12 (1.06 to 1.18) for each SD of knee extensor strength and 1.18 (1.15 to 1.21) per 5 kg of body weight. Fifteen per cent of the increase in OA risk due to higher knee extensor strength could be attributed to knee injury and adult occupation.

Conclusion Higher knee extensor strength in adolescent men was associated with increased risk of knee OA by middle age, challenging the current tenet of low muscle strength being a risk factor for OA. We confirmed higher weight to be a strong risk factor for knee OA.

INTRODUCTION

Knee extensor muscle weakness is well recognised in individuals with knee osteoarthritis (OA).[1–5] The reported mechanisms behind this association include reduced physical activity due to joint pain leading to muscle atrophy and/or impaired muscle activation.[4] However, the underlying mechanisms behind any association between muscle strength and incident knee OA are not fully understood. Knee extensor muscle weakness might precede the development of radiographic or symptomatic knee OA, but results are inconclusive. Segal *et al*[6] reported a decreased risk of symptomatic, but not radiographic, knee OA in Multicenter Osteoarthritis Study participants with higher when compared with lower knee extensor strength. In the same cohort, higher muscle strength was reported to be a protective factor for knee OA when investigating persons with meniscal pathology only.[7] Slemenda *et al*[8] reported a weak association between low muscle strength and incident radiographic knee OA, but only in women. In other studies, weak or no association between low knee extensor strength and incident knee OA was found among young persons after anterior cruciate ligament reconstruction,[9 10] while in a sample of persons having chronic knee pain, fewer single-leg rises, indicative of weak lower extremity strength, were associated with higher risk for radiographic OA.[11] Importantly, in the literature, there is a paucity of studies on the role of lower extremity muscle strength in *young* individuals for knee OA development. There is also a lack of studies using population-based samples *not* enriched with pre-existing mild disease or with multiple knee OA risk factors as in most of the studies mentioned above. In addition, the common practice of defining strength exposure variables as a ratio of force by body weight might have resulted in finding spurious associations.[12] Furthermore, possible mediators of the association, such as joint injury or occupational factors have not been evaluated. Therefore, the aims of this study were (1) to evaluate to what extent isometric knee extensor strength and body weight in adolescence are associated with the risk of knee OA by middle age in a comprehensive, population-based cohort of Swedish men and (2) to evaluate the role of knee injury and adult occupation behind an association between knee extensor strength and knee OA.

METHODS

Data sources and study sample

We included 41 886 men, typically aged 18 years, who underwent a mandatory military conscription examination in Sweden between September 1969 and May 1970. The examination was performed at six centres including standardised physical and mental tests as well as evaluations by a physician. At that time, only a severe disability could be a reason for exclusion from the examination. The data were registered in the Swedish Military Service Conscription Register.

For the included men, we retrieved individual level data from the Swedish National Patient Register containing information about every hospitalisation (from 1987 to 2010), 1-day surgery (from 1997 to 2010) and specialist outpatient care visits (from 2001 to 2010). We included the date of visit

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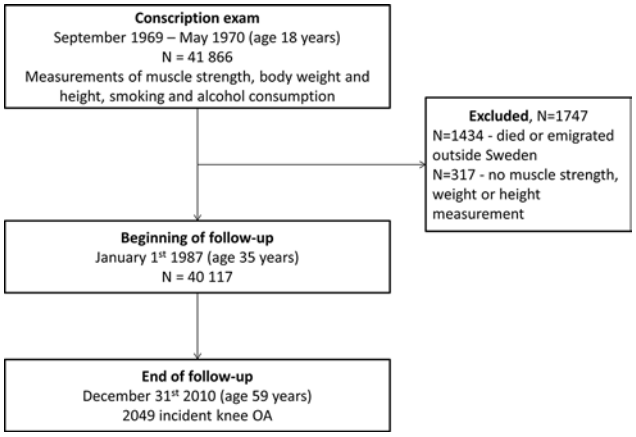

Figure 1 Flow chart of the study. OA, osteoarthritis.

and the diagnostic codes at the time of visit according to the International Classification of Diseases (ICD) system (ICD-9 up to year 1997 and ICD-10 from year 1998 and onwards). We further required the men to be alive and resident in Sweden by 1 January 1987, at the typical age of 35 years, corresponding to the start of the registration of diagnostic codes in the National Patient Register (figure 1).

In addition, we collected information about the highest level of parental education at the time of conscription and about occupation of the conscripts themselves in adulthood from Statistics Sweden.[13]

The study was approved by the Regional Ethical Review Board in Lund, Sweden.

Isometric knee extensor strength

Isometric knee extensor strength was measured using a validated protocol as previously described.[14] In brief, custom build chairs were used to measure knee extension in a sitting position (90° of hip flexion with the pelvis fixed by a strap to prevent concomitant extensions of the hip) with 90° knee flexion and arms crossed over the chest. Strength was measured using a dynamometer placed at the level of the lateral malleolus. The test was administered by trained personnel and performed at least three times with the highest value being recorded. Knee extensor strength in Newtons, multiplied by estimated shank length in metres (calculated as 0.246 times the body length), was used in the analysis.[15]

Covariates

Body weight was measured to the nearest kg, and body height was measured to the nearest cm. Body mass index (BMI) was calculated and WHO thresholds of 18.5 and 25 were used to define underweight and overweight.[16] Smoking status was self-reported in categories through questionnaires: non-smoker (reference category), 1–5 cigarettes per day, 6–10 cigarettes per day, 11–20 cigarettes per day and 21 or more cigarettes per day. Alcohol consumption was self-reported as frequency and amount of consumed beer, wine or sprit per week. We recalculated the intake as grams of pure alcohol consumed per week.[17] The conscripts' adult occupation was categorised into (1) farmers or mining or oil industry workers, (2) production workers and machine operators, (3) physically demanding service job workers (police, fire department, hotel, household chores and similar) or (4) other (reference category) based on the ISCO-68 international classification. The parental highest level of education (maximum from mother and father) was categorised into two categories: high school or less (reference category) and higher education.

We defined knee injury as (at least one) diagnostic code of dislocation of knee (ICD-9 code: 836, ICD-10 code: S83), knee contusion (924B, S80.0), fracture of patellae (822, S82.0) or internal derangement of knee (717, M23) during the follow-up period.

Definition of knee OA

We defined incident knee OA as the first record of knee OA registered in inpatient or specialist care between the year 1987 (typical age 35 years) and 2010 (typical age 59 years) with either the ICD-9 code 715 or the ICD-10 code M17. The validity of the diagnostic codes in Sweden has previously been reported to be high.[18–20] The positive predictive value of a knee OA diagnosis in a random sample of one of the largest regions in Sweden (Skåne, 1.3 million inhabitants) with respect to American College of Rheumatology clinical and radiographic criteria or radiographic knee OA (equivalent to Kellgren-Lawrence grade 2 or higher) was 88%.[19]

Statistical analysis

For descriptive purposes, we divided the study sample into four groups using quartiles of knee extensor strength. For our primary analyses, we used Cox proportional hazards regression models. We evaluated the proportional hazards assumption using tests and visual inspection of the zero-slope of Schoenfeld residuals. Follow-up for all included men was from 1 January 1987 (National Patient Register inception) until knee OA diagnosis, death, emigration or 31 December 2010, whichever occurred first (figure 1). The main exposure of interest was knee extensor strength in Nm included as a *continuous* variable. We performed a crude analysis and an analysis adjusted for pre-specified confounders, that is, parental education status (categorical), smoking (categorical), alcohol consumption (continuous) and body weight and height (both continuous). We evaluated the association between body weight and incident knee OA in a second model, adjusted for all the above covariates apart from knee extensor strength.

The percentage of persons who emigrated or died during the study period was low (9%), and results from an analysis ignoring time to event were essentially the same as from the Cox regression analysis. Thus, for the formal mediation analysis, we used logistic regression where we excluded persons who emigrated. The potential mediators evaluated were knee injury during follow-up and adult occupation. Only knee injuries diagnosed before knee OA were included. We used the method for decomposition of effects in nonlinear probability models developed by Karlson, Holm and Breen,[21] as implemented in the Stata command 'khb'.[22] The analysis was adjusted for the same confounders as those included in the Cox model. The statistical analysis was performed using Stata 14, StataCorp 2015 software.

Sensitivity analyses

We performed additional analyses where persons diagnosed with OA (joint not specified), rheumatoid arthritis (RA) or meniscal/cartilage pathology at the time of conscription were excluded.

We repeated our main analysis of the association between muscle strength and knee OA when excluding persons in the lowest or highest percentile of weight (ie, we included only those with weight between 49 and 95 kg to avoid potential bias due to non-positivity). Third, to evaluate the impact of our knee OA definition on the results, we expanded the definition to also

Table 1 Descriptive characteristics of the study sample divided into groups by quartiles of knee extensor strength

	Knee extensor strength quartiles				
	1	2	3	4	All
N	10 095	9983	10 018	10 021	40 117
Knee extensor strength in Nm, mean (SD)	177 (21.4)	217 (8.1)	246 (9.2)	295 (27.6)	234 (46.9)
Weight in kg, mean (SD)	61 (7.9)	65 (7.7)	68 (8.2)	72 (9.3)	66 (9.3)
Length in cm, mean (SD)	176 (6.4)	178 (6.0)	179 (6.0)	180 (6.0)	178 (6.3)
BMI, mean (SD)	19.8 (2.3)	20.6 (2.3)	21.2 (2.4)	22.2 (2.7)	21.0 (2.6)
Alcohol consumed per week in g,* median (IQR)	53 (23,101)	54 (26,102)	55 (27,102)	55 (27,105)	54 (25,102)
At least one parent with higher education,* n (%)	717 (7.1)	750 (7.5)	849 (8.5)	891 (8.9)	3207 (8.0)
OA, RA or meniscal/cartilage pathology, n (%)	72 (0.7)	56 (0.6)	69 (0.7)	70 (0.7)	267 (0.7)
Smoking, per day*					
Non-smoker	3884 (39)	3943 (40)	3971 (40)	4306 (44)	16 104 (41)
1–5 cigarettes	1110 (11)	1079 (11)	1201 (12)	1147 (12)	4537 (11)
6–10 cigarettes	2192 (22)	2151 (22)	2126 (22)	1935 (20)	8404 (21)
11–20 cigarettes	2398 (24)	2343 (24)	2204 (22)	2147 (22)	9092 (23)
21+ cigarettes	371 (4)	314 (3)	358 (4)	308 (3)	1351 (3)
Adult occupation*					
Farmers/miners	5789 (58)	5690 (58)	5621 (57)	5619 (57)	22 719 (57)
Production/machine operators	527 (5)	521 (5)	589 (6)	607 (6)	2244 (6)
Service, physically demanding	2876 (29)	2871 (29)	2893 (29)	2876 (29)	11 516 (29)
Other	762 (8)	776 (8)	810 (8)	824 (8)	3172 (8)
Knee injury during follow-up, n (%)	614 (6.1)	645 (6.5)	643 (6.4)	769 (7.7)	2671 (6.7)

*Missing data: alcohol consumption, 3.8%; parental education, 6.4%; smoking, 1.6%; adult occupation, 1.2%.
BMI, body mass index; OA, osteoarthritis; RA, rheumatoid arthritis; SD, standard deviation; IQR, interquartile range

include diagnoses of knee OA registered within primary care. These data were retrieved from regional healthcare registries (years 2000–2010) of two large regions in Sweden (Skåne and Västra Götaland) for a subset of 10 391 persons and yielded 24% more persons with knee OA. However, the date of diagnosis was not available and, thus, a logistic regression model was used for this analysis.

Finally, to evaluate if the results may reflect the propensity of individuals to seek healthcare, we repeated the analysis with *hip* OA as the outcome, as we expected not to find an association between knee extensor strength and hip OA.

RESULTS

Four per cent of persons were excluded from the analysis sample due to no exposure measurement, emigration or death before the follow-up start in 1987 (figure 1). These persons were on average heavier, consumed more alcohol and were more likely to have at least one parent with higher education. The mean (SD) knee extensor strength was 234 (47) Nm, and the mean (SD) body weight was 66 kg (9.3 kg). Their mean (SD) BMI was 21.0 (2.6), whereof 14% and 7% were underweight and overweight, respectively, according to the BMI thresholds of 18.5 and 25. The most common musculoskeletal disease diagnosed at the conscription examination was flat foot causing no or light reduction in function (in 7% of the men). Less than 1% of the men were diagnosed with OA, RA or meniscal/cartilage pathology at the conscription examination (table 1).

The mean (SD) follow-up time was 22.8 (3.8) years with a maximum of 25 years. Among the 40 117 included men, we identified 2049 incident knee OA cases, yielding an incidence rate of 22.4 (95% CI 21.5 to 23.4) per 10 000 person-years and an incidence proportion of 5% over 25 years (table 2). The mean age at the time of knee OA diagnosis was 53 years.

The crude HR for incident knee OA per 47 Nm (1 SD) of knee extensor strength was 1.26 (95% CI 1.20 to 1.31). After adjustment for body weight and height, the HR was 1.11 (95% CI 1.06 to 1.16). After additional adjustment for smoking, alcohol consumption and parental education, the results remained essentially the same with an HR 1.12 (95% CI 1.06 to 1.17) (figure 2). Higher body weight was associated with a higher risk of knee OA, with an HR of 1.18 (95% CI 1.15 to 1.21) per 5 kg body weight.

Investigating the mediating role of knee injury during follow-up on the association between knee extensor strength and knee OA, the ratio of total effect of knee extensor strength to direct effect was 1.13. In other words, 13% of the total effect

Table 2 Incidence of knee osteoarthritis (OA) by knee extensor strength quartiles

	Mean (SD) follow-up time, years	Number (%) of incident knee OA cases	Crude OA incidence (95% CI) per 10 000 person-years
Quartiles of knee extensor strength			
1	22.8 (3.8)	378 (3.7)	16.4 (14.8 to 18.1)
2	22.9 (3.8)	469 (4.7)	20.5 (18.8 to 22.5)
3	22.8 (3.8)	510 (5.1)	22.3 (20.5 to 24.3)
4	22.7 (3.8)	692 (6.9)	30.4 (28.3 to 32.8)
All	22.7 (3.9)	2049 (5.1)	22.4 (21.5 to 23.4)

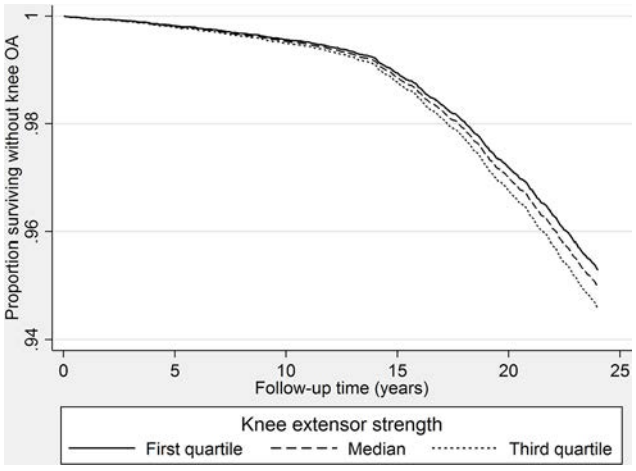

Figure 2 Survival function, estimated from the Cox regression model, at quartiles of quadriceps strength, adjusted for weight, height, parents' education, smoking and alcohol consumption. OA, osteoarthritis.

of knee extensor strength on knee OA was mediated by knee injury. Similarly, in the mediation analysis of adult occupation, the ratio of total effect to direct effect of knee extensor strength on knee OA was 1.02. Assessing both mediators in one model yielded similar results, where 15% of the effect of knee extensor strength was mediated by knee injury and occupation. On the absolute scale, the probability of having incident knee OA in this study sample was higher by 0.48 (95% CI 0.24 to 0.71) percentage points for a 1 SD of knee extensor strength. This was reduced to 0.42 (95% CI 0.19 to 0.66) percentage points after controlling for adult knee injury.

In the sensitivity analysis excluding 267 persons with OA, RA or meniscal/cartilage pathology diagnosed at the conscription examination, results remained similar (HR of knee OA per 1 SD of knee extensor strength was 1.11, 95% CI 1.06 to 1.17). Exclusion of persons with extreme weight had no essential impact on the results, HR of 1.11 (95% CI 1.06 to 1.17). When including knee OA diagnosed within primary care (in a subset of 10 391 persons), results were similar with an OR of 1.09 (95% CI 0.99 to 1.19). The association of knee extensor strength with *hip* OA adjusted for weight, height, parents' education, smoking and alcohol consumption was 1.04 (95% CI 0.97 to 1.12).

DISCUSSION

In this population-based study of young men, we found that higher knee extensor strength in adolescence was associated with an increased risk of incident knee OA by middle age. In absolute terms, the risk of incident knee OA over the follow-up time increased with 0.5% per 47 Nm higher knee extensor strength. Furthermore, 15% of this effect was estimated to be mediated by adult knee injury and adult occupation. Although the increase in risk was small, these results challenge the current belief of reduced muscle strength as a risk factor for knee OA.

Knee extensor weakness is well documented in individuals with knee OA and may often be secondary due to knee pain and/or reduced physical activity level.[3] However, knee extensor weakness has also been suggested to be an independent risk factor for the development of knee OA.[23] These results have been fairly consistent irrespective of the knee OA definition used, as recently reported in a systematic review.[23] However, these previous studies consisted of mainly middle-aged or elderly persons in cohorts enriched with risk factors for knee OA including frequent knee pain. In contrast, our study was population-based and included virtually all Swedish young men who were typically aged 18 years at the time of conscription, 35 years at the start of follow-up and 59 years at the end of follow-up. Thus, our study is less prone to sample selection bias than previous studies.[6 11 24–26] Furthermore, in a previous cohort study of persons without musculoskeletal problems at baseline, the authors reported that higher knee extensor strength divided by body weight was associated with lower risk of knee OA in women. Their analysis models were adjusted also for BMI (kg/m^2),[27] which implies a complicated function of body weight (with both weight and the inverse of weight included in the model). This makes interpretation of their findings and comparisons with other studies challenging. Furthermore, common practice of dividing muscle strength by body weight may lead to finding a protective effect of strength through inadequate control for the effect of body weight.[12] In our study, the relationship between knee extensor strength and risk of knee OA was roughly linear and persisted also after adjustment for several confounders and for body weight. We found body weight to be the strongest risk factor for knee OA, which is in accordance with previous evidence in younger adults.[28 29] Importantly, the absolute increase in risk due to higher knee extensor strength was rather small (~0.5% per one SD of knee extensor strength) and, thus, our results should not be interpreted as a discouragement of strengthening excises or physical activity in general.

The possible mechanisms behind an association between poor muscle strength and development of knee OA have mostly been hypothesised to be due to a protective role of strong muscles acting as shock absorbers and stabilisers of the joint. The interplay of muscle strength and other risk factors may be different in young adults when compared with middle-aged and elderly persons who may have several risk factors in addition to muscle weakness, that is, overweight or obesity. In a formal mediation analysis in the present work, we estimated the role of knee injury and adult occupation in the association between knee extensor strength and knee OA. Knee injury is the strongest known risk factor for the development of knee OA.[30] In our study, 13% of the increase in risk of knee OA associated with higher knee extensor strength was attributed to the role of knee injury. A limitation is that we were not able to identify knee injuries in young adulthood (before the age of 35 years), when such acute injuries often occur, or knee injuries that did not lead to a healthcare visit. Thus, our result is most likely an underestimation of the true role of knee injury behind the observed association between knee extensor strength in adolescence and knee OA by middle age. This reinforces the need for the prevention of joint injuries in young physically active individuals.[31]

There are some limitations related to the data sources that need to be acknowledged. First, we did not have any measurement of muscle strength during the follow-up. One hypothesis, still to be evaluated, may be that active persons with high muscle strength in adolescence, who later become less active and lose muscle strength, may be those at high risk of knee OA. Also, only knee OA diagnosed within inpatient or outpatient specialist care was included. Thus, persons with knee OA who have not sought healthcare, for example, those perceiving mild symptoms without substantial limitations in daily life, have not been captured. However, including knee OA diagnosed in primary care in a subset of our study sample yielded similar results as our main analysis. One could further speculate that our results reflect a higher propensity for healthcare seeking in persons with higher muscle strength, for example, due to higher body weight and overweight/obesity-related issues. This, however, is unlikely to have influenced our results, as we did not find a

similar association between knee extensor strength and incident *hip* OA. Similar constraints apply for possible misclassification of knee injury. Finally, only men were included in our study sample and, thus, we cannot draw any conclusions about women. Also, in the 1970s, many countries had compulsory military training and were in this respect similar to Sweden. Today, however, only a minority of countries have compulsory military service; thus, the generalisability to new generations of men may be uncertain.

CONCLUSION

In this large population-based sample of young men, we found evidence of increased risk of incident knee OA during middle age when having higher knee extensor strength or higher body weight, even after taking into account the role of adult knee injury and occupation, as well as several other confounders based on individual-level data. We could not confirm the previously reported increase in risk of knee OA in persons with knee extensor weakness. However, the vast majority of previous studies included older participants selected on the basis of having multiple risk factors for knee OA and, thus, are not directly comparable with our study sample. Thus, our results indicate that low muscle strength in young adulthood may not be a risk factor for the development of knee OA in men, while high body weight is.

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EXTENDED REPORT

Randomised controlled trial of prolonged treatment in the remission phase of ANCA-associated vasculitis

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ABSTRACT

Objectives A prospective randomised trial to compare two different durations of maintenance immunosuppressive therapy for the prevention of relapse in anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV).

Methods Patients with AAV were recruited 18–24 months after diagnosis if they were in stable remission after cyclophosphamide/prednisolone-based induction followed by azathioprine/prednisolone maintenance therapy. They were randomised (1:1) to receive continued azathioprine/prednisolone to 48 months from diagnosis (continuation group) or to withdraw azathioprine/prednisolone by 24 months (withdrawal group). The primary endpoint was the relapse risk, from randomisation to 48 months from diagnosis.

Results One hundred and seventeen patients were randomised and 110 remained to the trial end. At entry, median serum creatinine was 116 µmol/L (range 58–372), 53% were ANCA positive. The percentage of patients presenting with relapse was higher in the withdrawal than in the continuation treatment group (63% vs 22%, p<0.0001, OR 5.96, 95% CI 2.58 to 13.77). ANCA positivity at randomisation was associated with relapse risk (51% vs 29%, p=0.017, OR 2.57, 95% CI 1.16 to 5.68). Renal function, ANCA specificity, vasculitis type and age were not predictive of relapse. Severe adverse events were more frequent in the continuation than withdrawal groups (nine vs three events), but the continuation group had better renal outcome (0 vs 4 cases of end-stage renal disease), with no difference in patient survival.

Conclusions Prolonged remission maintenance therapy with azathioprine/prednisolone, beyond 24 months after diagnosis reduces relapse risk out to 48 months and improves renal survival in AAV.

Trial registration number ISRCTN13739474

INTRODUCTION

Anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV) are a group of autoimmune systemic diseases that are associated with a necrotising, pauci-immune, vasculitis of small blood vessels and the presence of circulating autoantibodies to myeloperoxidase (MPO-ANCA) or proteinase 3 (PR3-ANCA). The major subgroups of AAV are microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA, Wegener's) and eosinophilic GPA.[1] Renal involvement is manifested by a necrotising, crescentic glomerulonephritis and results in end-stage renal disease (ESRD) in up to 20% of patients.[2]

Therapeutic management of MPA and GPA is divided into induction and maintenance phases. Remission induction is achieved in most cases with cyclophosphamide or rituximab in combination with high-dose glucocorticoids and sometimes plasma exchanges, while remission maintenance regimens have employed an oral immunosuppressive, such as azathioprine or methotrexate, or repeat-dose rituximab, with or without low-dose glucocorticoids.[3 4] The optimal duration of remission maintenance therapy remains unknown, with current consensus recommendations suggesting at least 24 months, once remission has been obtained.[5]

Relapse occurs in 30%–50% of patients by 5 years and has been associated with a diagnosis of GPA, PR3-ANCA specificity, the presence of ear nose and throat involvement, persisting ANCA positivity after induction therapy, a lower serum creatinine at diagnosis and withdrawal of glucocorticoids or immunosuppressives.[6] The consequences of relapse are additional accrual of disease and treatment-related damage and morbidity, and renal relapse is associated with an increased risk of ESRD.[7] Continuing remission therapy increases exposure to the toxicities of immunosuppressives and glucocorticoids.

This study tested whether continued azathioprine/prednisolone was more effective in preventing relapse than their withdrawal at 24 months from diagnosis in patients with AAV.

METHODS

Study design and patients

We hypothesised that prolonged maintenance therapy with low-dose prednisolone and azathioprine reduces the frequency of relapse, when compared with withdrawal of immunosuppression 2 years after diagnosis. This trial (REMAIN, prolonged REmission-MAINtenance therapy in systemic vasculitis) was conducted by the European Vasculitis Society (EUVAS) and recruited patients from 33 centres in 11 European countries. The study was approved by the local ethics committee in each participating centre, and all patients provided written informed consent, according to the Declaration of Helsinki.

Criteria for inclusion were (1) a diagnosis of MPA, GPA or renal-limited vasculitis; (2) renal involvement and/or other threatened loss of function of a vital organ (lung, brain, eye, motor nerve or gut) and

ANCA positivity, and ANCA-negative patients were eligible for enrolment in the study only when there was histological confirmation of pauci-immune vasculitis; (3) remission-induction therapy with cyclophosphamide and prednisolone for at least 3 months, with or without plasma exchanges; and (4) stable remission on azathioprine/prednisolone. They were recruited and randomised 18–24 months from commencement of therapy.

Exclusion criteria were age under 18 years, pregnancy, previous malignancy, known HIV infection, previous life-threatening relapse, ESRD at inclusion and allergy to study medications. Patients not in stable remission for at least 6 months at 18 months after commencement of therapy and patients who had discontinued azathioprine and/or prednisolone were excluded from the study.

Disease definitions

Diagnostic definitions were initially based on the 1994 Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitides[8] and re-evaluated after completion of the study, according to the recently revised criteria.[1] Remission was defined as a 1994 Birmingham Vasculitis Activity Score (BVAS) of 0, indicating the absence of new or worse disease activity, with persistent disease activity for no more than one item.[9] Major relapse was defined by the recurrence or first appearance of at least 1 of the 24 items of the BVAS, which are indicative of threatened function of a vital organ (kidney, lung, brain, eye, motor nerve or gut) attributable to active vasculitis. Minor relapse was defined by the recurrence or first appearance of at least three other BVAS items. Determinations of remission and relapse were made by the investigator and validated retrospectively by an independent observer.

Drug regimens

Patients were randomly assigned at entry, 18–24 months after initiation of immunosuppression, in a 1:1 ratio, to continuation or withdrawal treatment groups (table 1).

Evaluations

Study assessments were performed at entry, then every 3 months until 30 months from entry for non-relapsing patients and until the time of relapse for relapsing patients. The assessments included BVAS,[9] serum creatinine, C-reactive protein, ANCA positivity (requiring both positivity by indirect immunofluorescence and by PR3-ANCA or MPO-ANCA assay, performed in local laboratories), drug doses and adverse events. Glomerular filtration rate (eGFR) was estimated using the modification of diet in renal disease (MDRD) formula.[10] All cause damage since the vasculitis diagnosis was assessed by the Vasculitis Damage Index (VDI)[11] at entry then every 6 months to the trial end.

Endpoints

The primary endpoint was the percentage of patients presenting a relapse of vasculitis, including major and minor relapses, during the study period. Secondary endpoints were incidence of major and minor relapses, mortality, adverse events of therapy, rise in cumulative damage score (VDI), deterioration of eGFR, incidence of ESRD and ANCA status during follow-up.

Statistical analysis

Randomisation was performed centrally. Treatment allocation was done by block randomisation (permuted blocks of four) per country. Primary data were collected locally in record books and subsequently submitted for centralised validation and analysis.

Based on a one-tailed design, with a significance level of 5% and a power of 0.8, the inclusion of 116 patients was required to demonstrate a 20% lower relapse rate in the maintenance therapy group during the study period (assuming a threefold increase of relapse risk in the discontinuation arm).

The demographic characteristics of the two groups were compared with the use of Student's t-test or a Wilcoxon rank-sum test for continuous measures and a χ^2 test or Fisher's exact test for categorical variables. The effect of treatment on time to relapse was examined by Kaplan-Meier analysis, with the use of the log-rank test. The two groups were compared in terms of the secondary endpoints reached between the time of remission and the end of the study. The rates of adverse events were compared with the use of two-by-two tables and Fisher's exact test. The values of eGFR and VDI were compared with the use of the Wilcoxon rank-sum test.

Table 1 Drug doses according to treatment arm

	Continuation arm		Withdrawal arm	
Months from randomisation	Prednisolone mg/day	Azathioprine mg/kg/day	Prednisolone mg/day	Azathioprine mg/kg/day
0	5–7.5	1	5	0.75
1	5–7.5	1	4	0.75
2	5–7.5	1	4	0.75
3	5	1	2	0
4	5	1	1	0
5	5	1	0	0
12	5	1	0	0
18	4	1	0	0
19	4	1	0	0
20	3	1	0	0
21	3	1	0	0
22	2	1	0	0
23	1	1	0	0
24	0	1	0	0
30	0	1	0	0

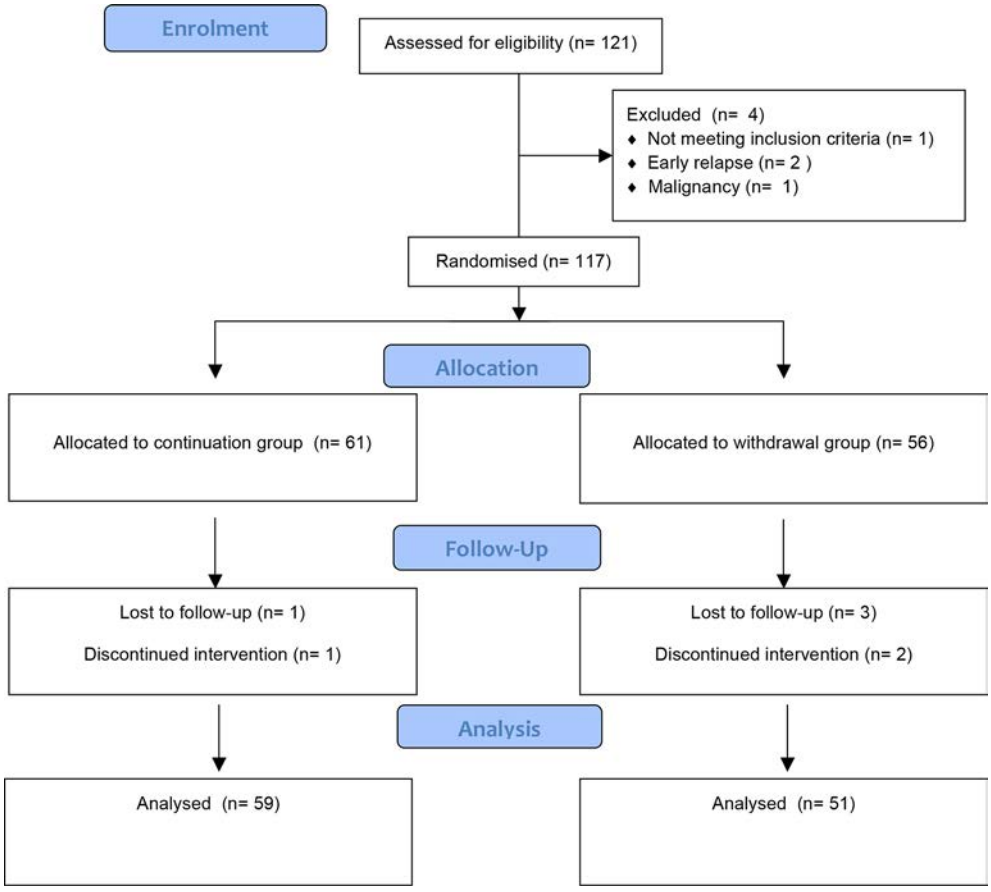

Figure 1 Flow diagram of the REMAIN study, summarising enrolment, intervention allocation, follow-up and data analysis.

RESULTS

Patients

Between September 1998 and March 2010, 121 patients were enrolled. Four were excluded due to ineligibility, because of early relapse (n=3) or malignancy (n=1). One hundred and seventeen patients were randomised with a mean period of 18.8±1.8 months after initiation of induction therapy: 61 to the continuation and 56 to the withdrawal groups. During the study, three patients withdrew (patient's choice (n=2) or physician's decision (n=1)) and four patients were lost to follow-up (figure 1). Complete data for analysis were available for 110 patients.

Demographic characteristics of the study population are detailed in table 2. Fifty-two patients (47%) had GPA and 58 (53%) had MPA. ANCA specificity was PR3 in 52% of cases and MPO in 44%, whereas ANCA were negative or without specificity in 4%. Almost all patients (96%) were enrolled in this study after the first remission of newly diagnosed AAV. The median follow-up was 925 days (IQR 878–970), after randomisation.

Protocol treatment

At randomisation, the mean daily azathioprine dose was 99±37 mg and the mean daily prednisolone dose was 5.9±2.2 mg. Immunosuppression was rapidly tapered according to the study protocol in the withdrawal group, whereas it was continued until the end of study in the continuation group. Median and mean daily doses of azathioprine and prednisolone are detailed in supplementary figures 1a and 1b.

Efficacy assessment

Primary endpoint

Thirty-two patients (62.7%) in the withdrawal group experienced a relapse as compared with 13 (22.0%) in the continuation group (log rank test p<0.0001) (figure 2a). The patients in the withdrawal group had a 2.84-fold higher relative risk of relapse (95% CI 1.72 to 4.9) when compared with patients continuing immunosuppression. Interestingly, 78% of relapses in the withdrawal group occurred after removal of azathioprine versus 8% in the continuation group. The median daily azathioprine dose at relapse was 75 mg (IQR 50–100) mg in the continuation group versus 0 mg (IQR 0–0) in the withdrawal group.

Of note, primary endpoint difference between treatment groups remained highly significant (p<0.001) if we excluded patients of the withdrawal group that continued small doses of azathioprine beyond month 6 (n=5) or if we included patients that withdrew their consent or were lost to follow-up (n=7).

Secondary endpoints

A major relapse occurred in 18 patients (35.3%) of the withdrawal and in eight patients (13.5%) of the continuation groups (p=0.007) (figure 2b). The eGFR at last follow-up was 52.5±26.7 mL/min/1.73 m^2 in the withdrawal and 54.1±24.7 mL/min/1.73 m^2 in the continuation groups (p=0.78). Nevertheless, the ΔeGFR between randomisation and end of study in the withdrawal group was −3.3±14.9 mL/min/1.73 m^2, whereas it was +2.5±9.8 mL/min/1.73 m^2 in the continuation group (p=0.01). This difference was not found

Table 2 Demographics of randomised patients according to treatment arm, 18–24 months after diagnosis

Variable	Continuation group (n=59)	Withdrawal group (n=51)	p Value
Age (years)	57.7±14.1	57.4±14.3	0.89
Sex (%)			0.69
Male	49	53	
Female	51	47	
AAV type (%)			0.96
GPA	47	47	
MPA	53	53	
ANCA at diagnosis (%)			0.11
PR3	46	59	
MPO	47	41	
Negative	7	0	
Delay from diagnosis (months)	18.6±0.2	19.0±0.2	0.28
Serum creatinine (µmol/L)	140±67	129±54	0.34
eGFR (mL/min/1.73 m^2)	51.6±23.0	55.8±23.4	0.34
ANCA			0.59
Positive	51%	56%	
Negative	49%	44%	
Prednisolone dose (mg/day)	5.8±2.3	5.9±2.1	0.61
Azathioprine dose (mg/day)	102±35	95±39	0.27
VDI	1.8±0.2	1.8±0.2	0.98

Values are given as mean±SD.
AAV, ANCA-associated vasculitis; eGFR, estimated glomerular filtration rate; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; PR3, proteinase 3; MPO, myeloperoxidase; VDI, Vasculitis Damage Index.

when the analysis was restricted to patients that had completed follow-up without relapse (+0.8±15.4 vs +2.6±8.0 mL/min/1.73 m^2, p=0.53). Four patients (7.8%) of the withdrawal group developed ESRD during follow-up as compared with none in the continuation group (p=0.012). Of note, median eGFR at randomisation, for these four patients, was 29.5 mL/min/1.73 m^2 (range 15–59). There were two deaths (3.9%) in the withdrawal and five (8.5%) in the continuation groups (p=0.32). Causes of death were cancer in three cases, cardiovascular disease in 2 and undetermined for two patients.

Although there was no difference in the percentage of ANCA-positive patients at randomisation (51% in the continuation vs 56% in the withdrawal groups), patients in the withdrawal group had more frequent reappearance of ANCA after randomisation. By month 6, 72% of patients in the withdrawal group were ANCA positive compared with 52% in the continuation group (p=0.04) (see online supplementary figure 2a). There was no difference in the final VDI score between the two groups (see online supplementary figure 2b).

Predictors of relapse

Univariate analysis revealed that withdrawal of immunosuppression (p<0.0001) and ANCA positivity at randomisation (p=0.017) were the only predictors of relapse during follow-up (figure 2c and table 3). Multivariate analysis confirmed that these factors were independently associated with risk of relapse. ANCA specificity at diagnosis (PR3 vs MPO), disease phenotype (GPA vs MPA), age or renal function at randomisation were not predictive of relapse in this study. Similar results were obtained when studying the risk of major relapse during follow-up.

Kaplan-Meier analysis shows that the difference between survival according to treatment arm persists across different subgroups of patients, such as patients with MPO-ANCA or PR3-ANCA specificity (see online supplementary figure 3a), as

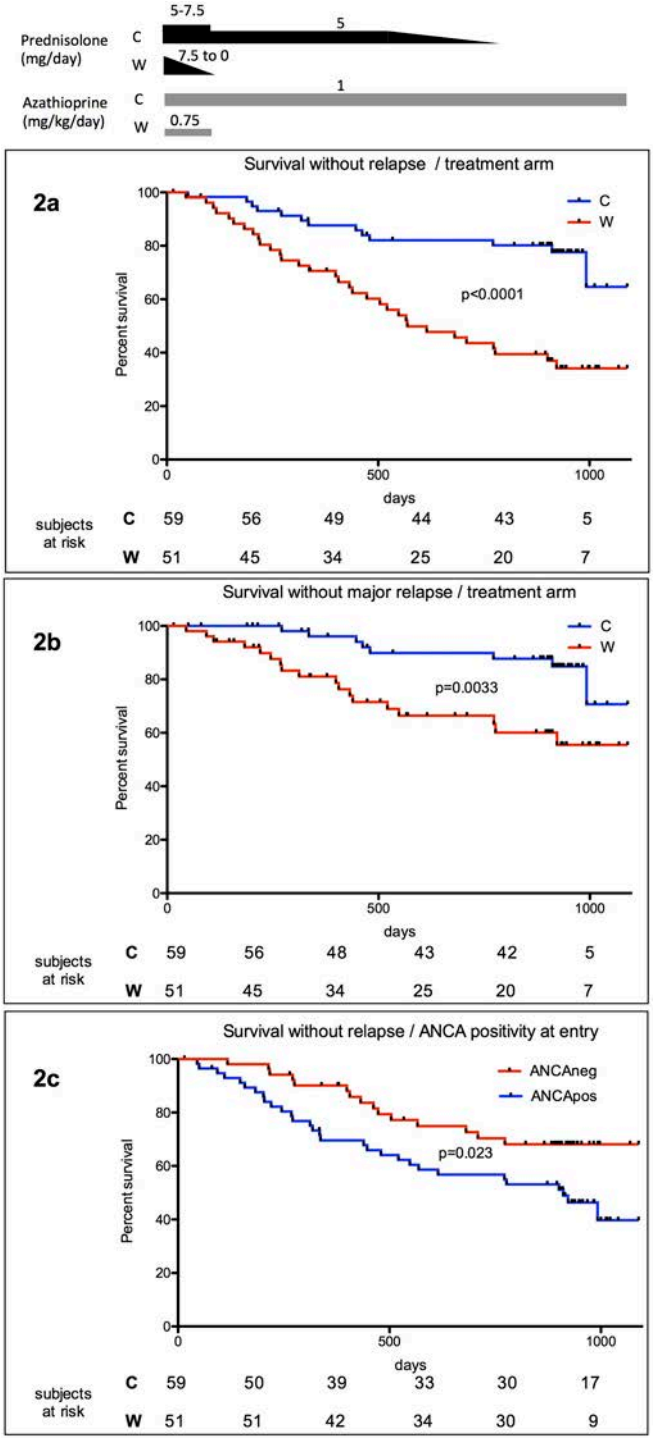

Figure 2 Kaplan-Meier analysis of the study population showing relapse-free survival according to treatment group (2a), major relapse-free survival according to treatment group (2b) and relapse-free survival according to positivity or negativity of ANCA at randomisation (2c). Daily dose of prednisolone and azathioprine in each group is shown above. C, continuation subgroup; W, withdrawal subgroup.

Table 3 Risk factors associated with AAV relapse

	Subgroup	Relapse risk	p Value	OR (95% CI)
Treatment arm	W	32/51 (63%)	<0.0001	5.96 (2.58 to 13.77)
	C	13/59 (22%)		
ANCA specificity at diagnosis	PR3	28/57 (49%)	0.13	1.82 (0.83 to 3.98)
	MPO	17/49 (35%)		
ANCA testing at randomisation	Positive	30/58 (51%)	0.017	2.57 (1.16 to 5.68)
	Negative	15/51 (29%)		
Disease	MPA	22/58 (38%)	0.5	0.77 (0.36 to 1.65)
	GPA	23/52 (44%)		

AAV, ANCA-associated vasculitis; C, continuation subgroup; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; MPO, myeloperoxidase; PR3, proteinase 3; W, withdrawal subgroup.

well as in patients with or without ANCA positivity at randomisation (see online supplementary figure 3b).

Severity of relapse

Characteristics of the relapses occurring during the study period are detailed in supplementary table 1. Relapse severity was not different between treatment arms. Mean BVAS at relapse was 7.1±4.1 in the withdrawal group and 8.7±4.4 in the continuation group (p=0.29). Organ involvement at relapse was not different between the two groups, except eye, nose, throat (ENT) involvement, which was more frequent in the continuation group (69% vs 34%, p=0.048).

Adverse events

Seventy-one adverse events were reported in 46 patients (table 4). Severe or life-threatening adverse events occurred in 13 patients (12%). There was no statistical difference between the two groups in the prevalence or severity of adverse events. The most frequent events were infections, occurring in 13 (22%) patients of the continuation and 10 (19%) of the withdrawal group. Haematological disorders and cardiovascular events were more frequent among patients of the continuation group.

DISCUSSION

ANCA-associated vasculitis carries a substantial and often unpredictable risk of relapse, and prolonged relapse prevention therapy is recommended that may itself contribute to organ damage, morbidity and patient mortality.[7] We have shown that continuation of treatment with azathioprine and prednisolone beyond 24 months from diagnosis was more effective at preventing relapse than withdrawal of these agents for AAV patients with GPA/MPA. These results confirm the ability of the azathioprine/prednisolone combination to influence relapse risk and suggest that treatment should be continued for at least 48 months from diagnosis, especially in those with persistent ANCA positivity after induction therapy.

Despite the use of azathioprine/prednisolone-based remission maintenance therapy, the risk of relapse after induction of remission with cyclophosphamide was 38% at 5 years in the recent meta-analysis of previous EUVAS trials.[6] This risk remains high when rituximab is given for induction of remission instead of cyclophosphamide, with a 32% risk of relapse at month 18.[12] Previous relapse prevention studies in AAV have demonstrated an equivalence of methotrexate to azathioprine, a lower efficacy with mycophenolate mofetil, and no efficacy of etanercept.[13–15] In view of the high proportion of patients with renal disease in this study, azathioprine was selected over methotrexate. Repeat-dose rituximab has recently been shown to be superior at preventing relapse than azathioprine,[16] but this drug was not is use in AAV at the time this trial was designed.

The optimal duration of immunosuppressive therapy after induction of remission is unknown. Only one previous study has compared different regimens, in patients with persistent anti-PR3-ANCA positivity at remission. Sanders *et al*[17] randomised 45 patients to receive either standard (1 year after diagnosis and subsequent tapering) or extended (4 years after diagnosis and tapered thereafter) azathioprine maintenance therapy. Although 46% of patients relapsed in the standard therapy group versus 24% in the extended therapy group, the difference was not statistically different.

This study clearly demonstrates that continuation of glucocorticoids and azathioprine beyond 2 years is associated with a threefold reduction of relapse risk. Moreover, extension of immunosuppression is associated with a better renal survival, as illustrated by the fact that all patients that reached ESRD during follow-up had discontinued azathioprine a few months before. Of note, previous studies have shown that every renal relapse is associated with an eGFR decrease of 8–12 mL/min[18 19] and that those patients are 4.7 times more likely to progress to ESRD.[20]

The overall frequency of relapse seen in this study, 41% at 48 months, was similar to that reported in other AAV studies (46% in the IMPROVE trial,[14] 35% in the WEGENT trial,[13] 45% in the azathioprine arm of the MAINRITSAN trial).[16] In the present study, relapse risk (62.7%) in the control group was higher than usually reported, but in previous trials the follow-up period after cessation of immunosuppression was significantly shorter. Interestingly, there was good compliance of clinicians to the study drug regimen in the withdrawal group and even a trend to underdosing in the continuation group that may have reduced the magnitude of the treatment effect of azathioprine/prednisolone. The relative contributions of azathioprine or prednisolone to the treatment effect are not known, although an earlier systematic review has highlighted the increase in relapse risk that follows glucocorticoid withdrawal.[6]

In our study, continuation of immunosuppressive therapy was associated with more frequent adverse events such as malignancies or infections. The safety profile was not statistically different between the two groups, but our study was underpowered to detect significant differences in adverse events between groups. Importantly, patient survival as well as VDI score, which reflects the cumulative organ damage due to vasculitis and/or treatment, was similar. Of note, the final VDI score in the whole study

Table 4 Adverse events (AEs)

Variable	Continuation group (n=59)	Withdrawal group (n=51)	p Value
Total number of AEs	43	28	0.07
Number (%) of patients with at least one AE	26 (44%)	20 (39%)	0.69
Number (%) of patients with ≥ grade 3 AE	9 (15%)	3 (6%)	0.13
Type of AE			
Cancer	7	4	0.54
Non-melanoma skin cancer	2	2	0.99
Infection	17	13	0.83
Cytopaenia	7	1	0.066
Hepatitis	2	2	0.99
Cardiovascular events	5	0	0.060

AEs were classified according to the v3.0 Common Terminology Criteria for Adverse Events.

population was 2.2, similar to that described (2.66) in the long-term follow-up of the EUVAS trials.[7] Despite small numbers, we found that cytopaenias and cardiovascular complications were more common in the continuation group, possibly in relation with the known toxicity of azathioprine and the metabolic effects of glucocorticoids.

The main question raised by this study is whether we should recommend an extended duration of immunosuppression for all patients with AAV after achievement of sustained remission. Numerous studies have demonstrated that the risk of relapse is more important among patients with GPA and/or anti-PR3,[6 21] suggesting that this subgroup of patients should receive prolonged immunosuppressive therapy. Our study was underpowered to confirm this hypothesis (see online supplementary figure 3a). Nevertheless, our data show that persistent ANCA positivity, 2 years after initiation of immunosuppression predicts a higher risk of relapse, suggest that this subgroup of patient may require a different immunosuppressive regimen. Other studies have observed a reduced risk of relapse was associated with negativity of ANCA at the time of switching to maintenance therapy.[22 23] However, in our study, relapse occurred in 29% of patients with negative ANCAs at randomisation, when assigned to azathioprine discontinuation (supplementary figure 3b), revealing that even those patients have an important risk of relapse when stopping the immunosuppressive drugs too early. Of note, 83% of relapsing patients with ANCA negativity at inclusion had positive ANCA testing at relapse.

Our study has several limitations. First, this trial was open label and the absence of placebo might have led to overestimation of the relapse rate in patients having discontinued azathioprine and corticosteroids. BVAS is a semiobjective tool, although many relapses were renal assessed by objective criteria. Nevertheless, prognosis was still different between the two groups, even when robust criteria were used to define severe flare of disease or ESRD. Second, this study was designed and conducted before widespread use of rituximab, and it is difficult to extrapolate these results to patients receiving B-cell depleting agents as induction and/or maintenance therapy. Further studies are determining if, similarly to what we have shown with azathioprine, an 18-month duration is less effective than a 48-month maintenance therapy with rituximab, for prevention of AAV relapse (MAINRITSAN 3three trial, NCT02433522). Third, the study design may have induced some bias in the study population, excluding patients with intolerance to azathioprine and patients with more severe disease, such as patients with life-threatening vasculitis or patients who experienced early relapse, during the initial 18 months azathioprine therapy preceding randomisation. We did not have detailed information from diagnosis to precisely describe organ distribution such as ENT or cardiovascular involvement, baseline renal function, cyclophosphamide exposure (total dose, intravenous/oral administration), factors known to influence relapse risk. Fourth, the 12 years' duration of study enrolment could have influenced the results. Nevertheless, the relapse rate was stable throughout the study period, and patients who entered the study before 2006 had an overall relapse rate of 40% versus 42% for those who were enrolled after this date. Finally, we were not able to calculate and compare cumulative glucocorticoid dose in each group, as treatment data were not collected for all patients, after occurrence of relapse. This point is important as it has been shown that part of the long-term metabolic and cardiovascular toxicity of immunosuppression is due to excessive cumulative doses of glucocorticoids, given either as a preventive remission maintenance therapy or as curative induction treatment during repeated flares of AAV.[24]

In conclusion, we suggest that at least some of the patients that have reached remission of AAV require long-term immunosuppressive therapy to prevent recurrence of the disease. The challenge of future studies will be to define the best immunosuppressive scheme, providing both efficacy and limited toxicity and to find clinical or biological markers that will identify high-risk patients who will require prolonged therapy.

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Contributors AK contributed to data collection, data analysis and interpretation,manuscript preparation and review. CP contributed to data generation, collection,analysis and interpretation, manuscript preparation and review. MH contributed to study design and set-up, data generation, analysis and interpretation,manuscript preparation and review. KdG contributed to data generation, analysis and interpretation, manuscript preparation and review. XP contributed to data generation, analysis and interpretation, manuscript preparation and review. JWCT contributed to data generation, analysis and interpretation, manuscript preparation and review. MS contributed to study design and set-up, data generation, analysis and interpretation, manuscript preparation and review. LG contributed to data generation, analysis and interpretation, manuscript preparation and review. DJ contributed to study design and set-up, data generation and collection, analysis and interpretation, manuscript preparation and review.

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Patient consent Patient.

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Data sharing statement All study data are included in this manuscript.

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EXTENDED REPORT

Comparative effectiveness of allopurinol versus febuxostat for preventing incident renal disease in older adults: an analysis of Medicare claims data

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ABSTRACT

Objective To assess the comparative effectiveness of allopurinol versus febuxostat for preventing incident renal disease in elderly.

Methods In a retrospective cohort study using 2006–2012 Medicare claims data, we included patients newly treated with allopurinol or febuxostat (baseline period of 183 days without either medication). We used 5:1 propensity-matched Cox regression analyses to compare the HR of incident renal disease with allopurinol use (and dose) versus febuxostat (reference). Sensitivity analyses included multivariable-adjusted regression models.

Results There were 31 465 new allopurinol or febuxostat treatment episodes in 26 443 patients; 8570 ended in incident renal disease. Crude rates of incident renal disease per 1000 person-years were 192 with allopurinol versus 338 with febuxostat. Crude rates of incident renal disease per 1000 person-years were lower with higher daily dose: allopurinol <200, 200–299 and ≥300 mg/day with 238, 176 and 155; and febuxostat 40 and 80 mg/day with 341 and 326, respectively. In propensity-matched analyses, compared with febuxostat, allopurinol use was associated with lower HR of incident renal disease, 0.61 (95% CI 0.49 to 0.77). Compared with febuxostat 40 mg/day, allopurinol doses <200, 200–299 and ≥300 mg/day were associated with lower HR of incident renal disease, 0.75 (95% CI 0.65 to 0.86), 0.61 (95% CI 0.52 to 0.73) and 0.48 (95% CI 0.41 to 0.55), respectively. Sensitivity analyses using multivariable-adjusted regression confirmed these findings.

Conclusions Allopurinol was associated with a lower risk of incident renal disease in elderly patients than febuxostat. Future studies need to examine the mechanism of this potential renal benefit of allopurinol.

INTRODUCTION

Chronic kidney disease (CKD) is a common chronic medical condition with a significant public health impact. One in 10 American adults have CKD.[1] CKD incidence in the US elderly increased significantly from 2% in 2000 to 4.5% in 2008 and the prevalence increased from 18.8% in 1988—94% to 24.5% to 2003–2006.[2] There has been a long-standing 'chicken or the egg' controversy regarding the relationship of hyperuricemia with renal disease.[3] Since uric acid is primarily excreted as well as reabsorbed in the kidney, many have argued that hyperuricemia is merely a result of slowly progressive CKD.[4 5] Others argued that based on animal studies and other human data hyperuricemia may be causative to CKD.[6–10] Allopurinol and febuxostat, two urate-lowering therapies (ULTs), have been the focus of recent interest for their potential role in preserving renal function.[11 12]

Xanthine oxidase (XO) is a form of the enzyme xanthine oxido-reductase (XOR) that generates reactive oxygen species.[13] XO catalyses the oxidation of hypoxanthine to xanthine and xanthine to uric acid.[13] Allopurinol is a purine analogue that inhibits XO, thereby lowers serum urate (sUA) and possibly reduces oxidative stress. Febuxostat is a non-purine analogue that selectively inhibits XO and lowers sUA.[14] In an animal model of oxonic acid-induced hyperuricemia, hyperuricemia-induced renal arteriopathy and hypertension developed and macula densa NO synthase decreased[15]; co-administration of allopurinol or L-arginine (stimulation of NO synthesis) in this animal model prevented hypertension and kidney damage.[6 15] XOR-derived superoxide and hydrogen peroxide lead to biomolecule oxidation including the formation of F2-isoprostanes and the oxidation of amino acids. Both lipid and protein oxidation end products are elevated in clinical and experimental kidney injury.[16 17] Oxidative stress reduction is more prominent with febuxostat than with allopurinol.[18 19] Thus, while evidence supports a detrimental role of oxidative stress in kidney injury and suggests that allopurinol may be protective against renal injury, and febuxostat likely even more protective, conclusive evidence supporting a protective effect is lacking.

Evidence from well-designed observational studies indicated that allopurinol use was associated with improved renal function or slower decline in renal function in some,[20–22] but not all,[23–26] observational studies. These studies differed in population examined and confounders adjusted in analyses. Our recent observational study showed that higher allopurinol dose and a longer duration of use was independently associated with lower risk of incident renal disease in allopurinol users 65 years or older.[27] Observational studies showed that febuxostat use was associated with a slower decline in renal function.[28 29] Allopurinol or febuxostat use was associated with a slightly improved renal function in 137 hypertensive patients with hyperuricemia 1–3 months post treatment.[30]

Large-scale randomised studies are under way to assess whether compared with placebo each XO inhibitor, allopurinol[31] or febuxostat,[32] can prevent renal function loss. This evidence is needed and will confirm their nephroprotective

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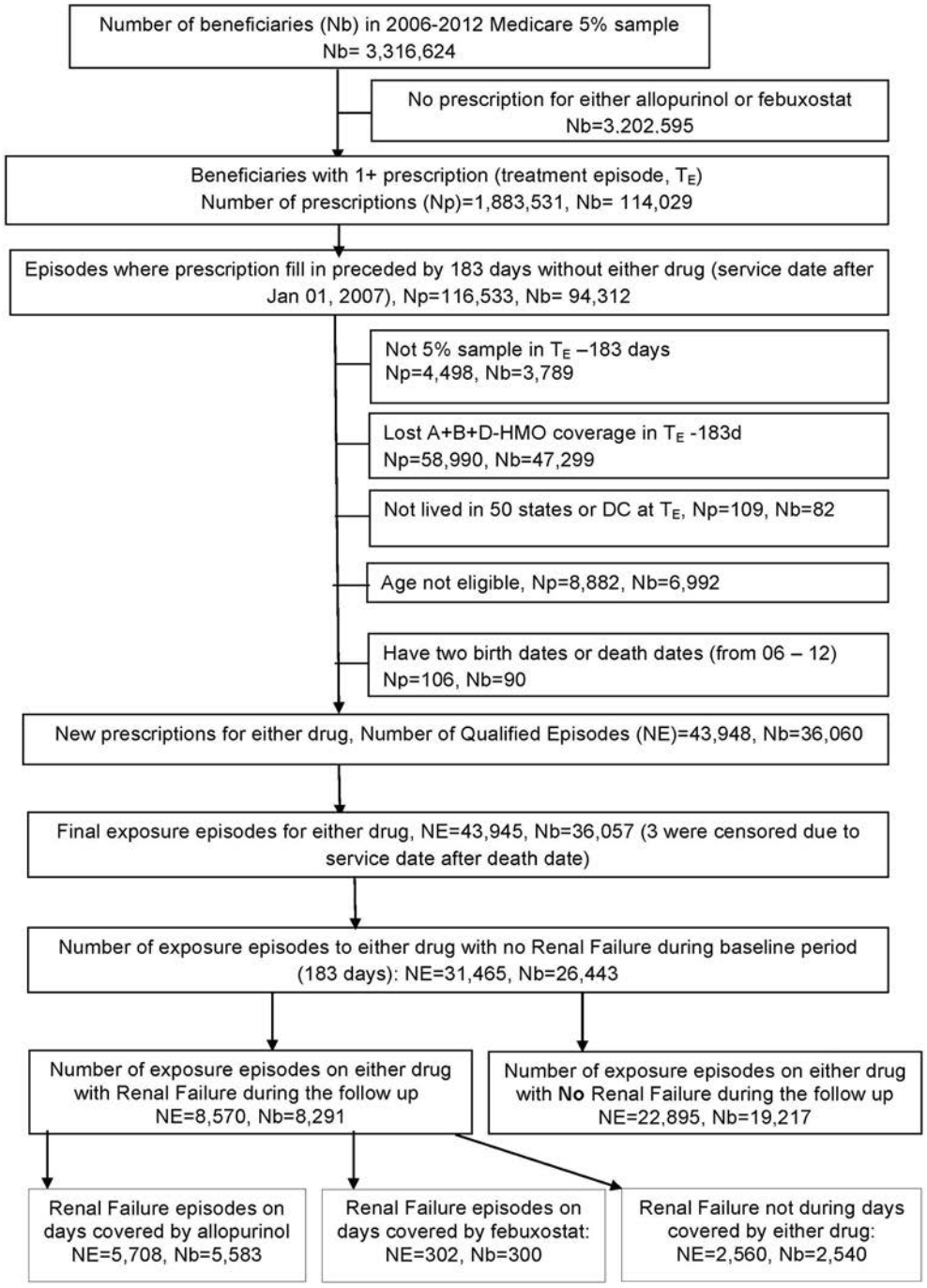

Figure 1 The flow chart shows the selection of new allopurinol or febuxostat exposure episodes after applying all the eligibility criteria including an absence of renal disease and the absence of any allopurinol-filled prescription in the baseline period of 183 days (new user design). We found 31 465 new allopurinol or febuxostat exposure episodes in 26 443 patients. Of these, 8570 episodes ended in incident renal disease and 22 895 ended without incident renal disease. We followed each eligible patient with a new filled allopurinol or febuxostat prescription until the patient lost full Medicare coverage, had incident renal disease (the outcome of interest), died or reached the each of the study period on 31 December 12, whichever came first. Since some beneficiaries contributed multiple episodes, some resulting and some not resulting in incident renal disease, therefore the total exceeds the boxes right above (third and second from the bottom of the figure). HMO, Health Maintenance Organization; DC, District of Columbia.

potential. However, neither study will answer a key question: does the renal protective effect of allopurinol differ from that of febuxostat? Systematic reviews have mostly failed to show any superiority of febuxostat over allopurinol for non-renal beneficial outcomes in chronic gout.[33 34] A finding of greater renal protection with febuxostat or allopurinol can provide important evidence for preferring one medication to the other. Therefore, a real-world comparative effectiveness study is needed to address this question.

Our objective was to conduct a comparative effectiveness study of renal function preservation with allopurinol versus febuxostat. We hypothesised that in the US elderly, (1) compared with allopurinol, febuxostat use (and dose/duration of use) will be associated with a lower risk of incident renal disease, and (2)

Table 1 Demographic and clinical characteristics of episodes of incident renal disease (baseline of 183 days with no renal disease)

	All episodes	Renal disease during the follow-up		p Value
		Yes	No	
Total, N (episodes)	31 465	8570	22 895	
Age, mean (SD), in years	75.8 (7.39)	77.4 (7.55)	75.1 (7.23)	<0.0001
Gender, N (%)				<0.0001
Male	16 410 (52.2%)	4181 (48.8%)	12 229 (53.4%)	
Female	15 055 (47.8%)	4389 (51.2%)	10 666 (46.6%)	
Race/ethnicity, N (%)				<0.0001
White	25 000 (79.5%)	6687 (78.0%)	18 313 (73.3%)	
Black	3503 (11.1%)	1107 (12.9%)	2396 (10.5%)	
Hispanic	634 (2.0%)	175 (2.0%)	459 (2.0%)	
Asian	1564 (5.0%)	429 (5.0%)	1135 (5.0%)	
Native American	90 (0.3%)	24 (0.3%)	66 (0.3%)	
Other/unknown	674 (2.1%)	148 (1.7%)	526 (2.3%)	
Region, N (%)				0.26
Midwest	7811 (24.8%)	2168 (25.3%)	5643 (24.7%)	
Northeast	5022 (16.0%)	1377 (16.1%)	3645 (15.9%)	
South	12 723 (40.4%)	3474 (40.5%)	9249 (40.4%)	
West	5909 (18.8%)	1551 (18.1%)	4358 (19.0%)	
Charlson-Romano comorbidity score, mean (SD)	1.52 (1.94)	1.91 (2.07)	1.38 (1.87)	<0.0001

that renal function preservation will be greater with a higher allopurinol dose or longer duration use.

METHODS

Study design and data sources

This retrospective cohort study used claims data from the 5% random sample of Medicare beneficiaries for the years 2006–2012. These data were obtained from the Centers for Medicare and Medicaid Services Chronic Condition Data Warehouse. For each beneficiary, we abstracted demographic and insurance coverage data; claims for inpatient, outpatient, skilled nursing facility, non-institutional provider, home health, hospice, durable medical equipment services; and claims for prescription drugs. The Institutional Review Board at the University of Alabama at Birmingham approved the study.

Table 2 Crude Incidence rates of incident renal disease with allopurinol or febuxostat exposure* by use, duration of use and dose

	Person-days of follow-up	Cases of renal disease (n)	Renal disease incidence rate (95% CI) per 1000 person-years
Allopurinol	10 871 816	5708	192 (187 to 197)
Febuxostat	326 051	302	338 (301 to 378)
Allopurinol duration of use			
1–180 days	5 231 119	6177	431 (420 to 442)
181–365 days	2 088 383	947	166 (155 to 176)
>1 year	3 552 314	1254	129 (122 to 136)
Febuxostat duration of use			
1–180 days	201 663	224	406 (354 to 462)
181–365 days	68 191	44	236 (171 to 316)
>1 year	56 197	34	221 (153 to 309)
Allopurinol dose (mg/day)			
<200	4 295 869	2806	238 (230 to 247)
200–299	1 814 587	877	176 (165 to 188)
≥300	4 761 360	2025	155 (148 to 162)
Febuxostat dose (mg/day)			
40	262 193	245	341 (300 to 387)
80	63 858	57	326 (247 to 422)

*Drug exposure up to 30 days after last day of medication fill/refill; baseline period was 183 days, that is, each new exposure was defined as no previous exposure in the baseline. 95% CIs were calculated using Fischer's exact test.

Eligible population and covariates

Medicare beneficiaries 65 years of age or older (age for becoming eligible for the Medicare programme) were eligible for this cohort study if they met the following criteria: (1) enrolled continuously in the traditional Medicare fee-for-service and pharmacy coverage (parts A, B and D) and not enrolled in the Medicare Advantage Plan; (2) newly treated with allopurinol and/or febuxostat and (3) lived in the USA. We defined a new episode of treatment with allopurinol (or febuxostat) as a filled allopurinol (or febuxostat) prescription after a clean baseline period of 183 days during which no allopurinol (or febuxostat) prescription was filled, respectively. The baseline period did not apply to the time of switching from one medication to the other since these patients already had a 183-day medication-free baseline period.

We used the Medicare denominator file to capture baseline patient characteristics including age, gender, race/ethnicity, residence (northeast, south, midwest and west). We calculated Charlson-Romano comorbidity index score, a validated weighted comorbidity index developed by the adaptation of the Charlson index[35] for administrative and claims data analysis,[36] for the baseline period for each episode. Charlson-Romano index includes myocardial infarction, cerebrovascular disease, heart failure, diabetes, liver disease, pulmonary disease, and so on. We included important covariates, that is, age at start of each episode, sex, race/ethnicity, region, Charlson-Romano comorbidity score, gout medications (colchicine, probenecid, non-steroidal anti-inflammatory drugs (NSAIDs)), gout duration, risk factors for renal disease (hypertension, tobacco use,

Table 3 Demographic and clinical characteristics of renal disease episodes by drug exposure

	No renal disease	Episodes with renal disease			
	NE=22 895	Not on either NE=2560	On allopurinol NE=5708	On febuxostat NE=302	p Value
Age*, mean (SD)	75.1 (7.23)	77.3 (7.38)	77.5 (7.63)	77.3 (7.36)	NS*
Gender, N (%)					0.72†
Male	12 229 (53.4%)	1232 (48.1%)	2802 (49.1%)	147 (48.7%)	
Female	10 666 (46.6%)	1328 (51.9%)	2906 (50.9%)	155 (51.3%)	
Race, N (%)					<0.0001†
White	18 313 (73.3%)	1933 (75.5%)	4510 (79.0%)	244 (80.8%)	
Black	2396 (10.5%)	341 (13.3%)	737 (12.9%)	29 (9.6%)	
Others	2186 (9.5%)	286 (11.2%)	461 (8.1%)	29 (9.6%)	
Charlson-Romano comorbidity score†, mean (SD)	1.38 (1.87)	1.85 (1.99)	1.94 (2.11)	1.74 (1.83)	NS‡
Charlson-Romano comorbidity score†, N (%)					0.33
0	10 828 (47.3%)	870 (34.0%)	1948 (34.1%)	105 (34.8%)	
1	3367 (14.7%)	433 (16.9%)	904 (15.8%)	59 (19.5%)	
≥2	8700 (38.0%)	1257 (49.0%)	2856 (50.0%)	138 (45.7%)	
Region					0.023
Midwest	5643 (24.7%)	616 (24.1%)	1485 (26.0%)	67 (22.2%)	
Northeast	3645 (15.9%)	385 (15.0%)	946 (16.6%)	46 (15.2%)	
South	9249 (40.4%)	1062 (41.5%)	2290 (40.1%)	122 (40.4%)	
West	4358 (19.0%)	497 (19.4%)	987 (17.3%)	67 (22.2%)	

*Pairwise comparisons for episodes with renal disease between neither versus allopurinol, allopurinol versus febuxostat or febuxostat versus neither showed non-significant differences, 0.40, 0.99 and 0.85, respectively.
†p Values denote the results of χ^2 tests of difference between the three categories for episodes with renal disease.
‡Pairwise comparisons for episodes with renal disease between neither versus allopurinol, allopurinol versus febuxostat or febuxostat versus neither showed non-significant differences, 0.18, 0.64 and 0.23, respectively.
NE, number of episodes; NS, not significant.

hyperlipidaemia, etc) and the use of medications for cardiovascular or renal disease or medications that can impact renal function including statins, beta-blockers, diuretics, aspirin, ACE inhibitors, angiotensin receptor blockers (ARBs) and calcium channel blockers.

Study outcomes

The first occurrence of incident renal disease during the follow-up, with an absence of this diagnosis in the previous 183 days (baseline period), identified with the presence of a diagnostic code, was the primary outcome of interest. Renal disease was identified by the presence of any of the following International Classification of Diseases, ninth revision, common modification (ICD-9-CM) codes, 582.xx, 583.xx, 585.xx, 586.xx or 588.xx. This approach has been used to assess renal disease in the validated Charlson-Romano comorbidity index[36] (commonly used comorbidity index), is valid with high positive predictive value and specificity,[37 38] has been used in several high-quality studies[27 39–41] and is being currently used by the US Renal Data System Coordinating Center[42]; a similar set of codes is also used in Deyo-Charlson index.[43] These codes correspond to moderate or severe renal disease/insufficiency, but no direct equivalence to creatinine clearance has been provided. The study follow-up for each renal disease episode began on the earliest allopurinol (or febuxostat) initiation date during the study period and ended on the earliest of the following dates—the first date of an ICD-9-CM code for incident renal disease, losing full Medicare coverage, switching of medication (allopurinol to febuxostat or vice versa), the date of death or the end of the study period (31 December 2012). Each patient was allowed to contribute multiple allopurinol and/or febuxostat treatment episodes during the study period.

Predictor of interest: new allopurinol versus new febuxostat treatment

We defined a new filled prescription of allopurinol or febuxostat as new allopurinol or new febuxostat treatment. Based on the days' supply for prescription in pharmacy records and the stock carry over not exceeding 30 days, we assigned the days of exposure for each allopurinol or febuxostat episode. If a patient had prescriptions for both drugs, then they were considered exposed to the one that was prescribed second; for example, if a patient were taking febuxostat and got a new prescription of allopurinol, then they were considered to be on allopurinol only as of the allopurinol fill date. We considered patients exposed to allopurinol (or febuxostat) for 30 days after the end of days' supply to capture the attributable events since some residual biological effects of allopurinol (or febuxostat) could extend beyond the last day of use and most patients have some extra days of medication supply due to imperfect adherence. If there were >30 days between prescription fills, a new allopurinol (or febuxostat) exposure was started. If the patient switched medications, the 30-day latency did not apply and they were immediately classified as exposed to the new medication only. This was done since the ULTs achieve significant blood and tissue concentrations soon after initiation.

The main predictor of interest was allopurinol use, with febuxostat use as the reference category. Additionally, we examined the effect of all allopurinol doses and the higher febuxostat dose (80 mg/day), with febuxostat 40 mg/day as the reference category. Allopurinol daily dose was calculated as the mean daily use for each continuous allopurinol episode and categorised as <200, 200–299 and ≥300 mg/day; febuxostat daily dose was calculated similarly, and categorised as 40 and 80 mg/day. We calculated the duration of allopurinol

Table 4 Association of allopurinol versus febuxostat for the hazard of incident renal disease in propensity score*-matched analysis in patients with no baseline renal disease before the index allopurinol or febuxostat prescription

	HR (95% CI)	p Value
Type of ULT		
Febuxostat	Ref	
Allopurinol	0.61 (0.54 to 0.69)	<0.0001
Daily ULT dose		
Febuxostat (mg/day)		
40	Ref	
>40	0.97 (0.73 to 1.29)	0.83
Allopurinol (mg/day)		
<200	0.75 (0.65 to 0.86)	<0.0001
200–299	0.61 (0.52 to 0.73)	<0.0001
≥300	0.48 (0.41 to 0.55)	<0.0001
Duration of ULT use		
1–180 days— febuxostat	Ref	
181–365 days—febuxostat	0.86 (0.60 to 1.23)	0.42
>1 year—febuxostat	1.05 (0.71 to 1.57)	0.81
1–180 days—allopurinol	0.59 (0.51 to 0.68)	<0.0001
181–365 days—allopurinol	0.62 (0.49 to 0.78)	<0.0001
>1 year—allopurinol	0.64 (0.50 to 0.81)	0.0002
Comparisons for dose and duration within each ULT, allopurinol or febuxostat		
Daily ULT dose		
Febuxostat (mg/day)		
40	Ref	
>40	0.97 (0.73 to 1.29)	0.83
Allopurinol (mg/day)		
<200	Ref	
200–299	0.79 (0.69 to 0.91)	0.0009
≥300	0.63 (0.57 to 0.70)	<0.0001
Duration of ULT use		
1–180 days—febuxostat	Ref	
181–365 days—febuxostat	0.86 (0.60 to 1.23)	0.42
>1 year—febuxostat	1.05 (0.71 to 1.57)	0.81
1–180 days—allopurinol	Ref	
181–365 days—allopurinol	0.97 (0.80 to 1.19)	0.77
>1 year—allopurinol	1.17 (0.94 to 1.47)	0.16

*Propensity score-matched analyses: we matched allopurinol to febuxostat in a 1:5 ratio, matching for age, gender, race, region, Charlson index comorbidities, cardiovascular disease medications (statins, beta-blockers, diuretics, ACE inhibitors, aspirin, angiotensin receptor blockers and calcium channel blockers), gout medications (colchicine, probenecid, non-steroidal anti-inflammatory drugs), gout duration and risk factors for renal disease (hypertension, tobacco use, hyperlipidaemia, etc).
Ref, referent category; ULT, urate-lowering therapy.

(or febuxostat) treatment for each episode. Duration of allopurinol (or febuxostat) use was categorised as 1–180 days, 181 days to 365 days and >1 year. This arbitrary categorisation of the duration of use was based on clinical relevance, with 1–180, 181–365 and >365 days corresponding to short-term, intermediate-term and long-term use. It also allowed for enough exposure, that is, 6 months, in the first category to observe a biological phenomenon of renal function preservation. Since 90-day prescriptions are the most common duration, these groups corresponded to 2, 3–4 or >4, 90-day filled prescriptions. Sensitivity analyses were done with 1–90 days, 91–180 days, 181–365 days and >1-year durations, corresponding to 1, 2, 3–4 or >4, 90-day filled prescriptions.

Statistical analysis

We compared baseline characteristics between allopurinol (or febuxostat) episodes during which patients did versus did not develop incident renal disease in the follow-up period. We calculated crude incidence rates for renal disease per 1000 person-years of exposure; 95% CIs were calculated using Fischer's exact test.

We performed propensity score-matched analyses (age, gender, race, region, Charlson index comorbidities, cardiovascular disease medications (statins, beta-blockers, diuretics, ACE inhibitors, aspirin, ARBs and calcium channel blockers), gout medications (colchicine, probenecid, NSAIDs), gout duration, risk factors for renal disease (hypertension, tobacco use, hyperlipidaemia, etc)) to control for differences between patients exposed to allopurinol versus febuxostat to examine comparative effectiveness of allopurinol versus febuxostat. Groups were matched in 1:5 for the propensity score-matched analysis with greedy matching (five allopurinol-exposed matched to one febuxostat-exposed), to ensure enough events for analyses. We used Cox proportional hazard regression models and calculated the HR of incident renal disease for allopurinol use versus febuxostat use (reference category); analyses for daily allopurinol (or febuxostat) dose used febuxostat 40 mg daily dose as the reference category.

In addition to the propensity-matched analyses described above, we performed traditional multivariable Cox regression models without propensity adjustments as sensitivity analyses. Models were adjusted for age, gender, race, Charlson-Romano comorbidity score and the use of medications for cardiovascular diseases (statins, beta-blockers, diuretics, ACE inhibitors, aspirin, ARBs and calcium channel blockers). Separate multivariable models assessed allopurinol versus febuxostat daily dose. We used the Huber-White 'Sandwich' variance estimator to account for correlations between observations from the same patient and calculated robust SEs for all estimates since patients could possibly contribute more than one episode of new allopurinol (or febuxostat) use.[44] We also explored the association of allopurinol use duration with incident renal disease with febuxostat use duration as the reference category. Analyses were done using SAS V.9.4.

We conducted additional sensitivity analyses to test the robustness of findings from our main multivariable-adjusted analyses for allopurinol use and allopurinol daily dose by (1) adjusting for the presence of gout; (2) adjusting for gout duration; (3) adjusting for colchicine and probenecid use; (4) limiting to patients who never crossed over from allopurinol to febuxostat or vice versa; (5) limited to renal disease codes of ICD-9 codes 582.xx and 585.xx, more specific for CKD; and (6) changing baseline period from 183 days to 365 days.

RESULTS

Clinical characteristics of study population

There were 31 465 new allopurinol or new febuxostat treatment episodes with no renal disease during the baseline period that met the study eligibility criteria. Of these, 8570 episodes ended with incident renal disease events (of which 5708 occurred during days of allopurinol exposure, 302 during febuxostat exposure and 2560 during non-exposed periods for both) and 22 895 episodes ended without incident renal disease (figure 1). Compared with episodes without incident renal disease, incident renal disease episodes were more frequent in individuals who were older, female or black and had higher Charlson-Romano comorbidity score (table 1).

Table 5 Multivariable-adjusted association of allopurinol versus febuxostat and other risk factors with incident renal disease in patients who received allopurinol or febuxostat

	Multivariable-adjusted (model 1)*		Multivariable-adjusted (model 2)*		Multivariable-adjusted (model 3)*	
	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
Age (years)						
65 to <75	Ref		Ref		Ref	
75 to <85	**1.43 (1.35 to 1.52)**	**<0.0001**	**1.40 (1.32 to 1.48)**	**<0.0001**	**1.43 (1.35 to 1.52)**	**<0.0001**
≥85	**1.86 (1.73 to 2.00)**	**<0.0001**	**1.76 (1.63 to 1.89)**	**<0.0001**	**1.86 (1.73 to 2.00)**	**<0.0001**
Gender						
Male	Ref		Ref		Ref	
Female	1.03 (0.98 to 1.09)	0.22	1.00 (0.95 to 1.06)	0.95	1.03 (0.98 to 1.09)	0.22
Race						
White	Ref		Ref		Ref	
Black	**1.35 (1.25 to 1.46)**	**<0.0001**	**1.35 (1.25 to 1.46)**	**<0.0001**	**1.35 (1.25 to 1.46)**	**<0.0001**
Other	1.03 (0.94 to 1.13)	0.5	1.02 (0.93 to 1.12)	0.74	1.03 (0.94 to 1.13)	0.51
Charlson-Romano score, per unit change	**1.14 (1.13 to 1.15)**	**<0.0001**	**1.14 (1.12 to 1.15)**	**<0.0001**	**1.14 (1.13 to 1.15)**	**<0.0001**
Statins	0.95 (0.86 to 1.05)	0.34	0.95 (0.86 to 1.05)	0.3	0.96 (0.86 to 1.06)	0.37
Beta-blockers	1.08 (0.98 to 1.19)	0.13	1.08 (0.98 to 1.18)	0.14	1.08 (0.98 to 1.19)	0.1
Diuretics	**1.22 (1.11 to 1.34)**	**<0.0001**	**1.22 (1.11 to 1.34)**	**<0.0001**	**1.21 (1.10 to 1.33)**	**<0.0001**
ACE inhibitor	1.02 (0.91 to 1.15)	0.71	1.02 (0.91 to 1.14)	0.74	1.03 (0.92 to 1.15)	0.65
Aspirin	1.37 (0.76 to 2.47)	0.3	1.37 (0.76 to 2.47)	0.3	1.37 (0.76 to 2.47)	0.30
Calcium channel blockers	1.09 (0.97 to 1.23)	0.14	1.09 (0.97 to 1.22)	0.15	1.09 (0.97 to 1.23)	0.15
ARB	1.14 (0.99 to 1.31)	0.06	1.14 (0.99 to 1.30)	0.07	1.14 (0.99 to 1.31)	0.06
ULT type						
Febuxostat	Ref		–		–	
Allopurinol	**0.66 (0.59 to 0.75)**	**<0.0001**				
Daily ULT dose	–					
Febuxostat (mg/day)						
40			Ref		–	
>40			1.06 (0.80 to 1.42)	0.68		
Allopurinol (mg/day)						
<200			**0.79 (0.69 to 0.90)**	**0.001**		
200–299			**0.64 (0.55 to 0.74)**	**<0.0001**		
≥300			**0.56 (0.49 to 0.65)**	**<0.0001**		
Duration of ULT use	–					
1–180 days—febuxostat					Ref	
1–180 days—allopurinol					**0.66 (0.58 to 0.75)**	**<0.0001**
181–365 days—febuxostat					0.89 (0.64 to 1.25)	0.50
181–365 days—allopurinol					**0.65 (0.55 to 0.77)**	**<0.0001**
>1 year—febuxostat					1.02 (0.70 to 1.48)	0.94
>1 year—allopurinol					**0.63 (0.53 to 0.75)**	**<0.0001**

*Multivariable models were adjusted for the following variables to assess independent association of allopurinol versus febuxostat and incident renal disease.
Model 1 = ULT type + age + race + gender + Charlson-Romano score + beta-blockers + diuretics + ACE inhibitors + statins.
Model 2 = ULT dose + age + race + gender + Charlson-Romano score + beta-blockers + diuretics + ACE inhibitors + statins.
Model 3 = ULT use duration + age + race + gender + Charlson-Romano score + beta-blockers + diuretics + ACE inhibitors + statins.
Bold indicates statistically significant.
ARB, angiotensin receptor blockers; Ref, referent category; ULT, urate-lowering therapy.

Crude incidence rates of renal disease with allopurinol or febuxostat were 192 and 338 per 1000 person-years, respectively (table 2). Crude incidence rates of renal disease were lower with longer allopurinol or febuxostat use durations of 1–180 days, 181–365 days and >1 year, respectively: allopurinol, 431, 166 and 129, and febuxostat, 406, 236 and 221 per 1000 person-years (table 2). Similarly, crude incidence rates of renal disease were lower with higher allopurinol or febuxostat dose: 238, 176 and 155 with allopurinol doses <200, 200–299 and ≥300 mg/day; and 341 and 326 per 1000 person-years with febuxostat doses 40 and 80 mg/day, respectively (table 2).

Among patients with incident renal disease, the mean age, gender and Charlson comorbidity scores did not differ significantly by whether the episodes occurred after an exposure to allopurinol, febuxostat or neither, but race/ethnicity did ($p<0.0001$; table 3).

Propensity-adjusted hazards of incident renal disease

In a 1:5 propensity-matched multivariable-adjusted analyses, compared with febuxostat use, allopurinol use was associated with lower HR of incident renal disease, 0.61 (95% CI 0.49 to 0.77) (table 4). Compared with febuxostat 40 mg/day,

Table 6 Sensitivity analysis of allopurinol use and allopurinol dose compared with febuxostat on the hazard of incident renal disease in patients who received allopurinol or febuxostat using multivariable-adjusted* Cox regression models

	Multivariable-adjusted: model 1			Multivariable-adjusted: model 2	
	HR (95% CI)	p Value		HR (95% CI)	p Value
Sensitivity analysis 1: additionally adjusted for the presence of a diagnosis of **gout**					
ULT type			ULT dose (mg/day)		
Febuxostat	Ref		Febuxostat 40	Ref	
Allopurinol	**0.67 (0.60 to 0.75)**	**<0.0001**	Febuxostat >40	1.06 (0.80 to 1.42)	0.69
			Allopurinol <200	**0.80 (0.70 to 0.91)**	**0.001**
			Allopurinol 200–299	**0.64 (0.56 to 0.74)**	**<0.0001**
			Allopurinol ≥300	**0.57 (0.50 to 0.65)**	**<0.0001**
Sensitivity analysis 2: additionally adjusted for the **duration of gout** *diagnosis*					
ULT type			ULT dose (mg/day)		
Febuxostat	Ref		Febuxostat 40	Ref	
Allopurinol	**0.66 (0.59 to 0.74)**	**<0.0001**	Febuxostat >40	1.07 (0.80 to 1.42)	0.66
			Allopurinol <200	**0.79 (0.69 to 0.90)**	**0.001**
			Allopurinol 200–299	**0.64 (0.55 to 0.73)**	**<0.0001**
			Allopurinol ≥300	**0.57 (0.49 to 0.65)**	**<0.0001**
Sensitivity analysis 3: additionally adjusted for presence of diagnosis of **gout and additional drugs for gout (colchicine, probenecid)**					
ULT type			ULT dose (mg/day)		
Febuxostat	Ref		Febuxostat 40	Ref	
Allopurinol	**0.67 (0.60 to 0.75)**	**<0.0001**	Febuxostat>40	1.06 (0.80 to 1.42)	0.69
			Allopurinol<200	**0.80 (0.70 to 0.91)**	**0.001**
			Allopurinol 200–299	**0.64 (0.56 to 0.74)**	**<0.0001**
			Allopurinol≥300	**0.57 (0.50 to 0.65)**	**<0.0001**
Sensitivity analysis 4: analyses in a subset of patients **who never crossed over (switched) from one ULT to the other ULT**					
ULT type			ULT dose (mg/day)		
Febuxostat	Ref		Febuxostat 40	Ref	
Allopurinol	**0.58 (0.50 to 0.67)**	**<0.0001**	Febuxostat >40	1.05 (0.71 to 1.58)	0.8
			Allopurinol <200	**0.69 (0.59 to 0.81)**	**<0.0001**
			Allopurinol 200–299	**0.55 (0.47 to 0.65)**	**<0.0001**
			Allopurinol ≥300	**0.49 (0.42 to 0.58)**	**<0.0001**
Sensitivity analysis 5: analyses for a subset of patients with codes for renal disease limited to **ICD-9 codes 582.xx and 585.xx (more specific codes for chronic kidney disease)**					
ULT type			ULT dose (mg/day)		
Febuxostat	Ref		Febuxostat 40	Ref	
Allopurinol	**0.62 (0.55 to 0.70)**	**<0.0001**	Febuxostat >40	1.06 (0.80 to 1.42)	0.68
			Allopurinol <200	**0.75 (0.66 to 0.86)**	**<0.0001**
			Allopurinol 200–299	**0.60 (0.52 to 0.69)**	**<0.0001**
			Allopurinol ≥300	**0.52 (0.45 to 0.59)**	**<0.0001**
Sensitivity analysis 6: analyses for a subset of patients **with 365-day baseline period instead of 183** *days*					
ULT type			ULT dose (mg/day)		
Febuxostat	Ref		Febuxostat 40	Ref	
Allopurinol	**0.69 (0.61 to 0.78)**	**<0.0001**	Febuxostat 80	1.03 (0.75 to 1.41)	0.87
			Allopurinol <200	**0.81 (0.70 to 0.93)**	**0.003**
			Allopurinol 200–299	**0.65 (0.56 to 0.76)**	**<0.0001**
			Allopurinol ≥300	**0.59 (0.51 to 0.68)**	**<0.0001**

*Multivariable-adjusted Cox regression models were adjusted for the following variables.
Model 1 = ULT type + age + race + gender + Charlson-Romano score + beta-blockers + diuretics + ACE inhibitors + statins.
Model 2 = ULT dose + age + race + gender + Charlson-Romano score + beta-blockers + diuretics + ACE inhibitors + statins.
Bold values indicate statistically significant with p-value <0.05.
ICD-9, International Classification of Diseases, ninth revision; Ref, referent category; ULT, urate-lowering therapy.

allopurinol doses <200, 200–299 and ≥300 mg/day were associated with lower HR of incident renal disease, 0.75 (95% CI 0.65 to 0.86), 0.61 (95% CI 0.52 to 0.73) and 0.48 (95% CI 0.41 to 0.55), respectively (table 4). Compared with febuxostat use 1–180 days, all allopurinol durations of use were significantly associated with a lower HR of incident renal disease (table 4). Pre-match and post-match distribution of variables is shown in online supplementary appendices 1 and 2; most characteristics were well matched in the propensity-matched cohorts. Sensitivity analyses for use durations 1–90 days, 91–180 days, 181–365 days and >1 year confirmed main study findings (see online supplementary appendix 3).

Comparing within each medication dose/duration, we found that compared with respective low dose and short duration of use of 1–180 days, higher febuxostat dose, longer febuxostat use duration and longer allopurinol use duration were not significantly associated, but higher allopurinol doses (200–299 and ≥300 mg/day) were significantly associated with 21% and 37% lower hazard of incident renal disease (table 4).

Sensitivity analyses: traditional multivariable-adjusted hazards of incident renal disease

Multivariable-adjusted models showed that compared with febuxostat, allopurinol use was associated with a significantly lower HR for incident renal disease, 0.66 (95% CI 0.59 to 0.75) (table 5). Older age, black race/ethnicity, beta-blockers and diuretics were associated with significantly higher hazards of incident renal disease. In a separate multivariable-adjusted model, compared with febuxostat 40 mg daily, all allopurinol doses were significantly associated with a lower HR of incident renal disease, but febuxostat doses >40 mg daily were not significantly associated (table 5). Univariate analyses showed that among other factors, compared with febuxostat, allopurinol use was associated with a lower HR of 0.66 (95% CI 0.58 to 0.74) (see online supplementary appendix 4). Similar to that noted in the propensity-matched analyses, compared with febuxostat use 1–180 days, all allopurinol use durations were associated with a lower HR of incident renal disease, both in patients with versus without gout (see online supplementary appendix 5).

We performed several sensitivity analyses (adjusted for presence of gout; gout duration; colchicine and probenecid use; limited to patients who never switched from allopurinol to febuxostat or vice versa; limited to more specific codes for CKD and changed baseline period from 183 to 365 days) that confirmed the findings from the main multivariable-adjusted analyses for both allopurinol use and allopurinol doses with incident renal disease (table 6). There was minimal or no attenuation of HR and all results stayed significant.

DISCUSSION

In this comparative effectiveness study of 5% random sample of US Medicare beneficiaries 65 years or older, we were surprised to find that compared with febuxostat, allopurinol was independently associated with significantly lower hazard (39%) of incident renal disease (opposite to our hypothesis). Compared with febuxostat 40 mg/day dose, we found that all allopurinol doses (<200, 200–299 and ≥300 mg/day), but not febuxostat >40 mg/day, were associated with significantly lower HRs of incident renal disease. Theoretically, an alternate explanation might be a higher risk of renal failure with febuxostat, but it is inconsistent with evidence from randomised controlled trials and observational studies that have shown possible beneficial effect of febuxostat for renal function.[28 29 45] We observed a dose–response relationship with increasing allopurinol doses and risk reduction. Our study provided the crude incidence rates of incident renal disease in older adults on allopurinol or febuxostat. Several study findings merit further discussion.

What is known in this area and what does our study add? In a propensity-matched analysis of 873 matched pairs of treatment-naïve patients from a US commercial healthcare plan data from 143 237 patients with allopurinol or febuxostat use, the mean serum creatinine decreased significantly from pre-treatment to post-treatment with both ULTs, allopurinol, 1.3±0.6 to 1.2±0.6 mg/dL (p<0.001) and febuxostat, 1.4±0.6 to 1.3±0.6 mg/dL (p<0.001), with no significant difference between them.[28] In a single-centre 1-year retrospective study of 73 patients with hyperuricemia and an estimated glomerular filtration rate (eGFR) <45 mL/min, who were either switched from allopurinol to febuxostat (n=57) or continued on allopurinol (n=27), the (eGFR) decreased statistically more in the allopurinol continuation group, 1.6 versus 6.3 mL/min, respectively.[29] These studies differed in baseline renal function (normal renal function vs CKD stage 3 or 4), patient age (56 vs 67–73 years), gender (85% vs 61% men), allopurinol and febuxostat doses, follow-up duration (6–7 months vs 1 year) and the study setting (claims of the US privately insured population vs single-centre Japanese study). Thus, the evidence to date is unclear regarding whether febuxostat differs from allopurinol for preserving renal function.

Our study included a nationally representative cohort of Americans 65 years or older and found that compared with febuxostat, allopurinol was associated with 39% lower hazard of incident renal disease in multivariable-adjusted models. The hazard reduction was independent of age, gender, race, region, gout, medications for gout, medical comorbidities and the use of medications for cardiovascular or renal disease or those that can impact renal function, including ACE inhibitors, ARBs and diuretics. Allopurinol differs from febuxostat in being a purine analogue and non-selectively inhibiting XO and other enzymes in purine and pyrimidine pathways, which may possibly underlie these differences.[14] The estimate was robust as confirmed by using multivariable-adjusted regression, confirmed further by several sensitivity analyses. Our study is among the first robust studies to assess the comparative effectiveness of allopurinol versus febuxostat for incident renal disease in a nationally representative patient population. Our study differs from earlier studies in that we examined incident renal disease rather than small changes in eGFR, which is inherently clinically meaningful. Reproduction of these results in other independent data sets would further strengthen our confidence in these findings.

We noted a dose–response relationship between allopurinol and the reduction in the hazards of incident renal disease, with hazard reduction increasing from 21% to 36% to 44% for increasing allopurinol doses from <200 to 200–299 to ≥300 mg/day, a novel study finding. This finding further increases our confidence and fulfils another Bradford-Hill's criteria for causation versus association.[46]

What are the implications of our study findings? These findings indicate that allopurinol may be superior to febuxostat for renal function preservation in elderly Americans without significant baseline renal disease. Allopurinol dominates the treatment of hyperuricemia in the USA with >80–90% patients receiving allopurinol as the preferred ULT.[28 47 48] However, the commonly used allopurinol doses in patients with gout are low (mean dose, 230 mg/day),[47] often based on an erroneous interpretation of a pharmacokinetic study,[49] despite the recent evidence of clinical benefit and no increase in harm with appropriate allopurinol titration in patients with renal impairment to achieve target sUA.[50] Our study findings indicate that higher doses of allopurinol ≥300 mg/day were associated with less risk of renal disease, another potential benefit of the use of higher allopurinol dose, similar to the anti-ischaemic effect of allopurinol 600 mg/day.[51] A more effective inhibition of XO and reactive oxygen species and more optimal sUA reduction with higher doses of allopurinol[52 53] may be the mechanism of this benefit noted in our study and others. A higher allopurinol dose of about 400 mg/day is often required to achieve successful lowering of sUA

<6 mg/dL, an important goal for the management of gout, the most common indication for allopurinol.[54]

Our study has several limitations. Our observational study is at potential risk of residual confounding and channelling bias related to the study design. To avoid channelling bias, we conducted propensity-matched analyses matched for several factors including risk factors for renal disease. We also used a new user design and required all patients to be free of baseline renal disease. Due to non-availability of laboratory test results in Medicare, slight differences in the baseline renal function could not have existed between febuxostat versus allopurinol users. For example, in another propensity-matched study of allopurinol and febuxostat users, serum creatinine differed by 0.1 mg/dL (1.3 vs 1.4 mg/dL), a clinically insignificant difference.[28] We also confirmed our main study findings by conducting multivariable-adjusted regression that included several important potential confounders. We also conducted several sensitivity analyses that confirmed the robustness of results with minimal/no attenuation of estimates or significance. A higher unadjusted Charlson-Romano comorbidity index in allopurinol versus febuxostat users indicated that patients receiving allopurinol were sicker, not healthier, than those receiving febuxostat, which was contrary to our expectation. The use of claims database definition of renal disease raises the possibility of misclassification bias. However, this definition is used in the Charlson-Romano index,[36] has been shown to be valid previously[37 38] and has been used commonly previously in other studies[27 39–42 55 56] and by the US Renal Data System Coordinating Center.[42] Nevertheless, these findings must be confirmed in another large comparative effectiveness study. Our study does not answer the question whether allopurinol may be preferred over febuxostat in patients with existing renal dysfunction, that is, those with diabetes, hypertension or heart failure with concomitant CKD. Future studies including these populations are needed to address important questions.

The strengths of our study were that we studied a representative national US sample of older adults, the sample size was large, >6000 outcome events occurred during exposure to either allopurinol or febuxostat and analyses were adjusted for important confounders/covariates. Our findings were robust, as confirmed in multiple sensitivity analyses.

CONCLUSION

In conclusion, this comparative effectiveness study showed that allopurinol was more effective than febuxostat and was associated with greater reduction in the risk of incident kidney disease in a nationally representative sample of older Americans. The association of allopurinol with renal protection was dose-related, and possibly duration-related, with higher reduction of hazard of incident renal disease with higher allopurinol doses. A randomised trial is needed to confirm these findings. Future studies should also assess whether renal protection with allopurinol differs by the underlying aetiology of renal disease, that is, hypertension versus diabetes versus heart failure versus others. Translational studies are also needed to uncover the underlying mechanisms of this potential renal function benefit associated with allopurinol use.

Correction notice This paper has been amended since it was published Online First. Owing to a scripting error, some of the publisher names in the references were replaced with 'BMJ Publishing Group'. This only affected the full text version, not the PDF. We have since corrected these errors and the correct publishers have been inserted into the references.

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Patient consent The Ethics Committee waived the need for individual patient consent for this retrospective database study.

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EXTENDED REPORT

Efficacy and safety of the biosimilar ABP 501 compared with adalimumab in patients with moderate to severe rheumatoid arthritis: a randomised, double-blind, phase III equivalence study

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ABSTRACT

Objectives ABP 501 is a Food and Drug Administration-approved biosimilar to adalimumab; structural, functional and pharmacokinetic evaluations have shown that the two are highly similar. We report results from a phase III study comparing efficacy, safety and immunogenicity between ABP 501 and adalimumab.

Methods In this randomised, double-blind, active comparator-controlled, 26-week equivalence study, patients with moderate to severe active rheumatoid arthritis (RA) despite methotrexate were randomised (1:1) to ABP 501 or adalimumab (40 mg) every 2 weeks. Primary endpoint was risk ratio (RR) of ACR20 between groups at week 24. Primary hypothesis that the treatments were equivalent would be confirmed if the 90% CI for RR of ACR20 at week 24 fell between 0.738 and 1.355, demonstrating that ABP 501 is similar to adalimumab. Secondary endpoints included Disease Activity Score 28-joint count-C reactive protein (DAS28-CRP). Safety was assessed via adverse events (AEs) and laboratory evaluations. Antidrug antibodies were assessed to determine immunogenicity.

Results A total of 526 patients were randomised (n=264, ABP 501; n=262 adalimumab) and 494 completed the study. ACR20 response at week 24 was 74.6% (ABP 501) and 72.4% (adalimumab). At week 24, the RR of ACR20 (90% CI) between groups was 1.039 (0.954, 1.133), confirming the primary hypothesis. Changes from baseline in DAS28-CRP, ACR50 and ACR70 were similar. There were no clinically meaningful differences in AEs and laboratory abnormalities. A total of 38.3% (ABP 501) and 38.2% (adalimumab) of patients tested positive for binding antidrug antibodies.

Conclusions Results from this study demonstrate that ABP 501 is similar to adalimumab in clinical efficacy, safety and immunogenicity in patients with moderate to severe RA.

Trial registration number NCT01970475; Results.

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INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterised by synovial inflammation that results in joint damage. The introduction of biologics in 1998 resulted in improvements in outcomes with RA treatments.[1] Tumour necrosis factor (TNF) inhibitors were the first approved biological disease-modifying antirheumatic drugs (bDMARDs) for treatment of RA, followed by additional bDMARDs that had differing mechanisms of action.[1] The bDMARD adalimumab (AbbVie, Chicago, Illinois, USA) is a recombinant human IgG_1 monoclonal antibody that binds specifically to TNF-α. Adalimumab was approved for the treatment of moderate to severe RA and has been shown to have significant efficacy,[2] with improvements in patient's disease activity, quality of life and prevention of structural damage and disability. Safety concerns have been well delineated and are similar to other biologics, including risk of infections.[2] Adalimumab has been approved for other indications, including psoriasis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, inflammatory bowel disease, hidradenitis suppurativa and non-infectious intermediate and posterior uveitis and panuveitis; it is one of the most frequently prescribed biologics in clinical practice.[2–6] Adalimumab has been extensively studied in combination with methotrexate (MTX) and has been shown to improve outcomes versus placebo in patients with RA who demonstrate an incomplete response to MTX.[2 7 8]

Biosimilars, biological products that are similar to an already licensed reference product (such as adalimumab), are being developed.[9 10] Due to complexities involved in developing biological proteins, regulatory agencies have developed guidelines for demonstrating that proposed biosimilars are highly similar to the reference product and that no clinically meaningful differences exist between the proposed biosimilar and reference product in terms of safety, purity and potency.[9 11]

This pathway differs from innovator biologic product development and requires extensive structural and functional analysis to demonstrate that the biosimilar and originator molecule are highly similar in structure and effector function. Additionally, guidelines on biosimilars indicate that clinical trials should be conducted to compare the biosimilar and reference product in sensitive populations and with appropriate endpoints to enable detection of clinically meaningful differences, if any, between the proposed biosimilar and reference product.[12 13] Using this pathway, several biosimilars such as Inflectra™, Remsima™, Flixabi™

(infliximab biosimilars) and Benepali™ (etanercept biosimilar) have received marketing authorisation from the European Medicines Agency (EMA),[14–16] and the Food and Drug Administration (FDA) has recently approved biosimilars of filgrastim (Zarxio™), infliximab (Inflectra), etanercept (Erelzi™) and adalimumab (AMJEVITA™).[4 17–20]

ABP 501 (AMJEVITA) was approved as the first adalimumab biosimilar by the US FDA.[21] Analytical and biofunctional evaluations have demonstrated that ABP 501 and adalimumab are highly similar in their structural and functional properties, as well as biological activity.[22 23] A phase I, single-dose study of ABP 501 in healthy adults demonstrated pharmacokinetic equivalence to that of adalimumab.[24] To demonstrate similarity in clinical efficacy, safety and immunogenicity of ABP 501 compared with adalimumab, two phase III studies were conducted: one examined effects in patients with moderate to severe plaque psoriasis (NCT01970488) and one in patients with moderate to severe RA (NCT01970475).[24 25] Here, we report results from a phase III study designed to assess the clinical efficacy, safety and immunogenicity of ABP 501 compared with adalimumab for the treatment of moderate to severe RA.

METHODS

Study design

This was a randomised, double-blind, active comparator-controlled equivalence study designed to show clinical similarity between ABP 501 and adalimumab in adalimumab-naive adult patients with moderate to severe RA who had an inadequate response to MTX. The study was conducted in 12 countries and 100 centres across Europe, North America and Latin America (see online supplementary table 1). Following screening (≤4 weeks), patients were randomised 1:1 to receive either ABP 501 or adalimumab 40 mg subcutaneously on day 1 and then every 2 weeks until week 22. The primary endpoint assessments were conducted at week 24, followed by final safety and immunogenicity assessments at week 26 (see online supplementary methods for additional details on methodology and full protocol).

Study population

Patients ≥18 years to ≤80 years of age were included if they had a diagnosis of moderate to severe RA (per 2010 American College of Rheumatology/European League Against Rheumatism criteria) for ≥3 months. Patients were required to have active RA (≥6 swollen joints and ≥6 tender joints) at screening and baseline. Patients with an erythrocyte sedimentation rate ≥28 mm/hour or serum C reactive protein (CRP) >1.0 mg/dL and positivity for rheumatoid factor or anticyclic citrullinated peptide at screening were included. Patients were also required to have a negative test for tuberculosis at screening, defined as a negative purified protein derivative (<5 mm of induration at 48–72 hours after test) or a negative QuantiFERON (Qiagen, Hilden, Germany) test. Patients were required to have received MTX for ≥12 consecutive weeks and were on a stable oral dose of 7.5–25 mg/week for ≥8 weeks before receiving investigational product (IP). Patients were excluded if they previously used ≥2 biologic therapies for RA or had any previous use of adalimumab or an adalimumab biosimilar.

Concomitant therapies

Patients were required to receive a stable dose of MTX for the study duration, as prescribed by the treating physician. If a patient developed MTX-related side effects, a dose reduction was possible at the investigator's discretion. Patients were allowed to remain on oral corticosteroids (≤10 mg/day of prednisone or equivalent) if on a stable dose for ≥4 weeks prior to initiation of IP. Prohibited medications included non-biologic DMARDs (other than MTX) and biologic treatment for RA other than those being investigated.

Efficacy endpoints

The primary efficacy endpoint was the risk ratio (RR) of achieving a 20% improvement from baseline in the American College of Rheumatology core set of measurements[26] (ACR20) at week 24. Secondary efficacy endpoints included assessments of Disease Activity Score 28-joint count-CRP (DAS28-CRP), the RR for ACR20, ACR50 and ACR70 response (20%, 50%, 70% improvement in ACR core set of measurements) at various time points throughout the study. Additional endpoints included the risk differences (RD) for ACR20, ACR50 and ACR70.

Safety

Key safety endpoints included treatment-emergent adverse events (TEAEs), serious adverse events (SAEs) and incidence of antidrug antibodies (ADAs). Adverse events (AEs) of interest were also assessed based on the Standard Medical Dictionary for Regulatory Activities (MedDRA) queries.

ADAs were assessed at baseline and weeks 4, 12 and 26. In the current study, ADA status was assessed using a highly sensitive and drug tolerant assay based on the Meso Scale Discovery Electrochemiluminescent platform,[24 27] followed by a two-tiered test consisting of a screening and specificity assay. Assays were developed for each IP and each serum sample was tested using both assays. Of note, these assays differed from the original ELISA used for immunogenicity assessments of adalimumab.[2] Samples positive for binding ADAs were tested in a ligand-binding bioassay for neutralising activity. The sensitivity of the ADA detection assay was the same for both adalimumab and ABP 501. The assay was validated with a tolerance of 25 µg/mL of drug, and the highest observed maximum observed concentration (C_{max}) in this study was <6.0 mg/mL. Drug interference was, thus, not expected from the collected samples. The neutralising antibody cell-based bioassay was expected to detect all classes of antibodies that inhibit the biological activity of the drug, including monovalent IgG4 subclass antibodies.[24]

Statistical analyses

A sample size of approximately 500 patients was chosen to achieve 90% power to demonstrate equivalence between the ABP 501 and adalimumab groups for the primary efficacy endpoint, RR of ACR20 at week 24, with a two-sided significance level of 0.05 and equivalence margin of (0.738, 1.355). It assumed an expected ACR20 response of 63% at week 24 for each group and a 15% dropout by week 24.

All efficacy endpoints were analysed using the full analysis set, which included all randomised patients, based on patients' randomised treatment. Randomisation, conducted by an independent statistician, was computer-generated and was stratified by geographical region and prior biologic use (with prior biologic use capped at 40% of study population) for RA. The primary hypothesis, that there were no clinically meaningful differences between the ABP 501 and adalimumab groups, was tested by comparing the 2-sided 90% confidence interval (CI) of the RR of ACR20 at week 24 between ABP 501 and adalimumab to the equivalence margin of (0.738, 1.355). The rationale for the equivalence margin was based on considerations in the draft US FDA Non-inferiority Clinical Trials

Guidance for Industry.[28] The equivalence margin of 0.738, 1/0.738=1.355 for the RR of ACR20 responses was chosen based on a published relevant adequate and well-controlled trial[8] and was expected to preserve 50% of the estimated 80% upper confidence bound of the treatment effect of the reference product compared with placebo. The 90% CI for RR was estimated using a generalised linear model adjusted for the stratification factors. All other efficacy endpoints, including RD of ACR20, were analysed descriptively. For DAS28-CRP, treatment differences across time points for change from baseline in DAS28-CRP were evaluated using a mixed model for repeated-measures analysis, with stratification variables, visit, treatment group, treatment-by-visit interactions and baseline DAS28-CRP included in the model. For ACR50 and ACR70, treatment differences were estimated using the same model as described above for ACR20. Analyses of the RDs for ACR20, ACR50 and ACR70 between ABP 501 and adalimumab were descriptive in nature and their corresponding 90% CIs were estimated using the generalised linear model adjusted for stratification factors. For the primary analysis based on the full analysis set, missing values were imputed using the last observation carried forward method. As sensitivity analyses, efficacy endpoints were also analysed for the per-protocol analysis set, which included patients who completed the treatment period and did not have a protocol violation, based on observed cases. In addition, for binary endpoints, such as ACR20, another imputation was performed for sensitivity in which patients with a missing binary response at a certain visit were imputed as non-responders.

All safety data were analysed using the safety analysis set (all randomised patients who received ≥1 dose of IP) based on patients' actual treatment received. ADA data were analysed using the ADA analysis set, defined as the subset of patients in the safety analysis set who had ≥1 evaluable antibody test result (based on actual treatment received).

RESULTS

Patient disposition

A total of 526 patients were randomised and treated with IP (ABP 501, n=264; adalimumab, n=262) and 494 (93.9%) completed the study (ABP 501, n=243; adalimumab, n=251) (figure 1). The main reason for discontinuation in both groups was withdrawal of consent (ABP 501, 4.2%, n=11; adalimumab, 2.3%, n=6).

Baseline demographics and clinical characteristics

The majority of patients were female (81.0%) and white (95.1%), with a mean age of 55.9 years (range: 21–80 years) and a mean of 9.39 years and median of 7.09 years since diagnosis. Overall, baseline demographics and clinical characteristics were similar across groups, including mean (standard deviation [SD]) baseline DAS28-CRP scores, which were 5.66 (0.92) and 5.68 (0.91) for the ABP 501 and adalimumab groups, respectively (table 1).

Concomitant and previous medications

Prior use of biologics for RA and baseline RA medications was balanced across groups; the majority of patients (ABP 501, 73.1%; adalimumab, 71.8%) were treatment-naive for prior use of biologics for RA. Oral corticosteroids were used at baseline by 50.8% and 49.6% of patients in the ABP 501 and adalimumab groups, respectively. Similar percentages of patients used non-steroidal anti-inflammatory drugs in each group (ABP 501, 60.2%; adalimumab, 64.1%). Baseline mean MTX doses were similar across treatment groups (ABP 501, 16.89 mg/week; adalimumab, 16.56 mg/week).

Clinical efficacy

ACR20

At week 24, 74.6% (194/260) of subjects in the ABP 501 group and 72.4% (189/261) of subjects in the adalimumab group met the ACR20 response criteria (figure 2A, see online supplementary tables 2 and 3). The RR (2-sided 90% CI) of ACR20 at week 24 for ABP 501 versus adalimumab was 1.039 (0.954, 1.133). The 90% CI of (0.954, 1.133) was well within the predefined equivalence margin (0.738, 1.355), demonstrating clinical equivalence between ABP 501 and adalimumab (figure 2B). Additionally, the RD (two-sided 90% CI) between groups for ACR20 at week 24 was 2.604 (−3.728, 8.936).

The percentage of patients who achieved ACR20 at weeks 2 and 8 (secondary endpoints) were 35.4% (90/254) of patients who took ABP 501 and 24.5% (63/257) of patients who took

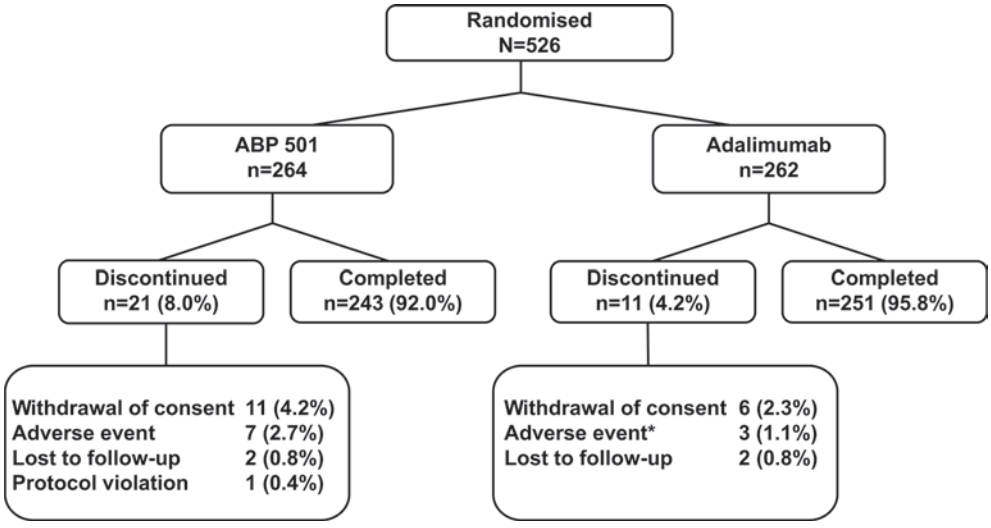


Figure 1 Patient disposition. *n=1 patient took prohibited concomitant medication due to an adverse event and was discontinued from the study. First patient was screened on 15 October 2013 and enrolled on 24 October 2013. Patients screened, n=747; per-protocol analysis set, n=463 (ABP 501, n=230; adalimumab, n=233).

Table 1 Baseline demographics and clinical characteristics (full analysis set)

Variable	ABP 501 (n=264)	Adalimumab (n=262)
Age, mean (SD), years	55.4 (11.9)	56.3 (11.5)
Women, n (%)	214 (81.1)	212 (80.9)
Race, n (%)		
White	251 (95.1)	249 (95.0)
Black or African American	9 (3.4)	12 (4.6)
Asian	3 (1.1)	0 (0.0)
Other	1 (0.4)	1 (0.4)
Region, n (%)		
Eastern Europe	169 (64.0)	168 (64.1)
Western Europe	22 (8.3)	20 (7.6)
North America	72 (27.3)	72 (27.5)
Latin America	1 (0.4)	2 (0.8)
Duration of RA, mean (SD), years	9.41 (8.08)	9.37 (8.05)
Duration of RA category, n (%)		
<5 years	101 (38.3)	90 (34.4)
≥5 years	163 (61.7)	172 (65.6)
Swollen joint count, mean (SD)	14.7 (9.1)	14.1 (8.0)
Tender joint count, mean (SD)	24.3 (14.4)	23.9 (13.5)
Subject Global Health Assessment, mean (SD)	6.5 (1.9)	6.6 (1.9)
Investigator Global Health Assessment, mean (SD)	6.8 (1.3)	6.7 (1.6)
HAQ-DI, mean (SD)*	1.482 (0.617)	1.498 (0.647)
Serum CRP, mean (SD), mg/L	13.881 (20.687)	14.678 (19.385)
Serum CRP, median, mg/L	6.140	7.630
DAS28-CRP, mean (SD)†	5.66 (0.92)	5.68 (0.91)
RF status, n (%)‡‡		
Positive	243 (92.0)	240 (91.6)
Anti-CCP status, n (%)‡		
Positive	212 (80.3)	230 (87.8)
Prior biologic use for RA, n (%)		
Yes	71 (26.9)	74 (28.2)
MTX dose, mean (SD), mg/week	16.89 (4.81)	16.56 (4.93)

*ABP 501, n=263; adalimumab, n=261; total, n=524.
†ABP 501, n=264; adalimumab, n=261; total, n=525.
‡At screening.
CCP, cyclic citrullinated peptide; CRP, C reactive protein; DAS28, Disease Activity Score 28-joint count; HAQ-DI, Health Assessment Questionnaire-Disability Index; MTX, methotrexate; RA, rheumatoid arthritis; RF, rheumatoid factor.

adalimumab at week 2 and 63.5% (165/260) versus 62.5% (163/261) of patients, respectively, at week 8 (figure 2A). The percentages of ACR20 responders were comparable across groups at all study time points, supporting clinical similarity between the ABP 501 and adalimumab groups.

ACR50 and ACR70

The percentages of patients who reached ACR50 response criteria at week 24 were 49.2% (120/244) and 52.0% (131/252) for the ABP 501 and adalimumab groups, respectively, with a RR (90% CI) for ABP 501 versus adalimumab of 0.948 (0.819, 1.097) and RD (90% CI) of −2.836% (−10.220%, 4.547%). The proportion of patients who achieved ACR50 was similar across treatment groups throughout the study. A total of 26.0% (64/246) and 22.9% (58/253) of patients reached ACR70 response criteria for ABP 501 and adalimumab at week 24, respectively. The RR (90% CI) for ACR70 at week 24 was 1.130 (0.872, 1.464) and the RD (90% CI) was 3.147% (−3.177%, 9.470%). The percentages of patients who achieved ACR70 were also similar across the ABP 501 and adalimumab groups at all study weeks.

DAS28-CRP

At week 24, the mean change from baseline in DAS28-CRP was −2.32 for both groups, with a difference between treatment groups (two-sided 90% CI) of −0.01 (−0.18, 0.17), further substantiating clinical efficacy equivalence between ABP 501 and adalimumab. Mean change from baseline in DAS28-CRP decreased similarly throughout the study (secondary endpoints) in both groups, indicating similar reduced disease activity (figure 3).

The percentage of patients who achieved DAS28-CRP remission increased over time for both groups from weeks 2 to 18 (range: 6.3%–31.1%, ABP 501; 2.8%–27.1%, adalimumab). At week 24, 30.5% and 35.5% of patients in the ABP 501 and adalimumab groups, respectively, reached DAS28-CRP remission.

The key efficacy data reported here were also analysed for the per-protocol analysis set (patients who completed the treatment period and did not have a protocol violation) as sensitivity analyses of key efficacy endpoints. For these endpoints, the per-protocol analysis set results were similar to that of the full analysis set (see online supplementary figure 1), which further confirms the similarity between ABP 501 and adalimumab.

Safety

Treatment-emergent adverse events

Overall, 52.3% of all patients had ≥1 TEAE during the study and the percentages of patients who reported TEAEs were similar for patients in the ABP 501 and adalimumab groups (50.0% and 54.6%, respectively) (table 2). TEAEs reported by >3% of patients in either group (ABP 501, adalimumab) were nasopharyngitis (6.4%, 7.3%), headache (4.5%, 4.2%), arthralgia (3.0%, 3.4%), cough (2.7%, 3.1%) and upper respiratory tract infection (1.5%, 3.8%). There were no differences between groups that were ≥5% for the percentage of patients who experienced a TEAE by preferred term. Findings from laboratory evaluations and vital sign examinations revealed no clinically significant changes in any outcome.

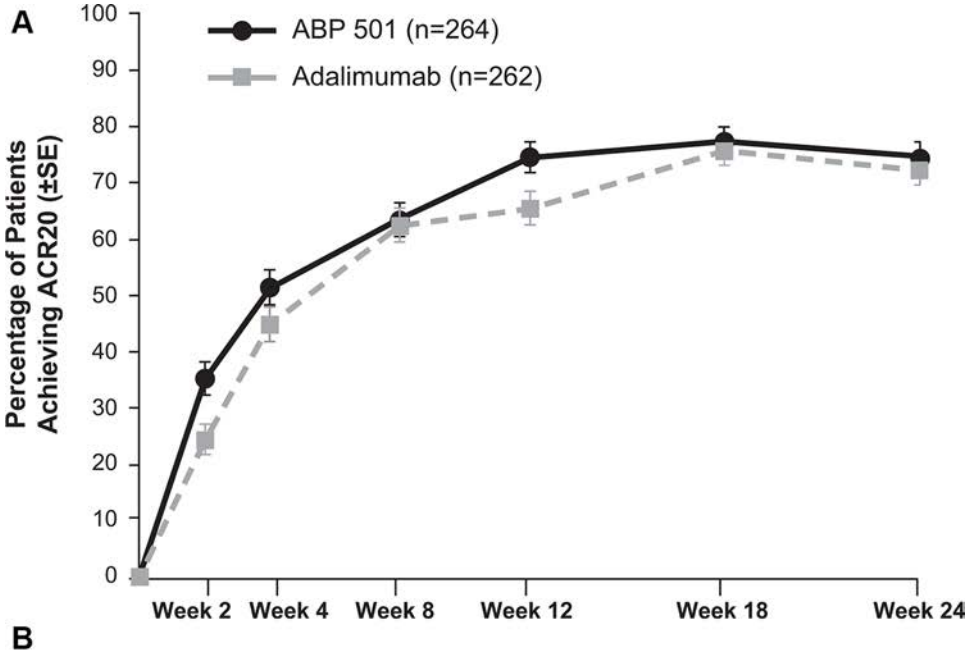
Serious adverse events

A total of 23 (4.4%) patients reported having 27 SAEs throughout the study and the percentages of patients were similar between the ABP 501 (n=10; 3.8%) and adalimumab (n=13; 5.0%) groups. The only SAE reported for >1 patient was sepsis (n=2; ABP 501) and both of these events had resolved by study end. Two patients in the ABP 501 group experienced >1 SAE at the same time. One patient had cardiopulmonary failure, pneumonia and sepsis and the other experienced peritoneal abscess, perforated appendicitis and secondary sepsis. No SAEs or treatment-related SAEs occurred in ≥2% of patients for either group by preferred term. No deaths occurred in this study.

Adverse events of interest

Standard searches for AEs of interest identified 80 (30.3%) patients in the ABP 501 group and 94 (35.9%) patients in the adalimumab group who had ≥1 event of interest (table 2). AEs of interest during the study included two malignancies, basal cell carcinoma and squamous cell carcinoma, reported in one subject in the ABP 501 group and squamous cell carcinoma of the skin reported in one subject from the adalimumab group. No cases of active tuberculosis were reported throughout the study. Reported hypersensitivities occurring in >2 patients overall included rash,

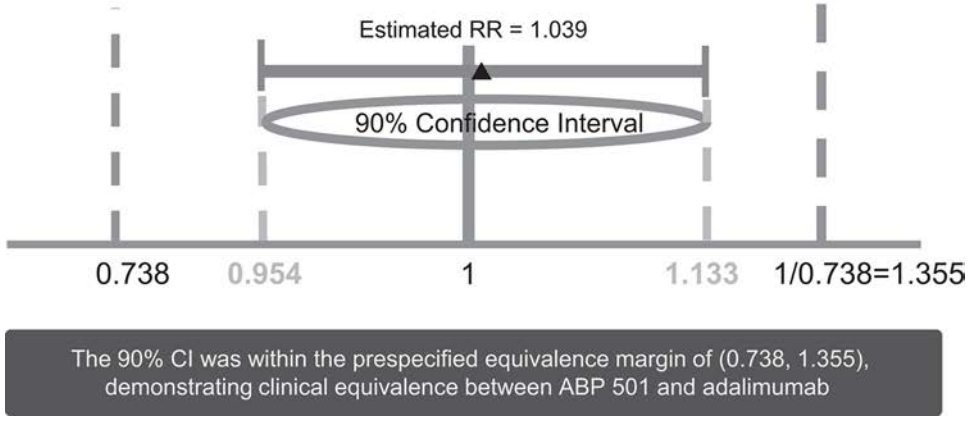
A



B

	ABP 501 (n=264)	Adalimumab (n=262)
ACR20 Response at week 24	194/260 (74.6%)	189/261 (72.4%)
RR of ACR20 (90% CI)	1.04 (0.95, 1.13)	

$$RR = \frac{\text{ACR20 Response Rate in ABP 501}}{\text{ACR20 Response Rate in Adalimumab}}$$



The 90% CI was within the prespecified equivalence margin of (0.738, 1.355), demonstrating clinical equivalence between ABP 501 and adalimumab

Figure 2 (A) Percentage of patients achieving ACR20 by study week (full analysis set). ACR20, 20% improvement from baseline in American College of Rheumatology core set measurements. (B) Ratio of ACR responses at week 24. ACR20, 20% improvement from baseline in American College of Rheumatology core set measurements. RR, risk ratio; 95% CI 0.938, 1.152.

erythematous rash and allergic dermatitis. Liver enzyme elevations were reported, however, none led to premature study discontinuation and none were associated with increases in bilirubin that would cause concern about drug-induced liver injuries according to Hy's law.[29] Injection-site reactions occurred in 2.3% and 5.0% of patients in the ABP 501 and adalimumab groups, respectively. Most events of interest were grade 1 or 2 in severity. Similar percentages of patients in the ABP 501 (n=3, 1.1%; infection, n=2; hypersensitivity, n=1; heart failure, n=1) and adalimumab (n=4, 1.5%; infections, n=3; heart failure, n=1) groups experienced SAEs of interest.

Immunogenicity

All 526 randomised and treated patients had ≥1 evaluable ADA result and were included in the antibody analysis. For the ABP 501 and adalimumab groups, 5 (1.9%) and 6 (2.3%) patients, respectively, tested positive for pre-existing binding antibodies and no patients tested positive for pre-existing neutralising antibodies. Overall, 201 (38.2%) patients tested positive for binding antibodies at weeks 4, 12, or 26 post-baseline, which was similar to the percentages in each group (ABP 501, n=101, 38.3%; adalimumab, n=100, 38.2%) (figure 4A). A total of 53 (10.1%) patients tested positive for neutralising

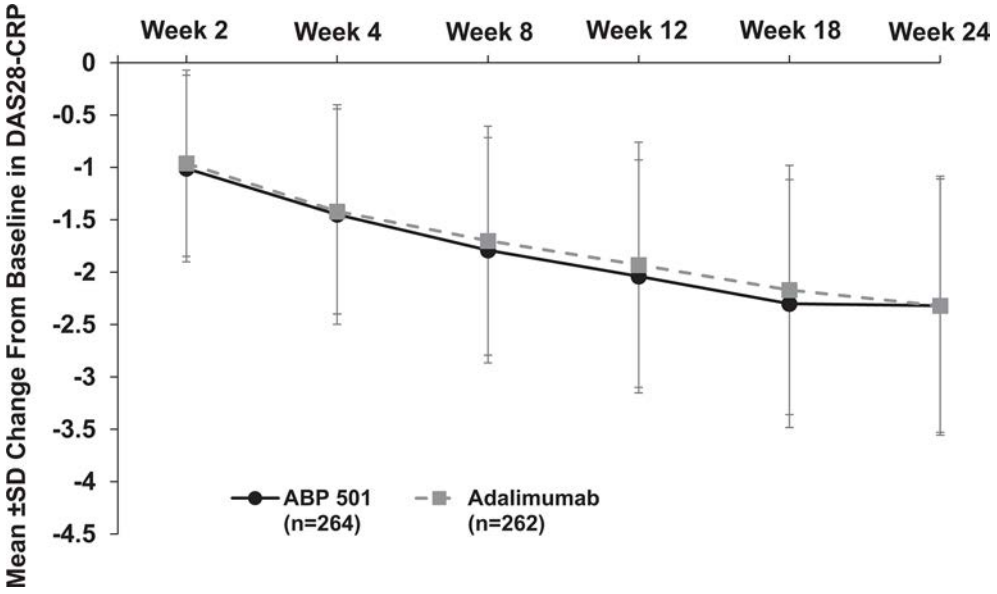


Figure 3 Mean±SD change from baseline in DAS28-CRP by study week (full analysis set). DAS28-CRP, Disease Activity Score 28-joint count-C reactive protein.

ADAs at weeks 4, 12, or 26 post-baseline, which was also similar to that for each treatment group (ABP 501, n=24, 9.1%; adalimumab, n=29, 11.1%).

Descriptive results for ADAs by visit and treatment indicate that the incidence of ADAs were similar in patients across both groups throughout the course of the study (see online supplementary table 4). In addition, the percentage of patients who demonstrated an ACR20 response throughout the study was found to be similar across treatment groups regardless of ADA status (figure 4B).

Pharmacokinetic results

All 526 randomised patients had at least one evaluable result for serum concentration of ABP 501 or adalimumab at any visit and were included in the pharmacokinetic analysis. Pharmacokinetic results revealed that trough serum concentrations were similar between groups across all study weeks, indicating that exposure was similar between treatment groups (see online supplementary table 5). The geometric mean trough serum concentrations at week 24 were 4844.16 ng/mL and 5210.75 ng/mL for ABP 501 and adalimumab, respectively, with similar concentrations across groups at all time points throughout the study (range: ABP 501, 2062.64–4844.16 ng/mL; adalimumab, 1936.11–5210.75 ng/mL).

Table 2 Overall safety and AEs of interest by treatment (safety population)

	ABP 501 (n=264)	Adalimumab (n=262)
	Number of patients, n (%)	Number of patients, n (%)
Any TEAE	132 (50.0)	143 (54.6)
Serious AEs	10 (3.8)	13 (5.0)
AEs leading to discontinuation of IP	5 (1.9)	2 (0.8)
AEs leading to study discontinuation	7 (2.7)	2 (0.8)
AEs of interest		
Any	80 (30.3)	94 (35.9)
Infections	61 (23.1)	68 (26.0)
Malignancies	1 (0.4)	1 (0.4)
Hypersensitivity	14 (5.3)	10 (3.8)
Haematological reactions	5 (1.9)	5 (1.9)
Heart failure	1 (0.4)	2 (0.8)
Liver enzyme elevations	13 (4.9)	10 (3.8)
Injection-site reactions	6 (2.3)	13 (5.0)

For each category, patients were included only once even if they had multiple events in that category. AEs coded using MedDRA V.17.1.
AE, adverse event; IP, investigational product; TEAE, treatment-emergent adverse event.

DISCUSSION

Development of biosimilars is a complex process that requires demonstration of similar efficacy, safety, immunogenicity and pharmacokinetics between the proposed biosimilar and reference product. For regulatory review, demonstration that there are no clinically meaningful differences between the proposed biosimilar and its reference product is necessary. FDA guidance on the development and approval of biosimilars requires that a stepwise, totality-of-evidence-based approach be used to generate data in support of biosimilarity and to evaluate any residual uncertainty.[13] Likewise, the EMA requires that biosimilars show similarity to the reference product in pharmacokinetics, pharmacodynamics, clinical efficacy and safety and that a risk management/pharmacovigilance plan is adopted in accordance with EU legislation.[12]

Analytical comparison has shown that ABP 501 and adalimumab are highly similar molecules with respect to physicochemical properties[22] and biological activity.[23] Pharmacokinetic equivalence of ABP 501 to adalimumab was also demonstrated in a phase I, single-dose study conducted in healthy adults.[24] Results from an additional clinical trial have shown that clinical efficacy and safety profiles for ABP 501 are similar to those of adalimumab in 350 patients with moderate to severe psoriasis (NCT01970488), which adds to the overall demonstration of similarity between the biosimilar and reference product.[25]

Results from this study indicate that ABP 501 is equivalent in efficacy to the reference product, adalimumab, in patients with RA. Similar efficacy results were observed for ABP 501

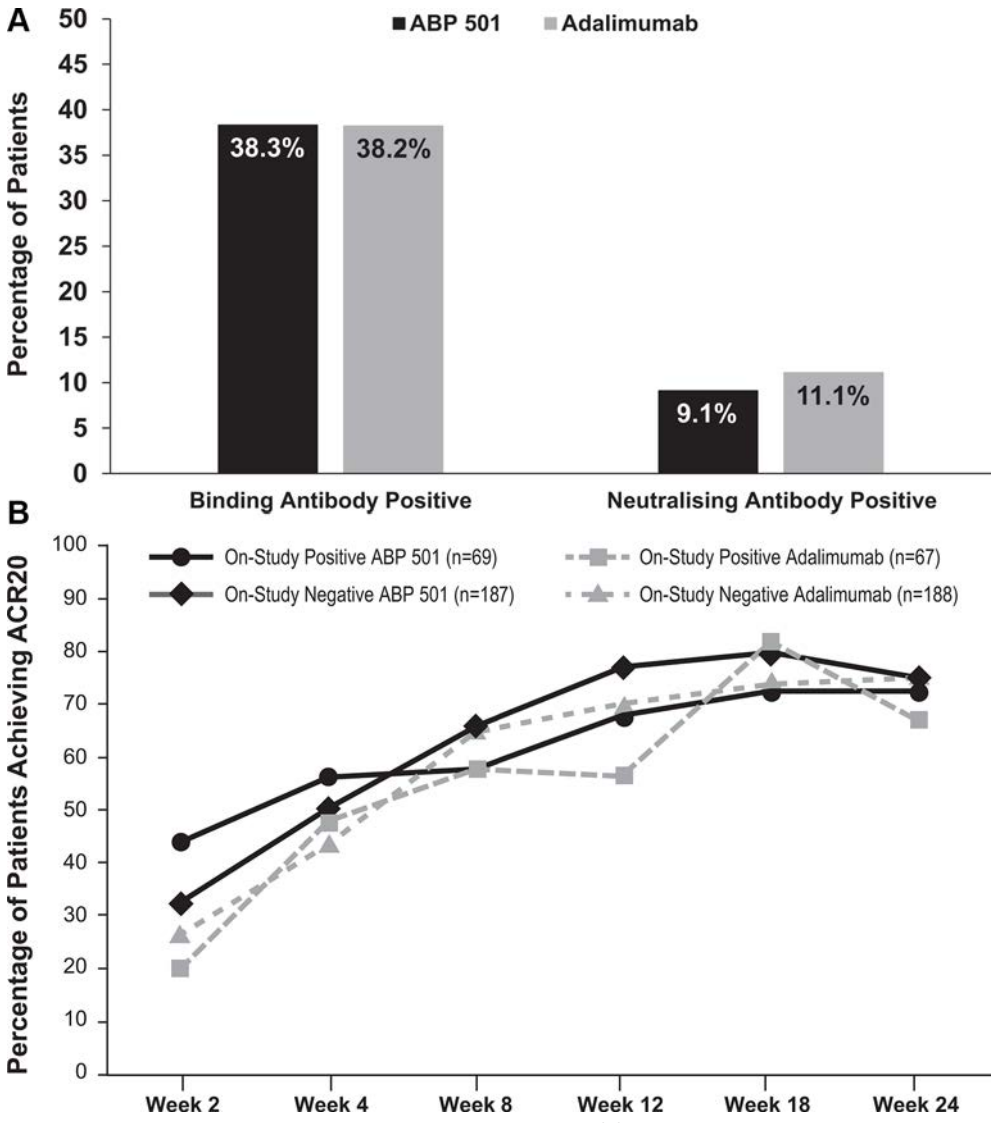


Figure 4 (A) Immunogenicity at any time point post-baseline throughout the study. (B) ACR20 responders by ADA status throughout the study (full analysis set). ACR20, 20% improvement from baseline in American College of Rheumatology core set measurements.

and adalimumab for ACR20 (primary endpoint), ACR50 and ACR70 (secondary endpoints). The mean changes from baseline in DAS28-CRP results were also similar between groups at all study time points.

The clinical safety results from this study indicate that ABP 501 and adalimumab have similar safety profiles. No new safety signals were detected in this study compared with other adalimumab clinical trials in patients with RA.[2] Hypersensitivity reactions were reported infrequently and occurred at a generally similar frequency in both treatment groups.

Since biosimilars are not identical molecules, the determination of immunogenicity by ADA formation is a major part of the registration programme for approval.[2] In the current study, a highly sensitive and drug tolerant electrochemiluminescent assay was used to assess ADA status.[27] In pivotal trials of adalimumab in RA, ADA status had been determined by ELISA assays that did not allow for detection of ADAs in the presence of drug and that measured ADA levels that were much lower than those reported in this trial.[2] Even though the levels of ADAs detected were higher due to a more sensitive assay than those reported from historical pivotal trials of adalimumab, the percentages of patients who tested positive for binding and neutralising antibodies in this trial were similar between the ABP 501 and adalimumab groups throughout the study. In a phase I study in which the relationship between pharmacokinetics and ADA status was assessed in healthy subjects receiving ABP 501 or adalimumab, results showed that overall exposure (area under the curve (AUC)) was approximately 20%–30% lower in subjects who were ADA positive versus those who were ADA negative.[24] Similarly, the elimination half lives ($t_{1/2}$) for ADA positive subjects were shorter (6–7 days) than patients who were ADA negative (12–15 days). This inverse correlation between ADA levels and serum concentrations of TNFα inhibitors has been previously shown in many studies and has been associated with decreased clinical efficacy.[30] Patients who were symptomatic seronegative were not included in this study as they may be misdiagnosed as having RA and would introduce more heterogeneity within the population. Additionally, as biosimilarity is proven via a totality-of-evidence-based approach, the demonstration of similarity between ABP 501 and adalimumab from analytical, functional, pharmacokinetic and clinical perspectives leaves no residual uncertainty that ABP 501 will behave differently in patients who were seronegative. In the present trial, the clinical responses for both ABP 501 and adalimumab were comparable to previously reported results suggesting that the majority of the antibodies

detected in these patients may have little or no clinical relevance.[2] Further, the percentage of patients who achieved an ACR20 response by ADA status indicates no change in efficacy throughout the study for either treatment group, regardless of ADA status.

Limitations of this study include the 6-month trial design; however, an open-label extension of this study, up to 72 weeks (NCT02114931), is on-going and will provide additional long-term safety and efficacy data for ABP 501 in patients with moderate to severe RA. Moreover, 52-week results from the previously mentioned trial in patients with psoriasis will also provide insights into the long-term immunogenicity and safety profile of ABP 501. Regulatory guidelines for biosimilars do not require monitoring of radiographic progression. While this may be an area of interest, detecting any clinically meaningful differences in radiographic progression would be challenging via studies designed to compare two active treatment groups.

Data from this study indicate that the clinical efficacy, safety and immunogenicity of ABP 501 are similar to that of adalimumab in patients with moderate to severe RA. Additionally, analytical, biofunctional and pharmacokinetic properties of ABP 501 have previously been shown to be highly similar to those of adalimumab.[22–24] Taken together, these data contribute to the totality-of-evidence-based requirements to demonstrate that ABP 501 is similar to adalimumab. The FDA has, thus, approved ABP 501 for use as a biosimilar to adalimumab,[20] making it a valuable new therapeutic option for the treatment of moderate to severe RA.

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Correction notice This paper has been amended since it was published Online First. Owing to a scripting error, some of the publisher names in the references were replaced with 'BMJ Publishing Group'. This only affected the full text version, not the PDF. We have since corrected these errors and the correct publishers have been inserted into the references.

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Patient consent Written informed consent was obtained from each patient prior to study enrolment.

Ethics approval This study was conducted in accordance with the current International Conference on Harmonization good clinical practice guidelines and the Declaration of Helsinki. The study protocol was approved by the institutional review board or independent ethics committee at each participating site and adhered to all local regulatory requirements including data protection requirements.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement All data from this study, published and unpublished, were made available to all authors. Further inquiry regarding availability of the data can be addressed to PK of Amgen, Inc.

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EXTENDED REPORT

The dynamics of response as measured by multiple composite outcome tools in the TIght COntrol of inflammation in early Psoriatic Arthritis (TICOPA) trial

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ABSTRACT

Background We aimed to evaluate the dynamics of treatment response with different composite measures in the TIght COntrol of inflammation in early Psoriatic Arthritis (TICOPA) trial.

Methods Participants with early disease-modifying antirheumatic drug-naïve psoriatic arthritis (PsA) were randomised 1:1 to either tight control (TC; 4 weekly review with therapy escalation if criteria not met) or standard care (SC; 12 weekly review). We calculated modified versions of the Psoriatic ArthritiS Disease Activity Score (PASDAS), Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA) Composite scorE (GRACE) and Composite Psoriatic Disease Activity Index (CPDAI) at baseline and 12 weekly to 48 weeks by blinded assessor. For missing data, we used the last observation carried forward. Comparison between groups was made by analysis of covariance and comparison of area under the curve (AUC).

Results 206 people were randomised to TC (n=101) or SC (n=105). Significant differences between treatment groups were seen (p<0.0001 for all composite measures). AUC analysis demonstrated a significant difference between groups for the PASDAS but not GRACE and CPDAI. For participants with oligoarthritis, a significant difference between groups was seen for each measure, although the significance levels were greatly diminished (PASDAS, p=0.04; GRACE p=0.01; CPDAI p=0.04). For oligoarthritis using AUC analysis, none of the measures could distinguish between groups.

Conclusions Composite measures of disease activity were able to distinguish between TICOPA treatment arms, although differences were diminished for those with oligoarthritis. Further data are needed to inform the preferred composite measure for use as the primary outcome in PsA trials.

Trial registration number ClinicalTrials.gov (NCT01106079) and ISCRCTN registry (ISCRCTN30147736).

INTRODUCTION

Psoriatic arthritis (PsA) is a heterogeneous disease which can manifest in several ways including arthritis, enthesitis, dactylitis, axial disease and skin/nail involvement. The lack of a specific validated target for PsA means that the primary outcome measure used in recent interventional studies has been the American College of Rheumatology 20% improvement (ACR20) criteria, a measure originally developed for rheumatoid arthritis focusing on peripheral joint activity.[1] However, new composite targets encompassing the complex manifestations of PsA have been developed. These include the Psoriatic Arthritis Disease Activity Score (PASDAS) and the GRAPPA CompositE Index (GRACE),[2] and, in addition, the Composite Psoriatic Disease Activity Index (CPDAI).[3] In addition to measuring disease activity at any point in time, these indices can also serve as responder indices and cut-offs for response have been developed.[4]

The TIght COntrol in Psoriatic Arthritis (TICOPA) study was the first study to demonstrate that tight control of disease utilising predefined activity levels to guide therapeutic changes resulted in significantly better clinical outcomes compared with standard care.[5] In the TICOPA study, the odds of achieving an ACR20 response at 48 weeks was twofold higher in the treat to target arm. However, the outcomes at intervening time points for each arm of the study were not described nor were validated composite disease activity measures for PsA reported. In this study, we evaluated treatment responses in the TICOPA study using the PASDAS, GRACE and CPDAI indices and compared their performance.

METHODS

The primary results of the TICOPA study have already been published.[5] In brief this randomised, controlled, parallel group, open label, multicentre clinical trial recruited people with early (less than 2 years), treatment-naive PsA. The full trial protocol is also available.[6] The primary objective of the trial was to compare tight control (TC) with standard care (SC), using minimal disease activity[7] as the treatment target. Participants received either TC or SC for a period of 48 weeks. Participants randomised to TC were seen every 4 weeks by the study physician and treated according to a predefined treatment protocol. Participants randomised to the SC arm were treated in a general rheumatology outpatient clinic supervised by a consultant rheumatologist. These patients were generally reviewed every 12 weeks but were seen more often if clinically indicated, with no formal measures of disease activity used in clinical decision making. A blinded assessor collected clinical assessments and patient reported outcomes every 12 weeks, and the composite disease activity measures were derived from these.

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Table 1 Comparison of composite measures with analysis of covariance and area under of curve for all patients

						Analysis of of covariance			Area under the curve	
		n	Tight control (n=101)	n	Standard care (n=105)	F	p	N	t	p
PASDAS	Baseline	88	5.36±1.42	85	5.09±1.33					
	12 weeks	87	4.08±1.37	85	4.44±1.64					
	24 weeks	88	3.60±1.58	85	4.25±1.88	13.4	<0.0001			
	36 weeks	88	3.34±1.74	85	4.21±1.73					
	48 weeks	88	3.17±1.64	85	4.02±1.80	18.0	<0.0001	173	2.57	0.01
GRACE	Baseline	97	4.37±1.68	96	4.06±1.64					
	12 weeks	96	3.27±1.70	96	3.44±1.78					
	24 weeks	97	2.76±1.77	96	3.21±1.98	7.5	0.007			
	36 weeks	97	2.55±1.97	96	3.21±2.03					
	48 weeks	96	2.32±1.96	96	2.98±2.00	14.5	<0.0001	192	1.44	0.15
CPDAI	Baseline	89	7.83±2.74	89	7.26±2.62					
	12 weeks	89	5.88±2.80	89	6.06±2.81					
	24 weeks	88	5.21±2.70	89	5.91±3.13	8.1	0.005			
	36 weeks	89	4.79±2.82	89	5.56±3.02					
	48 weeks	88	4.46±2.63	89	5.60±3.10	15.9	<0.0001	177	1.22	0.23

'F' and 't' are test statistics.
CPDAI, Composite Psoriatic Disease Activity Index; GRACE, GRAPPA Composite score; PASDAS, Psoriatic ArthritiS Disease Activity Score.

Composite measures

Derived PASDAS

The PASDAS is a weighted index comprising assessments of joints, function, acute phase response and quality of life and patient and physician Visual Analogue Scores (VAS). It is given by the formula:

PASDAS $=(((0.18\times\sqrt{}\text{physician global VAS})+(0.159\times\sqrt{}\text{patient global VAS})-(0.253\times\sqrt{}\text{SF36}-\text{PCS})+(0.101\times\text{LN (swollen joint count }+1))+(0.048\times\text{LN (tender joint count}+))+(0.23\times\text{LN (leeds enthesitis count}+1))+(0.377\text{ LN (dactylitis count}+1))+(0.102\times\text{ LN (CRP}+1))+2)^{*}1.5$.

Where LN = natural logarithm, PCS = Physical Component Summary Scale of 36-Item Short Form Health Survey (SF36), CRP=C reactive protein in mg/L. All VAS scores are 0–100 mm. Swollen joint count is 66 joints and tender joint count 68. In this study, the SF36 was not completed so an estimate of this outcome was calculated using the following formula: $\text{sf36pcs}=51.615-(6.52\times\text{HAQ})-(1.529\times\text{BASDAI})-(0.429\times\text{PsAQoL})$ where: sf36pcs is the estimated physical component score of the SF36, HAQ is the Heath Assessment Score (range 0–3),[8] BASDAI is the Bath Ankylosing spondylitis Disease Activity Index (range 0–10)[9] and PsAQoL is the Psoriatic Arthritis Quality of Life measure.[10] This formula was obtained by regression analysis using the GRACE data set[2] and explained 71% of the variance in sf36pcs scores (R^2adj=0.71).

The score range of the PASDAS is 0–10, with worse disease activity represented by higher scores.

Modified GRACE index

The GRACE is a composite score comprising assessments of joints, skin, pain, function and health-related quality of life. Each domain is transformed into a 'desirability' scale and the items then combined arithmetically.[4] The variables transformed are:

- 68 tender joint counts
- 66 swollen joint counts
- HAQ
- Patient global assessment of disease activity by VAS
- Patient VAS for skin
- Patient VAS for joints
- Psoriasis area and severity index (PASI)
- PsAQoL

In the TICOPA study, a VAS for skin was not collected and this item was thus omitted from the scale. Omitting the VAS for skin does not affect the score range as the score reflects the arithmetic mean of the individual components.

The GRACE index has a score range of 0–10 with worse disease activity represented by higher scores.

Modified CPDAI

This index measures disease activity in five domains: peripheral joints, skin, enthesitis, dactylitis and spine.[3] Within each domain, severity was graded as 0 (none), 1 (mild), 2 (moderate) and 3 (severe), according to predefined cut-offs (indicated in online supplementary table S1).

In the TICOPA study, the Ankylosing Spondylitis Quality of Life index was not obtained so this was substituted by the PsAQoL (range 0–20) using the same cut-off of 6. In addition, the DLQI was estimated using the following equation:

$\text{DLQI}=0.533+(1.98\times\text{HAQ})+(0.165\times\text{PSAQOL})+(0.405\times\text{PASI})$.

This formula was obtained by regression analysis using the GRACE data set[2] and explained 35% of the variance in DLQI scores (R^2adj=0.35).

STATISTICAL ANALYSIS

All statistical analyses were carried out in SPSS V.21. Where necessary, missing component data were replaced by carrying forward the last available observation. Comparison between groups at 48 weeks was made using an analysis of covariance with baseline values as the covariate. In addition, treatment groups were compared by calculating the area under the curve (AUC) and comparing the area with independent t-tests. We performed these analyses for all participants and, in addition, for those with oligoarthritis. Using previously defined cut-offs, we examined the proportion of people in each arm of the trial achieving good, moderate or poor outcome for each composite measure at 48 weeks,[4] comparing these proportions with the χ^2 test.

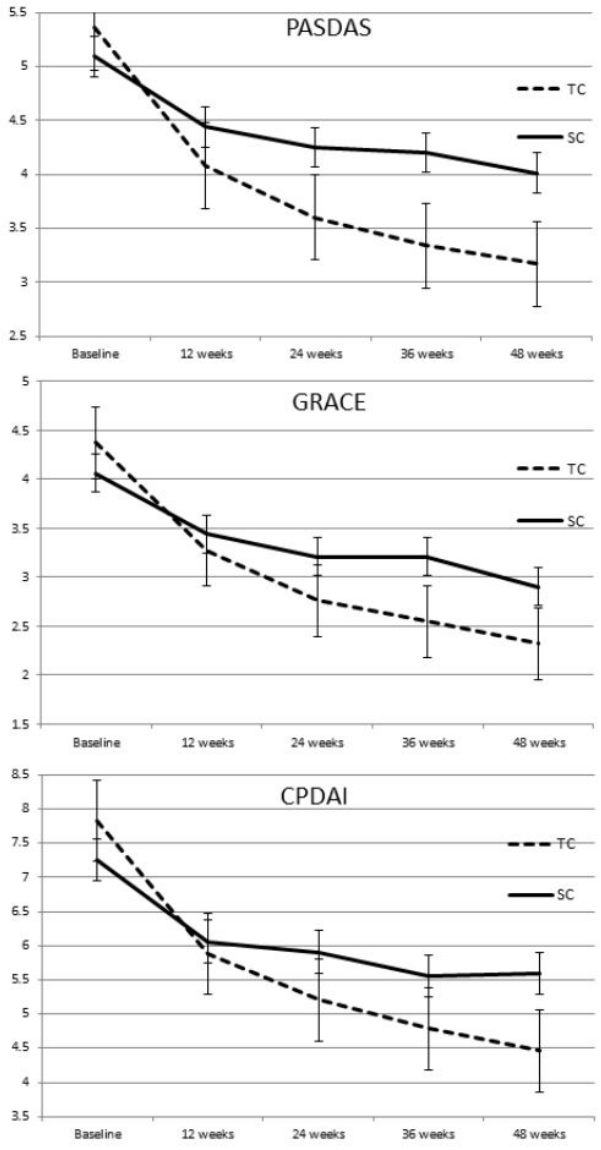

Figure 1 Change in scores for each composite measure in each treatment arm in the TICOPA study. TICOPA, TIght COntrol of inflammation in early Psoriatic Arthritis.

RESULTS

The study population consisted of 206 patients randomly assigned to either the TC (n=101, 49.0%) or SC (n=105, 51.0%). The baseline demographics of participants was, for TC and SC respectively, median age 46 years (range 18, 81) and 45 years (range 19, 71), males 53% and 52% and median duration of disease 0.9 months (range 0, 21.4) and 0.7 months (range 0, 23.6). In TC, 89.1% (n=90) of patients completed treatment and follow-up to week 48 with a similar proportion in SC (n=92, 87.6%).

Baseline composite scores differed, with the tight control having higher baseline scores. Mean scores for each measure at each major time point are given, along with the statistical analysis, in table 1. The baseline individual domain scores for each of the composite measures for the entire population are given in the online supplementary table S2. Analysis of covariance, adjusting for baseline data, for all available data demonstrated a highly significant difference for each of the composite measures. AUC analysis also showed significant differences between groups for PASDAS but not GRACE and CPDAI. The dynamics of change for each composite measure are illustrated in the figure 1. For all measures, there was separation between the groups at the first 'blinded' assessment point, with further divergence at subsequent time points. For information, the CPDAI is reported by domain at baseline and 48 weeks in the online supplementary table S3.

The data for patients with oligoarthritis at study entry are given in table 2. The baseline individual domain scores for the group with oligoarthritis are given in the online supplementary table S4. Although comparison of groups by analysis of covariance achieved significance, the statistics were smaller than those found for the entire cohort, and the mean values for each measure were reduced. Further, AUC analysis failed to show a difference between treatment arms for all of the measures tested but the number of participants for the analysis was small. Of note, the mean figures for each composite measure did not diverge until 24 weeks for this PsA subgroup.

The proportion of people achieving 'good', 'moderate' and 'poor' response at 48 weeks, for each measure, is given in table 3. For the PASDAS, 64.5% of people who achieved a 'good' response were in the TC arm, and 68.9% of those who achieved a 'poor' response were in the SC arm. The figures for the GRACE index were 63.6% and 66.3%, respectively, and for the CPDAI, 66.7% and 63.7%. For each measure, the difference in proportions was highly significant.

DISCUSSION

The TICOPA study was the first to show that a treat to target approach improved clinical outcomes for patients with early PsA. The primary outcome was the composite measure developed for rheumatoid arthritis clinical trials, the ACR20. However, secondary outcomes demonstrated benefits across both articular and skin domains, although not for dactylitis and enthesitis. The current study describes the treatment response in terms of disease activity using three validated composite disease activity measures, all of which assess disease activity across several domains. Early separation was seen between the treatment groups with significant differences at 48 weeks and significantly more patients in the TC arm achieving a good clinical outcome. However, it must be acknowledged that these three composite measures were not assessed in their entirety in the TICOPA study, and that adaptations had to be made for the scores to be obtained. If the required data had been available, it is possible that the measures would have behaved differently.

The early separation between treatment groups at 12 weeks was probably due to the more aggressive use of methotrexate. As reported in the original paper, subjects in the TC arm had rapid escalation of oral methotrexate to 25 mg weekly, and this was reflected in the numbers achieving that dose at 3 months (TC 82.2%; SC 7.6%). If the minimal disease activity target had not been reached at 12 weeks, sulfasalazine was added to methotrexate so that the continued and more pronounced separation of treatment groups between 12 and 24 weeks was partly attributable to this combination therapy. This is important as it would be advantageous to be able to predict who would respond to either methotrexate alone or combination conventional disease modifying therapy. Beyond 24 weeks, TC patients who continued to have active disease were eligible for biological therapy with tumour necrosis factor blockers and the further relative improvement seen in the TC arm beyond 24 months probably reflects this.

Table 2 Comparison of composite measures with analysis of covariance and area under the curve for patients with oligoarthritis at study entry

						Analysis of of covariance			Area under the curve	
		n	Tight control (n=27)	n	Standard care (n=30)	F	p	N	t	p
PASDAS	Baseline	21	4.14±1.10	23	4.15±0.80					
	12 weeks	20	3.30±1.33	23	3.24±1.04					
	24 weeks	21	2.82±1.35	23	3.49±1.65	2.8	0.10			
	36 weeks	21	2.97±1.87	23	3.45±1.49					
	48 weeks	21	2.61±1.50	23	3.48±1.53	4.7	0.04	44	0.94	0.35
GRACE	Baseline	26	2.77±1.06	28	2.74±1.09					
	12 weeks	25	2.23±1.41	28	2.21±1.10					
	24 weeks	26	1.80±1.42	28	2.24±1.51	1.0	0.33			
	36 weeks	26	1.76±1.86	28	2.40±1.75					
	48 weeks	26	1.39±1.49	28	2.29±1.61	6.6	0.01	54	0.97	0.34
CPDAI	Baseline	22	5.86±2.12	24	5.38±2.22					
	12 weeks	22	4.64±2.11	24	4.58±2.17					
	24 weeks	21	4.00±1.76	24	4.54±2.64	0.8	0.38			
	36 weeks	22	4.00±2.76	24	4.46±2.57					
	48 weeks	22	3.59±2.24	24	4.46±2.45	4.5	0.04	46	0.37	0.72

'F' and 't' are test statistics.
CPDAI, Composite Psoriatic Disease Activity Index; GRACE, GRAPPA Composite score; PASDAS, Psoriatic ArthritiS Disease Activity Score.

Table 3 Numbers achieving 'good', 'moderate' and 'poor' response at 48 weeks according to each measure and according to treatment group

	Tight control				Standard care					
	'Good' response	'Moderate' response	'Poor' response	Total	'Good' response	'Moderate' response	'Poor' response	Total	χ^2	p Value
PASDAS, n (%)	40 (46	29 (33)	19 (21)	88 (100)	22 (26)	21 (25)	42 (49)	85 (100)	15.1	0.001
GRACE, n (%)	35 (37)	32 (33)	29 (30)	96 (100)	20 (21)	19 (20)	57 (59)	96 (100)	16.5	0.0001
CPDAI, n (%)	34 (39)	15 (17)	39 (44)	88 (100)	17 (19)	9 (10)	63 (71)	89 (100)	14.0	0.001

CPDAI, Composite Psoriatic Disease Activity Index; GRACE, GRAPPA Composite score; PASDAS, Psoriatic ArthritiS Disease Activity Score.

In the subgroup of people with oligoarthritis, contrasting results were obtained. The composite scores were lower at baseline, as would be expected, but the early difference seen for the entire cohort was not seen for the oligoarthritis subgroup alone. This result may reflect the possibility that none of the composite measures is appropriate for assessing disease activity where joint counts are low. An alternative explanation is that there is a a lack of effect of methotrexate in patients with oligoarthritis. In this respect, it is worth noting that in the landmark methotrexate in psoriatic arthritis trial, better results were seen in the polyarticular subset of the disease.[11] More data are needed on the appropriate treatment strategy for this common subgroup of PsA.

At the time the TICOPA study was commenced, validated composite measures for psoriatic arthritis were unavailable so that not all appropriate outcomes were measured to allow calculation of the PASDAS, CPDAI and GRACE. However, we were able to make an allowance for the missing measures, either by estimating, using available measures or, in the case of the GRACE instrument, a modular measure, to omit the outcome (a skin VAS score). The effect of these modifications on the performance of the measures is difficult to assess: an independent data set in which all these variables are collected would inform this question. The new composite measures assess disease activity in domains other than the joints, and offer more responsiveness, together with larger effect sizes, in clinical trials.[12] In the future, it would be appropriate to use such measures as the primary outcome in clinical trials as it is likely that fewer patients will be needed to show a difference in treatment arms. The PASDAS is currently being used in this way.[13]

Which of the composite measures performs best in this study? In the overall patient population, there is probably little to choose between them, although the statistics for the PASDAS exceed those for the GRACE and CPDAI. Similarly, the PASDAS outperforms the GRACE and CPDAI using AUC analysis. Similar contrasts are evident in the analysis of the oligoarthritis patients. Each of the measures differs in construction: the PASDAS uses a weighted formula, the GRACE a modular scheme with each domain using a 'desirability' scale and the CPDAI also using a modular scheme in which patients are categorised within domain.[2] Each of the measures covers a similar range of domains but the CPDAI is the only measure addressing the five major domains of joints, skin, enthesitis, dactylitis and spine. The relative performance of the measures may have been a function of the patients enrolled in the study—spinal involvement was not prominent nor was the skin component—and the measures may perform relatively differently in alternative patient populations. In terms of outcome, all three measures gave a similar result (table 3), but it is worth noting that the cut-offs for outcome are still preliminary, although they have demonstrated good ability to distinguish radiographic progression in an alternative data set.[14]

In conclusion, the performance of several novel composite disease activity measures have been examined using data from the TICOPA trial. Each measure was able to distinguish between treatment arms, although all three showed diminished ability to distinguish treatment effect for patients with oligoarthritis. Further data are needed to guide the decision on selecting a preferred composite measure for use as the primary outcome in PsA clinical trials.

Contributors Study design and execution: the TICOPA study was conceived by PSH, LCC, PE and PGC. The study was designed by PSH and LCC. Study data and coordination were by Leeds Institute of Clinical Trials Research. Analysis and writing: the data were analysed by PSH who takes full responsibility for the results. All authors contributed to and approved the final version of the paper.

Competing interests None declared.

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EXTENDED REPORT

Low disease activity (DAS28≤3.2) reduces the risk of first cardiovascular event in rheumatoid arthritis: a time-dependent Cox regression analysis in a large cohort study

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ABSTRACT

Objective Systemic inflammation appears to contribute to the excess risk of cardiovascular disease (CVD) in rheumatoid arthritis (RA). The objective of this study was to investigate the effect of different levels of disease activity over time, particularly low disease activity and remission, on CVD risk in patients with RA.

Methods Data from the Nijmegen early RA inception cohort were used. The primary outcome was first CVD events within the first 10 years of follow-up. Cut points of the DAS28 for remission (<2.6) and low (≤3.2), moderate (3.2–5.1) and high (>5.1) disease activity were used. The effect of disease activity on CVD risk was analysed using Cox-proportional hazards regression with DAS28 as a time-dependent covariate and also conventionally with time-averaged DAS28 as the primary dependent variable.

Results Low DAS28 (≤3.2) was significantly associated with a reduced risk of CVD (HR 0.65, 95% CI 0.43 to 0.99) compared with DAS28 >3.2, both when included as a time-dependent covariate and as time-averaged DAS28 ≤3.2 (HR 0.52, 95% CI 0.33 to 0.81). Remission had a modest, non-significant protective effect against CVD (HR 0.67, 95% CI 0.43 to 1.07).

Conclusion Results of this study suggest that low disease activity is sufficient to achieve a protective effect against CVD in RA. Apparently, remission defined as DAS28 <2.6 has no additional protective effect against CVD compared with low disease activity. Our results strengthen the use of tight control strategies in daily clinical practice to achieve low stable disease activity or remission in patients with RA as soon as possible.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease that affects 0.5–1% of the population.[1] Patients with RA have an increased risk of cardiovascular disease (CVD).[2 3] Evidence suggests that the increased CVD risk is partly mediated by systemic inflammation, a characteristic for RA, in addition to traditional risk factors Inflammation may alter the effect of existing CVD risk factors or factors protective for CVD, leading to an increased risk of CVD.[4–6] Furthermore, inflammation may accelerate the atherosclerotic process[7 8] and lead to the formation of more severe coronary and carotid atherosclerotic plaques in patients with RA.[9–12] In comparison to healthy controls and patients with RA in remission, patients with active disease seem to have more unstable plaques, increasing the probability of CVD.[13] Consequently, the level of disease activity has been implicated as a contributing factor to the development of CVD. Conversely, clinical remission or the absence of inflammation may be associated with a reduced risk of CVD in RA. However, there is conflicting evidence concerning the association between the level of disease activity and CVD risk. The results from a case-control study showed no evidence that disease activity over time was associated with occurrence of myocardial infarction.[14] In another longitudinal study by our group, results indicated that very high disease activity over time or high disease activity at RA onset significantly contributes to the risk of CVD in RA.[15] In a recent study by Myasoedova *et al*, it was demonstrated that particularly exposure to disease activity flare-ups and increased cumulative burden of RA disease activity seems to contribute to this risk.[16] Furthermore, patients with active RA have significantly increased levels of biomarkers for CVD, while patients who were in remission did not.[17] Overall, these findings led to the hypothesis that achieving remission may reduce the risk of CVD in patients with RA. As a clinical consequence, tight control of disease activity could therefore have a beneficial effect on CVD risk.[18] It is unclear whether clinical remission needs to be achieved in order to eliminate or diminish the possible harmful effects of systemic inflammatory activity or if stable low disease activity over time is sufficient. Therefore, the primary objective of this study is to investigate the effect of different levels of disease activity over time, particularly low disease activity, on CVD risk in patients with RA. Second, the effect of remission over time on the risk of CVD is investigated.

PATIENTS AND METHODS

Study design and patients

The prospectively collected data from the Nijmegen early RA inception cohort were used. Patients were included at diagnosis of RA (baseline) in the outpatient clinic of the departments of rheumatology of the Radboud University Medical Centre (since 1985) or the Maartenskliniek in Nijmegen (since 1990). Patients with RA who fulfilled the 1987 American College of Rheumatology (ACR) (inclusion before 2010) or ACR/European League Against

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Rheumatism (EULAR) 2010 (inclusion after 2010) criteria for the classification of RA,[19] with disease duration of <1 year and who were disease modifying anti-rheumatic drug (DMARD) naïve, were included. All patients received written patient information and gave written informed consent. According to Dutch law and regulations, ethical review was not necessary for this observational study. Patients with confirmed CVD before inclusion and patients with a follow-up <12 months or patients with two or less DAS28 measurements were excluded for the current analyses. All disease activity measurements that were taken between the date of inclusion in the cohort and the date of a first CVD event or censoring were included in the analysis, with a maximum of 10 years of follow-up.

Data collection

The patients were seen during scheduled visits every 3–6 months. During these visits, disease activity was measured using the DAS28.[20 21] Baseline variables were retrieved from the cohort database: age (years), gender (male/female), rheumatoid factor (RF) positivity, anti-cyclic citrullinated peptide (anti-CCP) positivity, erythrocyte sedimentation rate (mm/hour) and C-reactive protein (CRP) (mg/L), Swollen Joint Count (SJC28), Tender Joint Count (TJC28) and the patient Visual Analogue Score for global disease activity (VAS), DAS28 and Health Assessment Questionnaire (HAQ). Data on traditional CVD risk factors at baseline were collected by review of medical charts and electronic patient files, including current smoking status (Y/N), blood pressure (mm Hg), height (m), weight (kg), diabetes mellitus (Y/N), hypertension (Y/N) and family history of CVD (Y/N). Non-fasting total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-c) concentrations (mmol/L) were measured using serum from frozen samples collected at baseline at laboratory facilities of Russells Hall Hospital, Dudley UK.[22]

Primary outcome

The primary outcome was occurrence of a first CVD event (physician diagnosed fatal or non-fatal cases of CVD), as retrieved from the database, by extensive review of medical charts and electronic patient files. The following were classified as CVD events: acute coronary syndrome, stable angina pectoris, cerebral vascular accident (CVA), transient ischaemic attack (TIA), peripheral artery disease and heart failure. Deaths due to CVD were verified from death certificates, provided by Statistics Netherlands,[23] including deaths due to CVD and CVA but excluding cerebral haemorrhage and non-coronary cardiac death.

Statistical analysis

Baseline variables were compared between the CVD event group and the non-event group by means of independent samples t-test, Wilcoxon or χ^2 statistics. The cut-off value for low disease activity was defined as DAS28 ≤3.2 and that for clinical remission was defined as DAS28 <2.6.[20 21 24] A Cox-proportional hazard regression model was chosen as the primary analysis. First, three analyses were performed with disease activity as the segmented time-dependent covariate and time to first CVD event (or disease duration) as the primary outcome. This type of analysis is suited to avoid bias introduced by analysing time course (non-baseline) variables in combination with survival time. Disease activity was added as a continuous variable and as a dichotomous variable: low disease activity (yes/no) or clinical remission (<yes/no). Disease activity was measured regularly, every 3–6 months, and a 6 month interval was maintained for the time-dependent covariate in the Cox-proportional hazard regression analyses. These time segments corresponded with DAS28 measurements at 6 month intervals. In case of one isolated missing measurement, the mean of the measurement prior and the measurement following the missing value was used. If more than one consecutive measurement was missing, subjects were censored. To repeat the analysis using a more readily interpretable reflection of disease activity, the next step was to analyse the time-averaged DAS28 as main dependent variable using conventional Cox-proportional hazard regression analysis. The time-averaged DAS28 was calculated by taking the area under the curve of the DAS28 score of the total follow-up period divided by the follow-up period. The analysis was performed again with the time-averaged DAS28 as a binary variable (time-averaged DAS28 ≤3.2 or 2.6).

In all analyses, sex and age were included in the model as confounders by default. The following potential confounders were considered; current smoking status, baseline measurements of systolic blood pressure (mm Hg), diastolic blood pressure (mm Hg) and body mass index (weight (kg)/height (m^2)), hypertension (physician diagnosis), diabetes mellitus (type 1 and 2), TC (mmol/L), HDL-c (mmol/L), family history of premature CVD, use of statins and use of anti-hypertensive medication (diuretics, ACE/angiotensin II inhibitors, beta-blockers or calcium blockers) at baseline, RF status, anti-CCP status, baseline DAS28, CRP and HAQ. Additionally, the effect of both remission and of low DAS28 over time on survival for CVD was assessed using Kaplan-Meier survival analysis. First, subgroups were made based on the time-averaged DAS28: remission (DAS28 <2.6), low (DAS28 2.6–3.2), intermediate (DAS28 3.2–5.1) and high (DAS28 >5.1). In the second analysis, patients were divided using to low time-averaged DAS28 (≤3.2) as the cut-off point. The survival distributions in both analyses were compared using log-rank testing. All analyses were performed using SPSS V.22.0.

RESULTS

There were 1157 patients included in the cohort. After exclusion of patients with a prior history of CVD, patients with a follow-up time <12 months or patients with two or less DAS28 measurements, 873 patients were included in the analyses. A total of 99 patients with RA developed a first CVD event during their first 10 years of follow-up: 44 (44%) cases of acute coronary syndrome (myocardial infarction or unstable angina pectoris), 18 (18%) cases of stable angina pectoris, 17 (17%) cases of CVA, 5 (5%) patients with a TIA, 10 (10%) cases of peripheral artery disease and 5 (5%) patients with heart failure. Out of all CVD events, 21% were fatal, mostly due to acute coronary syndrome (43%). Total follow-up time was 4560 patient years with a median (IQR) follow-up time of 5 (3–9) years. At baseline, there were differences between patients with and without CVD events (table 1). Patients with CVD events were on average older, and several other 'traditional' risk factors for CVD were raised including blood pressure, lipids and presence of diabetes. Patients who developed CVD were more frequently RF positive, not more frequently anti-CPP positive and had higher baseline disease activity (table 1). In total, 9151 DAS28 measurements were included during follow-up, and in 2738 (30%) of the visits, DAS28 was <2.6. Per patient, the percentage of their DAS28 measurements during follow-up that were scored <2.6 (time in remission) was in median (P25-P75) 17% (0.0–50%).

When disease activity was entered into the model as a continuous, segmented time-dependent variable, the results showed that disease activity had a significant effect on CVD risk after

Table 1 Patient characteristics at baseline

	Total cohort (n=873)	No CVD event (n=774)	CVD event (n=99)	p Value (CVD versus no CVD)
Age (years), mean±SD	54±14	53±14	62±9	<0.001
Sex (female), n (%)	574 (66)	524 (68)	50 (51)	0.001
Currently smoking, n (%)	272 (31)	235 (30)	69 (40)	0.156
BMI (weight(kg)/height (m²)), mean±SD	26±4	25±4	26±4	0.016
Systolic blood pressure (mm Hg), mean±SD	146±24	145±24	153±24	0.002
Diastolic blood pressure (mm Hg), mean±SD	84±12	83±12	86±11	0.026
Hypertension, n (%)	120 (14)	94 (12)	26 (26)	<0.001
Anti-hypertensives, n (%)	134 (15)	110 (14)	24 (24)	0.009
TC (mmol/L), mean±SD	5.2±1.2	5.2±1.2	5.3±1.4	0.448
HDL-c, mean±SD	1.3±0.3	1.3±0.3	1.2±0.3	0.040
TC:HDL-c ratio, mean±SD	4.1±1.0	4.1±1.0	4.4±1.0	0.013
LDL-c, mean±SD	3.2±1.1	3.1±1.0	3.2±1.2	0.357
Lipid lowering agents, n (%)	30 (3.4)	23(3)	7 (7)	0.035
Diabetes mellitus, n (%)	37 (4)	29 (4)	8 (8)	0.044
Family history of CVD, n (%)	265 (30)	232 (30)	33 (33)	0.494
Rheumatoid factor (positivity), n (%)	654 (75)	576 (74)	78 (79)	0.345
Anti-CCP (positivity), n (%)	554 (64)	493 (64)	61 (62)	0.686
DAS28, mean±SD	5.0±1.3	4.9±1.3	5.4±1.3	0.001
CRP, median (IQR)	14 (2-40)	13 (2-38)	21 (3-47)	0.083
HAQ, median (IQR)	0.6 (0.3–1.1)	0.6 (0.3–1.1)	0.7 (0.3–1.4)	0.468

Hypertension is defined as multiple measurements of elevated systolic blood pressure (>140 mm Hg) during multiple visits by a physician. Diabetes mellitus includes both type 1 and type 2. All variables represent baseline measures, except when otherwise stated.
anti-CCP, anti-cyclic citrullinated peptide; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; DAS28, 28-joint disease activity score; HAQ, Health Assessment Questionnaire; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TC, total cholesterol.

correction for confounders (table 2, panel B), indicating that CVD risk increases as DAS28 increases during follow-up. The HR, in table 2B, of 1.179 can be interpreted as an increase in risk of 18% if the DAS28 is one point higher. table 2, panel C, shows the results from the analysis with DAS28 ≤3.2 (yes/no) as a time-dependent variable after correction for confounders, indicating that CVD risk is significantly lower in patients with DAS28 ≤3.2 (HR 0.65, 95% CI 0.43 to 0.99).

The results from the Cox-proportional hazard analysis with remission (yes/no) as a time-dependent covariate showed a direction for a protective effect of time in remission against CVD (table 3, panel A and B) with an HR of 0.67. However, this effect did not reach statistical significance after correction for confounders (95% CI 0.43 to 1.07).

Mean±SD time-averaged DAS28 was 3.5±1.1 for the whole group, with a minimum and maximum time-averaged DAS28 of 0.7 and 7.3, respectively. The mean±SD time-averaged DAS28 was significantly lower in the non-event group compared with the event group (3.5±1.1 vs 3.9±1.2, respectively, with p<0.001). Results from a conventional Cox-proportional hazard regression analysis with time-averaged DAS28 as the main independent variable showed a significant effect on CVD with an HR of 1.60 (95% CI 1.28 to 1.99), as shown in table 4, panel B. After correction for confounders, the hazard of CVD notably increases with every point increase in time-averaged DAS28. The results from the following analysis (table 4, panel C) showed that, compared with patients with active disease, low time-averaged DAS28 (≤3.2) has a significant protective effect against CVD after correction for confounders (HR 0.53, 95% CI 0.34 to 0.84). Again a direction for a protective effect of time in remission against CVD was observed (not shown) with an HR of 0.78. However, this effect did not reach statistical significance after correction for confounders (95% CI 0.45 to 1.38). These results are in accordance with the results of the first set of analyses that included a time-dependent covariate.

For illustrative purposes, a Kaplan-Meier survival analysis was performed, investigating the effect of low disease activity and remission on time to first CVD event. Patients were divided into four groups; time-averaged DAS28 <2.6, 2.6–3.2, 3.2–5.1 and >5.1 for groups 1 through 4, respectively. Event rates were as follows: group 1 (n=189), 16 CVD events (8.5%); group 2 (n=163), 14 CVD events (8.6%); group 3 (n=444), 55 CVD events (12.4%) and group 4 (n=77), 14 CVD events (18%). Survival time (time to first CVD event) appears to decrease as time-averaged DAS28 increases (figure 1). Survival distributions differed significantly (p<0.027). Patients with the lowest survival rate (group 4) had the highest baseline DAS28 at diagnosis (mean±SD; 6.1±1.0) with 22% of patients diagnosed after the year 2000. The baseline DAS28 in groups 1, 2 and 3 was mean±SD: 4.1±1.3, 4.8±1.3 and 5.3±1.2, respectively. Of note, the survival distributions of patients with a time-averaged DAS28 <2.6 and a time-averaged DAS28 between 2.6 and 3.2 overlap indicating that these distributions do not differ significantly from each other. Figure 2 shows the survival distributions of patients with a time-averaged DAS28 ≤3.2 or very low disease activity over time and of patients with more active disease (time-averaged DAS28 >3.2). Survival distributions (figure 2) differed significantly (p=0.024).

DISCUSSION

Systemic inflammatory activity in RA has been suggested as an important contributing factor to the excess CVD risk in patients with RA. Therefore, it was hypothesised that achieving a state in which disease activity is low or nearly absent could have a beneficial effect on CVD risk. In this study, it is shown that low stable disease activity over time has a significant protective effect against CVD in RA. Although clinical remission (DAS28 <2.6) appears to have a protective effect against developing CVD, it did not reach statistical significance.

Table 2 Cox-proportional hazard regression analysis with time to first CVD event as the primary outcome and time-dependent DAS28 as the primary independent variable, before (panel A) and after (panel B) correction for confounders. Panel C shows results from the Cox-proportional hazards regression with DAS28 <3.2 (yes/no) as a time-dependent covariate after correction for confounders

				95% CI for Exp (B)	
	Beta	p Value	HR	Lower	Upper
Panel A: Crude model					
Time-dependent covariate (DAS28)	0.113	0.119	1.120	0.972	1.290
Age	0.064	<0.001	2.010	1.344	3.005
Gender	0.698	0.001	1.066	1.047	1.085
Panel B: Corrected model					
Time-dependent covariate (DAS28)	0.165	0.032	1.179	1.014	1.370
Age	0.062	<0.001	1.064	1.044	1.084
Gender	0.725	0.001	2.065	1.365	3.123
Hypertension baseline	1.036	<0.001	2.818	1.673	4.745
HDL-c	−0.736	0.043	0.466	0.222	0.977
CVD medication*	−0.515	0.026	0.597	0.379	0.940
DAS28 baseline	0.034	0.687	1.035	0.877	1.220
Panel C: Corrected model					
Time-dependent covariate (DAS28 <3.2)	−0.431	0.044	0.650	0.427	0.989
Age	0.064	<0.001	1.066	1.046	1.087
Gender	0.736	0.001	2.088	1.372	3.177
Hypertension	0.977	<0.001	2.656	1.547	4.559
HDL-c	−1.113	0.009	0.329	0.142	0.758
LDL-c	0.177	0.097	1.193	0.969	1.470
CVD medication*	−0.541	0.022	0.582	0.366	0.925
CRP	−0.001	0.538	0.999	0.994	1.003

*Anti-hypertensive medication, lipid lowering medication.
CRP, C-reactive protein; DAS28, 28-joint disease activity score; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.

Table 3 Cox-proportional hazard regression analysis with time to first CVD event as the primary outcome and remission as the time-dependent variable, before (panel A) and after (panel B) correction for confounders

				95% CI for Exp (B)	
	Beta	p Value	HR	Lower	Upper
Panel A: Crude model					
Time-dependent covariate (remission)	−0.211	0.358	0.810	0.516	1.270
Age	0.064	<0.001	1.066	1.047	1.086
Gender	0.665	0.001	1.945	1.304	2.901
Panel B: Corrected model					
Time-dependent covariate (remission)	−0.395	0.096	0.673	0.426	1.066
Age	0.063	<0.001	1.065	1.045	1.085
Gender	0.680	0.001	1.974	1.306	2.984
Hypertension	0.971	<0.001	2.640	1.540	4.528
HDL-c	−0.772	0.039	0.462	0.222	0.963
CVD medication*	−0.526	0.026	0.591	0.372	0.938

*Anti-hypertensive medication, lipid lowering medication.
CVD, cardiovascular disease; HDL-c, high-density lipoprotein cholesterol.

Previous studies demonstrated that inflammation contributes to accelerated atherosclerosis,[25–27] a cause of non-bleeding CVD. This study shows that active disease in RA is associated with an increased risk CVD. When looking at the overall trend of disease activity during follow-up, those patients who were able to achieve and maintain low disease activity over time appear to have a significantly lower risk of CVD than patients with more active disease. Interestingly, achieving remission did not offer any significant added value over sustained low disease activity, in terms of CVD risk reduction. Time to first CVD event was similar in patients with low disease activity and patients in remission and these patients had a significantly higher survival time compared with patients with more active disease. The results also showed that patients with the lowest survival times for CVD had the highest disease activity levels at baseline.

Several other studies have reported similar results with regard to increased disease activity in RA.[15 16 28 29] In another study, DAS28 did not appear to be significantly increased in patients with RA and myocardial infarction compared with RA controls.[29] However, only the first 6 months of follow-up after inclusion were incorporated for disease activity in this study. By contrast, Myasoedova *et al* have shown that particularly bouts of uncontrolled high disease activity are associated with a higher risk of CVD.[16] In another study that also included longitudinal disease activity, reduced time-averaged disease activity in RA was associated with fewer CVD events.[28] What our current study adds to that is notably longer follow-up with detailed data on determinants of CVD risk. Also, in this study, a Cox-proportional hazards regression with time-dependent covariates was used, as conventional Cox regression is potentially biassed.[30] Patients with RA in remission, defined as Clinical Disease Activity Index, or CDAI≤2.8, were found to have significantly lower levels of CVD risk markers compared with patients with active disease, supporting remission as a target for CVD risk management in RA.[17] Overall, patients who are able to achieve and maintain remission or low disease activity during follow-up, even sporadically, may be less likely to develop bouts of uncontrolled, sustained high systemic inflammation,

Table 4 Conventional Cox-proportional hazard regression analysis with time to first CVD event as the primary outcome and time-averaged DAS28 as the primary independent variable, before (panel A) and after (panel B) correction for confounders

				95% CI for Exp (B)	
	Beta	p Value	HR	Lower	Upper
Panel A: Crude model					
Time-averaged DAS28	0.383	<0.001	1.466	1.204	1.786
Age	0.060	<0.001	1.062	1.043	1.082
Gender	0.848	<0.001	2.336	1.549	3.521
Panel B: Full model					
Time-averaged DAS28	0.468	<0.000	1.597	1.279	1.994
Age	0.056	<0.001	1.057	1.037	1.077
Gender	0.954	<0.001	2.595	1.712	3.933
Hypertension baseline	0.920	<0.001	2.508	1.566	4.018
DAS28 baseline	−0.048	0.587	0.953	0.802	1.133
Panel D: Full model					
Time-averaged DAS28 binary; (≤3.2)	−0.630	0.007	0.533	0.337	0.843
Age	0.058	<0.001	1.060	1.040	1.080
Gender	0.803	<0.001	2.231	1.491	3.339
Hypertension baseline	0.882	<0.001	2.417	1.506	3.879
DAS28 baseline	0.042	0.614	1.043	0.886	1.228

DAS28, 28-joint disease activity score.

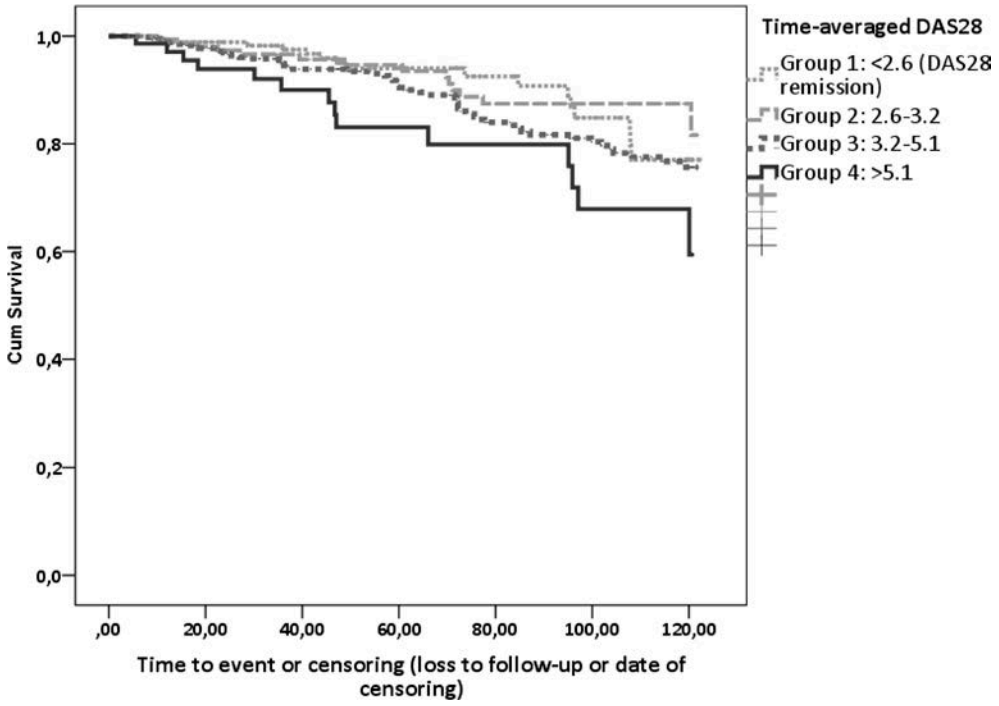

Figure 1 Survival distribution (time to first CVD event) for categories of time-averaged DAS28. Survival distributions differ significantly (p<0.027). Cumulative survival of CVD is depicted on the y-axis and time to a CVD event or censoring is depicted on the x-axis. CVD, cardiovascular disease; DAS28, 28-joint disease activity score.

a contributing factor to atherosclerosis and CVD. Patients with RA with very active disease at diagnosis, poor treatment response with more frequent flare-ups as a result, may form a subgroup within the RA population that is particularly at risk for developing CVD, significantly contributing to the excess CVD risk in this population.

Disease activity tends to fluctuate over the course for RA, which makes it difficult to accurately capture the level of exposure of a patient during follow-up. Also, as noted above, there may be a risk of bias as the patients who are able to stay event free the longest also have inherently more time to achieve remission or low disease activity, creating a

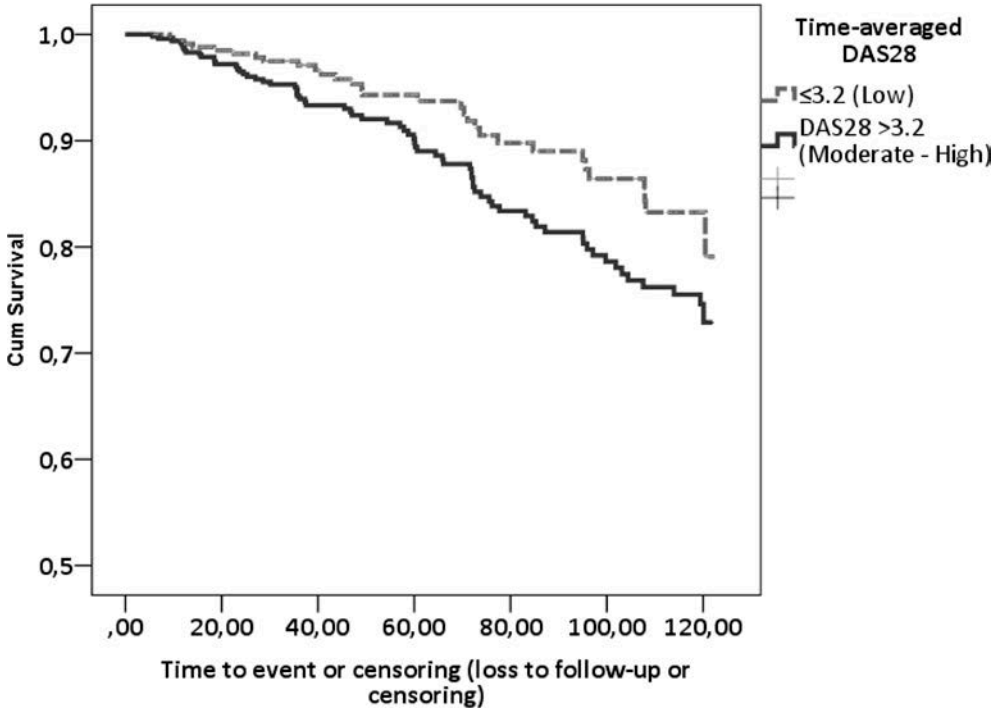

Figure 2 Survival distribution (time to first CVD event) for low (≤3.2) and moderate to high (>3.2) time-averaged DAS28. Survival distributions differ significantly (p<0.024). Cumulative survival of CVD is depicted on the y-axis and time to a CVD event or censoring is depicted on the x-axis. CVD, cardiovascular disease; DAS28, 28-joint disease activity score.

survivor bias or 'immortal time bias'. Immortal time bias can be avoided by integrating the changes in exposure status in the analysis.[30] Consequently, for this study, a Cox-proportional hazards regression with a segmented time-dependent covariate (DAS28) was chosen. For remission, there are a variety of definitions,[20 31–34] which were not all considered in this study. These definitions do appear to strongly correlate with each other;[32 33] however, including a different definition for remission could have an effect on results. DAS28 remission that was used in this study is defined as disease activity score <2.6 and this is not the same as the absolute absence of disease activity. On the other hand, remission according to the stricter ACR/EULAR remission criteria for RA is not prevalent, yet. Additional research in a larger cohort is needed to determine if clinical remission or the absence of inflammation has a significant added protective effect on CV risk compared with very low disease activity. Considering that the effect of low disease activity was found to be significant, it is likely that this effect is augmented for patients in remission. In a larger sample of patients, this effect may reach statistical significance. Finally, DMARDs were not included in study as it was hypothesised that DMARDs affect CVD risk through their effect on disease activity, the main independent variable in our analyses. Non-steroidal anti-inflammatory drugs may augment the risk of CVD. However, as they are often used intermittently, for short periods of time, while usage differs greatly between patients, accurately capturing the exposure is challenging. Determining their effect on CVD in RA may therefore be difficult. Also, not all NSAIDs may exert the same negative effect on the risk of CVD, complicating interpretation of results.[35]

In conclusion, this study shows that low DAS28 has a significant protective effect against the 10 year risk of CVD. Achieving sustained remission, here defined as DAS28 <2.6, is regarded as the ultimate treatment target and does not seem to provide a large advantage over low disease activity over time in terms further reducing CVD risk. Patients with RA with uncontrolled high disease activity appears to have the highest risk of developing CVD. Our results strengthen the use of tight control (treat-to-target) strategies in daily clinical practice to achieve low disease activity or remission in these patients, also with the aim to reduce CVD risk.

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Contributors All authors have given substantial contribution to the conception and design and/or analysis and interpretation of the data, have drafted and/or revised the manuscript critically for important intellectual content and have given final approval of the version to be submitted for publication. EEA had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the analysis.

Competing interests None delared.

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EXTENDED REPORT

A population-based cohort study of rheumatoid arthritis-associated interstitial lung disease: comorbidity and mortality

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ABSTRACT

Objectives To compare mortality risks in patients with rheumatoid arthritis-associated interstitial lung disease (RA-ILD) and patients with RA without ILD.

Design Matched cohort study.

Setting The study was conducted in Denmark, using nationwide, prospectively collected data.

Participants Among patients with RA diagnosed between 2004 and 2016, 679 patients with RA-ILD were matched for birth year, gender and age at RA diagnosis with 11 722 patients with RA but without ILD.

Main outcome measures Mortality risks were assessed using Kaplan-Meier mortality curves, and hazard rate ratios (HRRs) for death were estimated using Cox proportional hazards regression models.

Results The number of prevalent RA patients more than doubled from 15 352 to 35 362 individuals during the study period. RA-ILD was seen in 2.2% of incident RA patients. 34.0% of RA-ILD cases were diagnosed within 1 year prior to and 1 year after the RA diagnosis. One-year mortality was 13.9% (95% CI, 11.4% to 16.7%) in RA-ILD and 3.8% (95% CI, 3.5% to 4.2%) in non-ILD RA, 5-year mortality was 39.0% (34.4% to 43.5%) and 18.2% (17.3% to 19.1%) and 10-year mortality was 60.1% (52.9% to 66.5%) and 34.5% (32.8% to 36.1%), respectively. The HRRs for death were 2 to 10 times increased for RA-ILD compared with non-ILD RA, irrespective of follow-up period. Stratified analysis showed that the HRR for death was highest in the first months after the diagnosis of RA-ILD was made, especially in patients diagnosed with RA before diagnosis of ILD. HRR was higher in males and in patients without comorbidity as assessed by the Charlson Comorbidity Index.

Conclusions ILD is a serious complication in RA, with a significantly increased mortality compared with a large matched cohort of RA comparisons without ILD.

INTRODUCTION

The mortality of patients with rheumatoid arthritis (RA) has declined over the past decade, and the decline appears greater than that in the general population.[1–3] New treatment strategies for RA with early and effective intervention leading to reduced disease activity[4] are the likely cause for this improvement. However, mortality is still increased in RA,[1] and cardiovascular disease and interstitial lung disease (ILD) are the primary contributors to premature deaths.[5 6]

Subclinical interstitial lung abnormalities (ILAs) may be detected in 30%–50% of patients with RA,[7 8] but the individual risk of progression to clinically significant ILD is not known. However, ILAs are associated with increased mortality.[9] Clinically significant ILDs are seen in 5%–10% of patients with RA.[10–13] Reported estimates vary considerably due to differences in disease definition and diagnostic methods. Known risk factors for RA-ILD are tobacco smoking, male gender and high anti-citrullinated protein antibody (ACPA) levels.[10] In consecutive RA patients examined with high-resolution CT (HRCT), the prevalence of RA-ILD was 19%.[14] One-third of these patients showed significant progression during a 2-year period, and the strongest predictor of progression was pre-existing pulmonary function impairment.[15] The presence of usual interstitial pneumonia on HRCT has been associated with worse outcome than non-specific interstitial pneumonia.[16–19] However, a recent study[20] showed that pulmonary function, but not baseline HRCT pattern, independently predicted mortality after controlling for influential variables.

The reported median survival in previous RA-ILD studies ranges from 3 to 10 years.[11 12 20]

We designed the present study as an attempt to overcome the limitations of small RA-ILD cohorts, referral bias and changing management strategies over long study periods. We hypothesised that mortality in RA-ILD was increased compared with RA without ILD in a large population-based cohort of recent RA patients treated in accordance with current recommendations. The aim was to compare mortality among patients with RA-ILD with that in patients with RA without ILD and to investigate the impact of gender, age, seropositive RA and comorbidity on the risk of death in a matched cohort design.

METHODS

Setting

We conducted the study in Denmark, in a population of 5.6 million persons at risk (2016), using prospectively collected data from population-based medical databases.

Data sources

The Danish National Patient Registry (DNPR)[21] contains information about central person registry (CPR) number, dates of hospital admissions and discharges, diagnostic and surgical procedure codes

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and discharge diagnoses in all somatic hospitals in Denmark since 1977, and additionally, all outpatient hospital contacts and emergency department contacts since 1995. The International Classification of Diseases eighth edition (ICD-8) was used for discharge diagnoses coding until the end of 1993 and the ICD-10 codes thereafter. The DNPR was used as the source of information about all outpatient contacts and hospital admissions. Since the information is part of the reimbursement system for the hospitals, a high level of completeness is ensured.

The Danish Civil Registration System (CRS)[22] contains information about date of birth, place of residence, vital status and migration into or out of Denmark since 1968. The CPR number assigned to all Danish residents is a unique 10-digit personal identifier, which goes through all Danish registries including the CRS, enabling individual level linkage across all different registries. The system ensures complete follow- up with respect to mortality.

The Danish National Database of Reimbursed Prescriptions[23] includes data on all reimbursed prescriptions redeemed at Danish community pharmacies since 2004. The database contains no data on drugs dispensed during inpatient hospital stays and drugs dispensed directly to patients at hospital-based outpatient clinics.

Medication use for RA or RA-ILD were defined as reimbursement of at least two prescriptions for the same drug within 3 months before and 12 months after the index date. Data from 2004 until December 2015 were available.

The study was approved by the Danish Data Protection Agency (record number 1-16-02-277-16) and the Danish Health Data Authority.

Identification of patients with RA

We used the DNPR to identify all patients with a first-time diagnosis of RA as primary or secondary diagnosis during hospital admission or as outpatient contacts between 1 January 2004 and 1 July 2016. We used the ICD-10 codes M05 (seropositive RA) and M06 (other RA) for identification of RA. American College of Rheumatology (ACR) 1987 criteria[24] were used for the diagnosis of RA until 2009 and the ACR/European League Against Rheumatism (EULAR) criteria from 2010.[25] To ensure that only incident RA cases diagnosed after 2004 were included, we excluded all RA cases identified during the period 1977–2003 using ICD-10 codes from 1994 onward and ICD-8 codes before 1994 (712x RA and allied conditions).

Identification of RA patients with ILD (index cohort)

Information on primary and secondary ILD diagnoses was also obtained from the DNPR using all available hospital admission and outpatient contact information. ILD diagnoses prior to, synchronous with or after RA diagnosis were included. The ICD-10 codes used for the identification of ILD were J84 (other interstitial pulmonary diseases) and M05.1c (RA with pulmonary fibrosis). Screening for pulmonary involvement is not part of the follow-up programme for RA in Denmark, and HRCT scans are not performed systematically in asymptomatic patients. The index date was defined as the date when the patient had two diagnoses, RA and ILD, regardless of which one was assigned first.

We randomly selected matched comparisons with the same gender, year of birth and first time diagnosis of RA assigned ±1 year of the date of the RA diagnosis of the RA-ILD patients. The identification of the matched comparison cohort is described in detail in the online supplementary material.

MORTALITY

We obtained information on death from all causes from the CRS.[22]

COVARIATES

To obtain a complete hospitalisation history for the identified RA-ILD patients and matched RA comparisons without ILD, we used all inpatient and outpatient diagnoses recorded in the DNPR 5 years before the index date.

To measure comorbidity, we computed a Charlson Comorbidity Index (CCI) score[26] for each patient using the ICD-10 diagnostic codes listed in the online supplementary table 2s. RA and ILD diagnoses were not included in the CCI. Based on the score, we defined three comorbidity categories: 0 (no comorbidity, applied to patients with no previous record of conditions included in the CCI), 1 to 2 (moderate) and 3+ (severe).

Assessment of seropositivity was based on ICD-10 codes of either seropositive or seronegative/other RA and not on results of serological testing, which was not available from the national registries.

Data analysis

We described the cohort of RA-ILD patients and matched RA comparisons without ILD according to age, gender, CCI score, presence of ischaemic heart disease, cardiac failure, and diabetes, tabulating the number and proportion of patients overall. Cumulative mortality for the two groups were compared using Kaplan-Meier mortality curves. All individuals were followed from the index date until death, emigration or 1 July 2016, whichever came first. We used Cox proportional hazards regression to estimate hazard rate ratios (HRRs) for death and corresponding 95% CIs. Taking the matching into account, we computed crude HRRs and HRRs adjusted for CCI score and RA seropositivity. We computed the mortality risk and HRR during the different follow-up periods from the index date: 0 to 30 days, 31 day to 6 months, >6 months to 1 year, >1 year to 5 years and >5 years to 10 years. We reported the number of patient who were at risk of death at the start of each follow-up period and the number of patients who died during the same follow-up period. The assumption of the proportional hazards was assessed graphically.

In order to study potential differences in the association between ILD and mortality in subgroups of patients, we stratified all analyses on gender, age groups and comorbidity for different follow-up periods calculating both crude HRRs and HRRs adjusted for comorbidity and seropositive RA.

RESULTS

During the period 2004–2015, the incidence of RA remained stable, but a remarkable increase in the prevalence of RA was seen from 15 000 in 2004 to 35 000 individuals in 2016 (figure 1). A total of 31 333 incident RA patients diagnosed between 2004 and 2016 were included in the study. RA-ILD was seen in 679 (2.2%) of the incident RA patients. The distribution of gender, age and seropositivity of the entire RA cohort is shown in table 1. RA-ILD patients were older, more likely to be male and the frequency of seropositivity was higher.

The cohort of 679 RA-ILD patients was matched with a cohort of 11,722 RA patients without ILD. The demographics of the RA-ILD patients and the matched cohort of RA patients without ILD are presented in table 2. The distribution of the ICD-10 codes is listed in the online supplementary table 1s.

Fourteen per cent of ILD cases were diagnosed 1–5 years before RA. Thirty-four per cent were diagnosed within 1 year

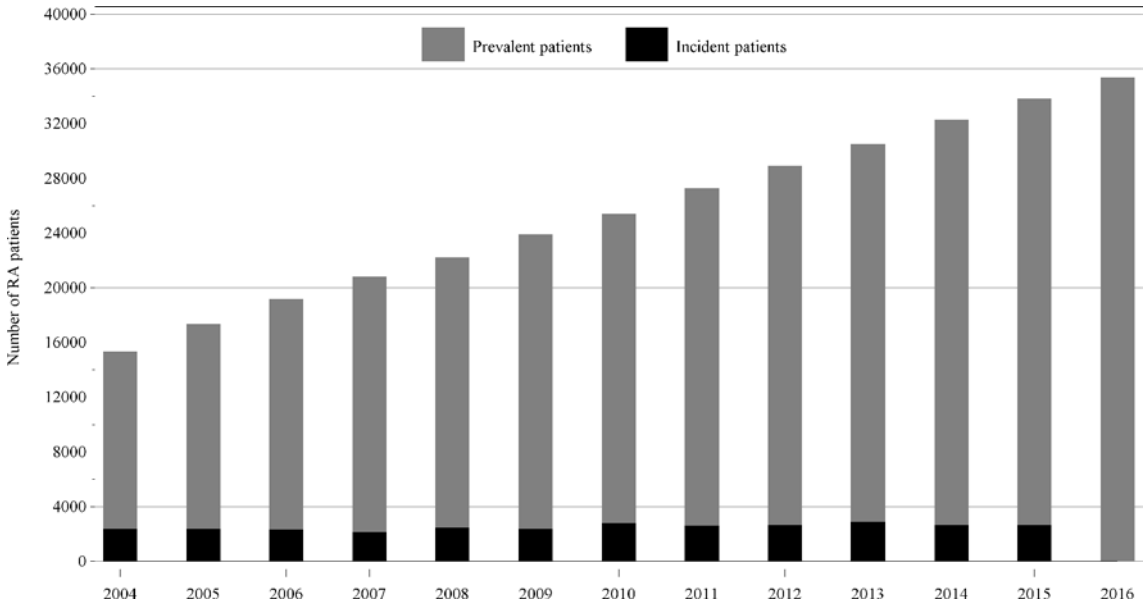

Figure 1 Incidence and prevalence of RA in Denmark from 2004 to 2016. RA, rheumatoid arthritis.

prior to the RA diagnosis, synchronous with the RA diagnosis, or within the first year after the RA diagnosis. Twenty-eight per cent were diagnosed 1 to 5 years after the RA diagnosis (see online supplementary table 3s).

The burden of comorbidity assessed by the Charlson Comorbidity Index was higher in the RA-ILD group (CCI ≥1 in 59.6% of RA-ILD patients and 38.0% of RA comparisons without ILD).

Individual comorbidity diagnoses of ischaemic heart disease, congestive heart failure and diabetes were also more frequent in the RA-ILD group, the difference being more pronounced for congestive heart failure (8.5% in the RA-ILD group and 4.4% in the non-ILD RA group). Table 3 shows the medication used for RA and RA-ILD based on reimbursed prescriptions in the first year after the RA diagnosis had been assigned. No data on hospital-dispensed medication were available.

Cumulative mortality in RA-ILD patients and matched comparisons without ILD is shown in figure 2. One-year mortality was 13.9% (95% CI, 11.4% to 16.7%) in RA-ILD patients and 3.8% (95% CI 3.5% to 4.2%) in matched comparisons, 5-year mortality was 39.0% (34.4% to 43.5%) and 18.2% (17.3% to 19.1%) and 10-year mortality was 60.1% (52.9% to 66.5%) and 34.5% (32.8% to 36.1%), respectively.

Median survival was 6.6 years in RA-ILD (95% CI 5.6 to 8.6 years). The median of survival was not reached in the matched RA cohort. The HRRs for death were increased for RA-ILD patients for all time periods during follow-up (table 4). Stratified analysis showed that HRR for death was higher in patients who were diagnosed with RA prior to ILD (see online supplementary table 4s).

The HRR for death within the first 30 days after the index date was 10.4 (95% CI 5.9% to 18.2%) in RA ILD patients (26 deaths in 679 patients) compared with matched comparisons (41 deaths in 11 722 patients). In stratified analyses, HRR for early death was even higher in males (HR 14.5, 95% CI (6.2% to 34.2%)) and higher in the age group 65 to 74 years. Seropositivity was not associated with differences in survival when compared with seronegativity/other RA. Assessment of the impact of the Charlson Comorbidity Index on risk of death in days 0–30 revealed that the HRR was 16.4 for CCI=0 and 10.0 for CCI=1–2, compared with 3.0 in the high comorbidity group (CCI=3+) (see online supplementary table 5s).

Table 1 Characteristics of RA patients with and without ILD

	All patients		Never ILD		Ever ILD	
Incident RA cohort	**n**	**%**	**n**	**%**	**n**	**%**
All	31 333	100.0	30 654	100.0	679	100.0
Female	21 775	69.5	21 403	69.8	372	54.8
Male	9558	30.5	9251	30.2	307	45.2
Age at RA diagnosis						
≤64 years	18 647	59.5	18 375	59.9	272	40.1
65–74 years	7060	22.5	6831	22.3	229	33.7
≥75 years	5626	18.0	5448	17.8	178	26.2
Mean age at RA diagnosis (SD)	59.3 (16.6)		59.1 (16.7)		66.5 (12.2)	
Median age at RA diagnosis	60.8		60.5		67.9	
Seropositive						
No	17 091	54.5	16 790	54.8	301	44.3
Yes	14 242	45.5	13 864	45.2	378	55.7

ILD, interstitial lung disease; RA, rheumatoid arthritis.

Table 2 Characteristics of RA-ILD patients and matched comparisons

Patient characteristics	Matched RA comparisons		RA patients with ILD	
Mean age at RA diagnosis (years)	66.2		66.5	
Median age at RA diagnosis (years)	67.6		67.9	
Mean age at index date* (years)	68.1		68.5	
Median age at index date* (years)	70.4		70.7	
	n	%	n	%
n	11 722	100.0	679	100.0
Age at index date*				
≤64 years	4233	36.1	242	35.6
65–74 years	3790	32.3	209	30.8
≥75 years	3699	31.6	228	33.6
Gender				
Female	6593	56.2	372	54.8
Male	5129	43.8	307	45.2
Seropositive (M05)				
No	6444	55.0	301	44.3
Yes	5278	45.0	378	55.7
Charlson Comorbidity Index				
Low (0)	7268	62.0	274	40.4
Medium (1-2)	3337	28.5	290	42.7
High (3+)	1117	9.5	115	16.9
Ischaemic heart disease				
No	10 528	89.8	591	87.0
Yes	1194	10.2	88	13.0
Congestive heart failure				
No	11 209	95.6	621	91.5
Yes	513	4.4	58	8.5
Diabetes				
No	10 858	92.6	612	90.1
Yes	864	7.4	67	9.9

*Index date: the date when both the RA and ILD diagnosis have been assigned for RA patients with ILD and the matching date for the matched RA cohort.
ILD, interstitial lung disease; RA, rheumatoid arthritis.

Table 3 Treatment based on at least two reimbursed prescriptions within 3 months before and 1 year after the index date

Patient characteristics	Matched RA comparisons		RA patients with ILD	
	n	%	n	%
n	9550	100.0	554	100.0
Corticosteroid				
No	7147	74.8	260	46.9
Yes	2403	25.2	294	53.1
Methotrexate oral*				
No	5976	62.6	421	76.0
Yes	3574	37.4	133	24.0
Salazopyrin				
No	8170	85.5	458	82.7
Yes	1380	14.5	96	17.3
Azathioprin				
No	9458	99.0	513	92.6
Yes	92	1.0	41	7.4
Hydroxychloroquin				
No	9002	94.3	508	91.7
Yes	548	5.7	46	8.3

Index date: the date when both the RA and ILD diagnosis have been assigned for RA patients with ILD and the matching date for the matched RA cohort.
*Information about hospital dispensed therapies; for example, subcutaneous methotrexate and biological therapies for RA are not available.
ILD, interstitial lung disease; RA, rheumatoid arthritis.

DISCUSSION

In this population-based cohort study from 2004 to 2016, the all-cause mortality among patients with RA-ILD was significantly higher than in a comparison cohort of RA patients without ILD matched for birth year, gender and age at RA diagnosis. The prevalence of RA increased during the study period, although the incidence remained stable. The main explanation to the increasing prevalence is the marked increase in survival among RA patients over the last decade.[1] This observation is in agreement with other recently published findings.[27 28] The 2010 change in diagnostic criteria for RA may have identified more patients with early RA.[25 29] However, the change did not affect the incidence of RA based on the DNPR data, and it is unlikely to have contributed to the increase in RA prevalence.

It is well established that smoking increases the risk of RA.[30] The prevalence of smokers in the Danish population has been stable since 2009 after decades of decrease.[31] If this smoking pattern can be generalised to the RA population, it may have contributed to the stability in the incidence of RA. It is not yet clear whether the systematic effort aimed at identifying and treating risk factors has prevented deaths from ischaemic heart disease in the RA population. The strategy used by rheumatologists in Denmark follows the EULAR recommendations.[32] Furthermore, an ongoing study compares the effect of a targeted, intensified, multifactorial intervention with that of conventional treatment of modifiable risk factors for cardiovascular disease in patients with early RA.[33]

The high risk of death within 30 days of diagnosis, particularly among patients who had been diagnosed with ILD after or at RA diagnosis, may be due to acute exacerbations in previously undiagnosed ILD. Previous cohort studies have shown that acute exacerbation as the first manifestation of ILD is common.[34 35] It is a serious complication in RA-ILD as well as in idiopathic ILD and has a very high mortality.[36–38] Diagnostic delay and severe disease at the time of ILD diagnosis has been described for other ILDs,[39 40] and may also contribute to the high initial mortality.

The difference in risk of death between RA-ILD and matched comparisons was especially high at short-term follow-up for RA-ILD patients with low CCI. RA-ILD patients with higher CCI and longer follow-up still have significant excess mortality compared with non-ILD RA patients, but the 'mortality gap' diminishes because of increasing mortality among the comparisons.

Stratified analysis based on the initial diagnosis of seropositive RA (M05), or other/seronegative RA (M06.0 and M06.9) showed no difference in mortality between the two groups. The frequency of seropositive RA may be under-reported in registry data. In RCT cohorts from Denmark,[41 42] the percentage of seropositivity equals the findings in other countries, with approximately 60% seropositive RA. A possible explanation to this difference may be the fact that some patients are assigned the diagnosis of RA at the first contact to the public health service when serological data may not yet be available. Seroconversion during the cause of follow-up may also occur.

In Denmark, RA is diagnosed by specialists in rheumatology, and thus, the validity of the registry data is likely to be high. This is supported by previous findings of a high positive predictive value of DNPR diagnoses for conditions included in the CCI.[43] In the same study, the positive predictive value of DNPR diagnoses of connective tissue disease, including RA, was 98%. A recent

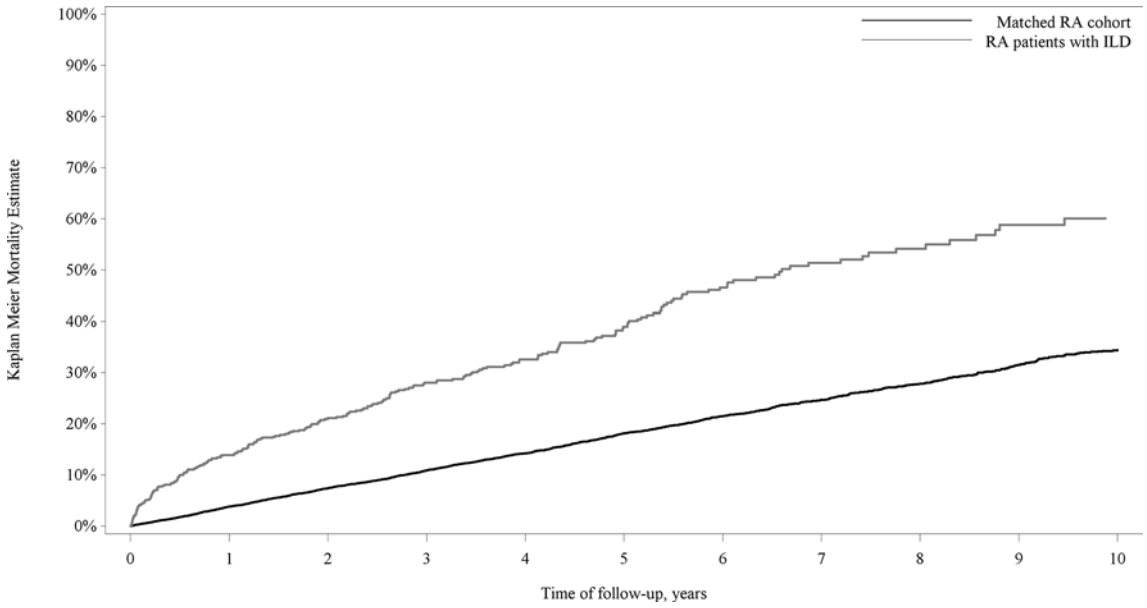

Figure 2 Kaplan-Meier survival curves for RA with and without ILD. ILD, interstitial lung disease; RA, rheumatoid arthritis.

study of the Swedish National Patient Registry demonstrated a validity of the registry-based RA diagnoses of 90% for incident as well as prevalent RA.[44] The majority of the remaining patients had other inflammatory rheumatic diseases. In the Nordic countries, hospital-based rheumatologists treat the majority of RA patients, and many similarities exist between healthcare systems. Therefore, the Swedish findings strongly support the validity of the RA diagnoses in the DNPR.

Future large studies are needed that include specific levels of IgM rheumatoid factor and anticyclic citrullinated peptide in the assessment of outcome in RA-ILD.

The frequency of ischaemic heart disease was only slightly higher in the RA-ILD group, and it is unlikely that this difference would account for the increased mortality. Other CCI groups, including cancer and lymphomas, showed an equal distribution between cases and comparisons. The main differences were seen in chronic pulmonary diseases, congestive heart failure, and connective tissue diseases. A previous study of patients with pulmonary fibrosis reported that almost half of the participants had been given an incorrect initial diagnosis of respiratory disease other than ILD.[45] This may contribute to the differences seen in the frequency of chronic respiratory diseases.

Strengths and limitations

The strength of the present study is the large, population-based RA cohort with complete follow-up of all patients and the match of 95% of patients with RA-ILD with at least 10 comparisons. Because of the unbalance between the RA-ILD and RA non-ILD groups, we used a matched cohort design in order to cope better with confounding. We achieved a good balance between the RA-ILD group and the comparison group. Furthermore, the matched design provided exact start of follow-up for RA patients without ILD.

The limitations are the lack of information about radiological ILD pattern, lung function impairment, smoking history, level of autoantibodies, RA activity and full therapy history, which are not available from the medical registries. No validation studies of registry ILD diagnoses exist. Rare diagnoses like ILD are likely to have a high positive predictive value, but they are also likely to be under-reported or misclassified as other, more common respiratory diseases. We used broad diagnostic codes for ILD based on our experience that J848 (other ILD) and J849 (unspecified ILD) are often used for RA-ILD. We also included J841 (ILD with fibrosis) to ensure the inclusion of patients misclassified with a diagnosis of idiopathic ILD, for example, idiopathic pulmonary fibrosis. RA-ILD is likely to be underdiagnosed, and thus, our study may overestimate the true mortality, but this should only encourage the identification of symptomatic patients and earlier diagnosis of RA-ILD.

In conclusion, our study showed a significantly increased risk of death in patients with RA-ILD compared with matched RA comparisons without ILD, especially in the first month after the final diagnosis of RA and ILD was made. Mortality remained significantly increased throughout the course of the disease.

Table 4 HRR for risk of death among RA patients with ILD compared with matched RA cohort

Time of follow-up	Matched RA comparisons, n deaths	Matched RA comparisons, n at risk	RA patients with ILD, n deaths	RA patients with ILD, n at risk	Crude HRR (95% CI)	Adjusted HRR (95% CI)*
0 to 30 days	41	11 722	26	679	10.0 (6.0 to 16.5)	10.4 (5.9 to 18.2)
>30 days to 6 months	162	11 577	40	646	4.1 (2.9 to 5.9)	4.0 (2.8 to 5.8)
>6 months to 1 year	214	10 831	24	572	2.1 (1.4 to 3.3)	1.9 (1.2 to 3.0)
>1 year to 5 years	1055	9707	107	500	2.3 (1.9 to 2.8)	2.0 (1.7 to 2.5)
>5 to 10 years	437	3944	38	170	2.9 (2.0 to 4.1)	2.7 (1.9 to 3.9)

*Adjustment made for seropositivity and Charlson Comorbidity Index.
HRR, hazard rate ratio; ILD, interstitial lung disease; RA, rheumatoid arthritis.

GENERALISABILITY AND CLINICAL IMPLICATIONS

The generalisability of the findings is likely to be very high, since the study is population based and uses high-quality health registry data with complete follow-up of all patients. It is the largest cohort described to date and represents recently diagnosed RA patients managed in accordance with currently recommended treatment strategies. Furthermore, the Danish welfare system provides the same high level of healthcare to the whole population. The results can be generalised to other populations with similar genetic backgrounds, environmental exposures and lifestyles.

The findings of this study, as well as the absence of RCT-based evidence to guide therapy in patients with RA-ILD,[46] emphasise the need of a close collaboration between rheumatologists and pulmonologists in the effort to improve the management of these patients.

Contributors CH, EB, OH and TE conceived the study idea and developed it in collaboration with ABP, SPU and AL. CH, ABP and SPU collected the data. CH, TE, EB, AL, AB, and OH reviewed the literature. ABP and SPU directed the initial analyses. These were developed further by the other coauthors and then carried out by ABP and SPU. CH, OH, ABP, SPU, AL, EB and TE participated in the discussion and interpretation of the results. CH organised the writing and wrote the initial draft. All authors critically revised the manuscript for intellectual content and approved the final version before submission. CH is the guarantor.

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Competing interests None declared.

Patient consent Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

Ethics approval The study was approved by the Danish Data Protection Agency (record number 1-16-02-277-16). As this study did not involve contact with patients or an intervention, it was not necessary to obtain permission from the Danish scientific ethical committee.

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EXTENDED REPORT

Magnetic resonance imaging assessed inflammation in the wrist is associated with patient-reported physical impairment, global assessment of disease activity and pain in early rheumatoid arthritis: longitudinal results from two randomised controlled trials

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ABSTRACT

Objectives To examine whether MRI assessed inflammation and damage in the wrist of patients with early rheumatoid arthritis (RA) are associated with patient-reported outcomes (PROs).

Methods Wrist and hand MRIs of 210 patients with early RA from two investigator-initiated, randomised controlled studies (CIMESTRA/OPERA) were assessed according to the Outcome Measures in Rheumatology RA MRI score (RAMRIS) for synovitis, tenosynovitis, osteitis, bone erosions and joint space narrowing (JSN) at baseline, 1 and 5 years follow-up. These features, and changes therein, were assessed for associations with health assessment questionnaires (HAQ), patient global visual analogue scales (VAS-PtGlobal) and VAS-pain using Spearman's correlations, generalised estimating equations and univariate/multivariable linear regression analyses. MRI features were further tested for trends against specific hand-related HAQ items using Jonckheere trend tests.

Results MRI inflammation, but not damage, showed statistically significant associations with HAQ, VAS-PtGlobal and VAS-pain for status and change scores, independently of C reactive protein and swollen joint count. MRI-assessed synovitis was most consistently associated with PROs, particularly VAS-PtGlobal and VAS-pain. MRI-assessed synovitis and tenosynovitis mean scores were positively associated with patient-reported difficulty to cut meat and open a milk carton ($p<0.01$), and similar patterns were seen for other hand-related HAQ items. Incorporating metacarpophalangeal joints in the analyses did not strengthen the associations between MRI pathology and PROs.

Conclusions MRI-assessed inflammation, but not damage, in early RA wrists is associated with patient-reported physical impairment, global assessment of disease activity and pain and influences the physical function in the hand.

Trial registration number NCT00660647.

INTRODUCTION

Patient-reported outcomes (PROs) are widely used in rheumatoid arthritis (RA) to assess the patients' evaluation of how the disease affects physical function, pain and global assessment of disease activity.

Little is known about the relationships between PROs and different pathological findings in the joint, such as inflammation and structural damage. Studies in established RA have shown that radiographic progression is associated with increasing health assessment questionnaire (HAQ)-score over time. However, most studies have failed to document this association in the early stage of the disease.[1 2] MRI can detect bone erosions earlier in the disease course and with higher sensitivity than conventional radiography.[3–5] MRI can also visualise joint space narrowing (JSN),[6] and soft tissue pathologies, including synovitis, tenosynovitis and osteitis.[5 7] Thereby, it seems plausible that MRI findings might shed more light than conventional radiography on the pathological processes underlying the PROs. The association between MRI features and PROs has previously been addressed longitudinally in an established RA cohort,[8] and cross-sectionally in early RA cohorts.[9 10]

In a post hoc analysis of pooled data from two randomised placebo-controlled trials of patients with early RA, we aimed to examine whether MRI-assessed inflammation and damage in the early RA wrist are associated with physical function, global assessment of disease activity and pain at initiation of treatment and after 1 and 5 years' follow-up.

MATERIALS AND METHODS

Primary studies

The patients assessed in this post hoc analysis participated in the investigator-initiated, multicentre, randomised, double-blind, parallel-group, placebo-controlled CIclosporin, Methotrexate, Steroid in

RA (CIMESTRA) or OPtimised treatment algorithm in Early RA (OPERA) studies. These studies aimed at achieving inflammatory control by use of conventional and/or biological disease-modifying antirheumatic drugs (DMARDs) combined with intra-articular injection of glucocorticoids, and have been described in detail previously.[11 12] All patients had RA according to the 1987 American College of Rheumatology (ACR) criteria, disease duration <6 months and were DMARD-naïve. In both studies, one treatment arm received methotrexate plus intra-articular glucocorticoids, while the other arm received additional ciclosporin (CIMESTRA) or adalimumab (OPERA). Glucocorticoid injections were performed after obtaining MRIs and completing questionnaires. MRI was performed in 129 of 160 patients in CIMESTRA and 85 of 180 patients in OPERA.

In both studies, 28 tender and swollen joint counts (TJC/SJC) and C reactive protein (CRP) were obtained at each visit. Furthermore, the patients completed HAQ and assessment of patient global and pain visual analogue scales (VAS-PtGlobal and VAS-pain). In this post hoc analysis, 125 patients from the CIMESTRA study (4 patients excluded due to missing clinical data) and 85 patients from the OPERA study were included.

MAGNETIC RESONANCE IMAGING

MRIs were obtained of the non-dominant (CIMESTRA) or right (OPERA) wrist and, if the field of view allowed, the second-fifth metacarpophalangeal (MCP) joints at baseline (CIMESTRA/OPERA, n=210), 1 year (CIMESTRA/OPERA, n=206) and 5 years (CIMESTRA, n=113) visits. T1-weighted sequences (either isometric three-dimensional sequences, allowing multiplanar reconstruction, or axial+coronal two-dimensional sequences) before and after injection of intravenous contrast and coronal short-tau inversion recovery sequences were obtained. Information on MRI units and imaging parameters has previously been published.[13 14]

MRIs were assessed chronologically by one experienced reader, blinded to patient data. The image-sets were scored for synovitis (0–3), osteitis (0–3) and bone erosions (0–10) according to the Outcome Measures in Rheumatology (OMERACT) RA MRI score (RAMRIS)[5] and for tenosynovitis (0–3) and JSN (0–4) according to recently published OMERACT scoring systems.[7 15] As an intrareader analysis, baseline and follow-up images of 37 patients were reanonymised and reread (see online supplementary table S1).

Conventional radiography

Radiographs of both hands and forefeet were assessed chronologically for bone erosions and JSN at baseline, 1-year and 5 years follow-up by a reader blinded to patient data, using the Sharp van der Heijde (SvH) method.[16]

Statistics

The data from the patients in the CIMESTRA and OPERA studies were pooled and analysed as a single cohort. Clinical and biochemical data, SvH-scores and PROs were analysed as observed. The MRI scores were summarised to wrist scores, MCP scores and total scores. The wrist score was used as the primary analysis since this joint region was covered by the largest number of MRIs (table 1). MCP scores and total scores were assessed in additional analyses. The scores were also combined to inflammation scores (synovitis+tenosynovitis+osteitis) and damage scores (bone erosion+JSN). Data imputations of MRI scores were only allowed when ≥70% of the data points within a parameter (eg, 70% of the tendons at wrist level for scoring tenosynovitis) at an individual time point was available. Hence, completely missing MRI data at a time point were not imputed. Data were imputed using last observation carried forward and backward for missing follow-up and baseline data, respectively, in subjects with only one time point observed. In subjects with two time points observed, linear interpolation/extrapolation was used. If imputed values exceeded the maximum score or were negative, the maximum value and 0 was used, respectively. Of all data points included in the analyses, the following percentages were imputed: synovitis: 0.24%, tenosynovitis: 0.28%, osteitis: 0.58%, bone erosion: 0.54%, JSN: 0.16%.

Change between baseline and follow-up was assessed using the Wilcoxon signed-rank test. Clinical and MRI wrist baseline data were compared between the CIMESTRA and OPERA cohorts using the Mann-Whitney U test for continuous data and χ^2 test for nominal data. Status and change in MRI scores were explored for associations with HAQ-scores, VAS-pain and VAS-PtGlobal using Spearman's correlation analysis. Additionally, the association between MRI parameters and HAQ, VAS-pain and VAS-PtGlobal status and change scores was assessed over all time points using univariate generalised estimating equations (GEE), where variables with a p value ≤0.10 were included in multivariable models. SJC and CRP were also included in these models to assess relationships between MRI measures and PROs independent of clinical assessments. Change scores were further assessed in univariate linear regression models where independent variables with a p value ≤0.10 were included in multivariable regression models with backwards selection, where SJC and CRP were forced into the model. Log-transformation was applied to achieve normal distribution for status scores, and due to statistical skewness, the damage parameters were dichotomised for the GEE analyses at the median value for status scores as follows: JSN: 0/>0, erosion: ≤1/>1, combined damage score: ≤2/>2. For change scores, all damage parameters were dichotomised as 0/>0.

The association between MRI features and HAQ at baseline was further explored by calculating the mean of the different MRI scores for each HAQ increment in separate items hypothesised to involve the hand (the ability to cut meat, open a new milk carton, open previously opened jars, turn faucets on and off and lift a full cup or glass to the mouth). These groups were assessed for trends using the Jonckheere trend test.

SvH-scores were assessed for associations with HAQ, VAS-PtGlobal and VAS-pain using Spearman's correlation and univariate linear regression analyses.

A p value <0.05 was considered statistically significant. The statistical analyses were carried out using the SPSS V.22 (SPSS, Chicago, Illinois, USA) and STATA V.14 (StataCorp, College Station, Texas, USA).

RESULTS

Patient characteristics

At 1-year follow-up, the HAQ, VAS-pain and VAS-PtGlobal scores had improved markedly (p<0.01). Notably, the HAQ score did not change between 1 and 5 years follow-up, and the level of VAS-pain and VAS-PtGlobal changed minimally. All MRI wrist inflammatory scores showed a statistically significant decrease between baseline and both follow-up visits (except osteitis at 5 years follow-up), while MRI wrist damage scores showed a statistically significant increase (table 1). No

Table 1 Baseline and follow-up characteristics of the patients

	Baseline	1-year follow-up	5 years follow-up
Age	52 (13.6) n=210		
Sex, females, n (%)	138 (66%) n=210		
Disease duration, days	99 (44.8) n=210		
IgM RF positivity, n (%)	145 (69%) n=210		
Anti-CCP positivity, n (%)	133 (63%) n=210		
Tender joint count, range 0–28	11 (7) n=210	2 (5)* n=199	1 (4)* n=113
Swollen joint count, range 0–28	9 (6) n=210	1 (1)* n=199	1 (2)* n=113
VAS-pain, 0–100 mm VAS	49.9 (23.9) n=209	18.7 (20.7)* n=198	15.1 (20.9)* n=109
VAS-PtGlobal, 0–100 mm	53.6 (25.6) n=210	20.3 (22.4)* n=198	21.0 (1–23)* n=109
HAQ, range 0–3	1.1 (0.7) n=210	0.4 (0.5)* n=199	0.4 (0.5)* n=110
Serum CRP, mg/L	30.1 (33.5) n=207	11.2 (15.8)* n=198	6.6 (12.7)* n=112
DAS28 score, CRP based	5.3 (1.1) n=207	2.5 (1.1)* n=197	2.1 (1.1)* n=108
Total SvH score	5.2 (6.6) n=204	6.0 (7.4)* n=203	10.3 (13.2)* n=107
Erosive (SvH erosion score>0), n (%)	115 (56%) n=204	128 (63%) n=203	71 (66%) n=107
MRI parameters			
MRI wrist synovitis score, range 0–9	4.5 (2.4) n=198	3.1 (1.9)* n=186	2.6 (2.0)* n=92
MRI wrist tenosynovitis score, range 0–27	4.1 (4.2) n=194	1.4 (2.3)* n=187	0.8 (1.9)* n=92
MRI wrist osteitis score, range 0–45	2.5 (6.3) n=207	1.8 (5.7)* n=195	1.3 (3.7) n=94
MRI wrist erosion score, range 0–150	1.7 (2.4) n=205	2.2 (2.8)* n=194	3.0 (5.5)* n=95
MRI wrist JSN score, range 0–68	0.5 (1.3) n=209	0.8 (1.5)* n=195	1.6 (3.3)* n=94
MRI wrist combined inflammation score, range 0–81	11.2 (10.1) n=194	6.4 (7.5)* n=186	4.6 (5.7)* n=91
MRI wrist combined damage score, range 0–218	2.2 (3.2) n=205	2.9 (3.8)* n=194	4.6 (8.6)* n=94
Erosive wrist (RAMRIS erosion score>0), n (%)	127 (62%) n=205	130 (67%) n=194	72 (76%) n=95
MRI MCP synovitis score, range 0–12	4.3 (2.5) n=178	2.9 (2.0)* n=164	2.3 (2.1)* n=66
MRI MCP tenosynovitis score, range 0–12	2.4 (2.5) n=181	0.9 (1.7)* n=164	0.5 (1.1)* n=66
MRI MCP osteitis score, range 0–24	0.3 (1.0) n=182	0.2 (0.7)* n=175	0.1 (0.4) n=77
MRI MCP erosion score, range 0–80	0.8 (1.0) n=181	0.9 (1.2) n=169	0.9 (1.5)* n=72
MRI MCP JSN score, range 0–16	0.1 (0.5) n=173	0.1 (0.5)* n=173	0.1 (0.4) n=76
MRI MCP combined inflammation score, range 0–48	7.0 (4.5) n=164	4.0 (3.3)* n=156	2.9 (3.0)* n=64
MRI MCP combined damage score, range 0–96	0.9 (1.3) n=158	1.0 (1.4)* n=168	0.9 (1.7)* n=69
Erosive MCP joints (RAMRIS erosion score<0), n (%)	80 (44%) n=181	84 (50%) n=169	31 (43%) n=72
MRI total synovitis score, range 0–21	8.8 (4.1) n=173	5.9 (3.0)* n=162	4.6 (3.2)* n=66
MRI total tenosynovitis score, range 0–39	6.7 (6.0) n=172	2.2 (3.6)* n=163	1.0 (1.8)* n=66
MRI total osteitis score, range 0–69	2.9 (6.7) n=185	1.8 (5.4)* n=178	0.9 (3.1)* n=77
MRI total erosion score, range 0–230	2.5 (2.8) n=178	3.1 (3.3)* n=169	4.1 (6.3)* n=72
MRI total JSN score, range 0–84	0.6 (1.4) n=190	0.9 (1.7)* n=177	1.7 (3.6)* n=81
MRI total combined inflammation score, range 0–129	17.9 (12.8) n=156	9.7 (8.1)* n=156	6.6 (6.3)* n=64
MRI total combined damage score, range 0–314	3.1 (3.7) n=177	4.0 (4.3)* n=169	5.9 (9.9)* n=69
Erosive total scores (RAMRIS erosion score>0), n (%)	131 (74%) n=178	131 (78%) n=169	62 (86%) n=72

*p<0.05 (Wilcoxon signed-rank test) for comparison to baseline.
Mean (SD) scores of clinical, biochemical and MRI data at baseline and after 1-year and 5 years follow-up.
CCP, cyclic citrullinated protein; CRP, C reactive protein; DAS, disease activity score; HAQ, health assessment questionnaire; JSN, joint space narrowing; PtGlobal, patient global; RAMRIS, rheumatoid arthritis MRI score; RF, rheumatoid factor; VAS, visual analogue scale.

statistically significant difference was found between the CIMESTRA and OPERA cohorts at baseline, except disease duration (108 vs 84 days in CIMESTRA and OPERA, respectively), MRI-assessed tenosynovitis and combined inflammation score (data not shown).

Cross-sectional associations between MRI features and PROs

Statistically significant correlations were primarily seen between MRI synovitis, tenosynovitis and combined inflammation score and HAQ at baseline. MRI damage features only showed statistically significant correlations between JSN and HAQ at 1-year follow-up and between combined damage score and HAQ at 5 years follow-up (table 2). The univariate GEE analyses showed statistically significant associations for all inflammatory parameters and PROs, except osteitis versus VAS-pain. Osteitis demonstrated the strongest independent association with HAQ. For VAS-PtGlobal and VAS-pain, no independent associations were seen (table 3).

Associations between changes in MRI features and changes in PROs

Changes in MRI inflammatory features showed statistically significant correlations with changes in PROs from baseline to 1 and 5 years, except tenosynovitis versus HAQ between baseline and 5 years follow-up and tenosynovitis versus VAS-PtGlobal at all time intervals (table 2). In GEE models, changes in synovitis were associated with changes in VAS-pain and VAS-PtGlobal scores. The change in tenosynovitis and osteitis were associated

Table 2 Correlation between wrist MRI features and patient-reported outcomes

	Baseline	1-year follow-up	5 years follow-up	Δ Baseline – 1-year follow-up	Δ Baseline – 5 years follow-up
HAQ					
MRI synovitis	**0.25*** **	0.09	0.13	**0.21** **	**0.22***
MRI tenosynovitis	**0.18*** **	−0.02	0.13	**0.24** **	0.19
MRI osteitis	0.12	**0.21** **	0.18	**0.16***	**0.32** **
MRI erosion	0.03	0.08	0.16	0.00	−0.07
MRI JSN	−0.05	**0.19***	0.13	−0.08	−0.15
MRI combined inflammation	**0.21** **	0.08	0.16	**0.26** **	**0.25***
MRI combined damage	0.02	0.13	**0.21***	−0.02	−0.09
VAS-PtGlobal					
MRI synovitis	0.09	−0.01	0.02	**0.22** **	**0.32** **
MRI tenosynovitis	−0.01	−0.03	0.15	0.13	0.17
MRI osteitis	0.12	0.09	−0.02	**0.25** **	**0.24***
MRI erosion	−0.06	0.03	0.09	0.05	−0.09
MRI JSN	0.02	0.05	0.09	0.02	−0.16
MRI combined inflammation	0.07	0.03	0.04	**0.24** **	**0.30** **
MRI combined damage	−0.03	0.04	0.16	0.06	−0.14
VAS-pain					
MRI synovitis	**0.18***	0.03	−0.02	**0.28*** **	**0.45*** **
MRI tenosynovitis	0.04	−0.03	0.18	**0.16***	**0.29** **
MRI osteitis	0.08	0.14	−0.06	**0.22** **	**0.29** **
MRI erosion	−0.02	0.01	0.04	0.03	−0.18
MRI JSN	0.05	0.07	0.03	0.01	−0.16
MRI combined inflammation	0.11	0.08	0.01	**0.28*** **	**0.40*** **
MRI combined damage	0.00	0.03	0.08	0.03	−0.18

Associations for status and change scores by Spearman's correlations. Statistically significant correlations are written in bold and are interpreted as follows: *p<0.05, **p<0.01, ***p<0.001.
Δ, change; HAQ, health assessment questionnaire; JSN, joint space narrowing; PtGlobal, patient global; VAS, visual analogue scale.

with changes in HAQ. The change in combined inflammation scores was significantly associated with change in all PROs (table 3). Change in synovitis, tenosynovitis and combined inflammation showed statistically significant or borderline significant association with change in HAQ, VAS-PtGlobal and VAS-pain at all time intervals in the univariate regression models. The association of change in synovitis with PROs was independent of change in CRP and SJC at all time intervals, except for HAQ between baseline and 1 year. The association of change in combined inflammation score was independent of change in CRP and SJC between baseline and 5 years follow-up. Change in erosion, JSN and combined damage score showed no statistically significant associations with change in PROs in any analyses (table 4).

Baseline associations between MRI scores and single HAQ items

The mean synovitis and tenosynovitis scores increased with each increment of the HAQ scale for the items regarding ability to cut meat and open a milk carton ($p<0.01$). Similar patterns were seen for the other hand-related HAQ items. The association between the mean osteitis score and HAQ items showed a varying pattern (figure 1). Damage parameters showed no statistically significant trends for any items (data not shown).

Conventional radiography

Greater SvH-scores for bone erosion were correlated with lower VAS-pain at baseline (−0.14, $p<0.05$) and there was an inverse relationship in linear regression for change in JSN with change in VAS-PtGlobal from baseline to 5 years follow-up (−1.91, 95% CI −3.28 to −0.54, $p=0.01$). No other statistically significant associations were found between SvH-scores and PROs (data not shown).

Influence of including MCP joints in the analyses

Univariate and multivariable analyses of the MRI scores in the MCP joints showed fewer statistically significant independent associations with PROs for linear regression analyses but not for GEEs (see online supplementary Tables S2 and S3). Using total scores (wrist+MCP joints) showed the same pattern, as when using the wrist only (see online supplementary Tables S4 and S5). A sensitivity analysis was performed, in wrist MRIs, limited to patients having both joint regions scanned (ie, subjects with total scores). This analysis showed similar findings as the primary wrist group (see online supplementary Tables S6 and S7).

Sensitivity analyses

By analyses of correlations, regression analyses and GEEs using MRI data without imputations, no originally statistically significant results became non-significant, except for the correlation between 0–5 years change in synovitis and change in HAQ (see online supplementary Tables S8-S10).

DISCUSSION

This post hoc analysis of patients with early RA showed a statistically significant association between MRI synovitis, tenosynovitis and osteitis in the wrist and HAQ, VAS-PtGlobal and VAS-pain but not between MRI/radiographic bone erosion and JSN and PROs. Synovitis was most consistently associated with PROs, particularly VAS-PtGlobal and VAS-pain. This association seemed weaker for HAQ. However, hand function, as measured by HAQ was significantly associated with synovitis and

Table 3 Univariate and multivariable generalised estimating equations for wrist scores

	Status				Change			
	Univariate		Multivariable		Univariate		Multivariable	
	β (95% CI)	p Value	β (95% CI)	p Value	β (95% CI)	p Value	β (95% CI)	p Value
HAQ†								
MRI synovitis	0.06 (0.04 to 0.07)	**<0.001**	0.00 (−0.02 to 0.02)	0.97	0.08 (0.04 to 0.12)	**<0.001**	−0.01 (−0.04 to 0.03)	0.72
MRI tenosynovitis	0.04 (0.03 to 0.05)	**<0.001**	0.01 (0.00 to 0.02)	0.14	0.07 (0.04 to 0.09)	**<0.001**	0.02 (0.00 to 0.04)	**0.05**
MRI osteitis	0.01 (0.01 to 0.02)	**<0.001**	0.04 (0.00 to 0.01)	**0.03**	0.02 (0.01 to 0.04)	**0.001**	0.01 (0.00 to 0.02)	**0.04**
MRI erosion	−0.03 (−0.11 to 0.05)	0.51			0.08 (−0.09 to 0.26)	0.35		
MRI JSN	−0.02 (−0.09 to 0.06)	0.67			−0.02 (−0.25 to 0.21)	0.84		
MRI combined inflammation*	0.02 (0.01 to 0.02)	**<0.001**	0.00 (0.00 to 0.01)	0.16	0.03 (0.01 to 0.04)	**<0.001**	0.01 (0.00 to 0.02)	**0.01**
MRI combined damage*	−0.06 (−0.12 to 0.02)	0.16			0.07 (−0.11 to 0.24)	0.44		
VAS-PtGlobal†								
MRI synovitis	0.10 (0.08 to 0.13)	**<0.001**	0.01 (−0.02 to 0.04)	0.48	4.13 (2.55 to 5.71)	**<0.001**	1.79 (0.34 to 3.23)	**0.02**
MRI tenosynovitis	0.06 (0.05 to 0.07)	**<0.001**	0.01 (−0.01 to 0.02)	0.29	2.12 (1.14 to 3.09)	**<0.001**	−0.08 (−1.08 to 0.92)	0.88
MRI osteitis	0.01 (0.00 to 0.02)	**0.004**	0.00 (−0.01 to 0.01)	0.92	0.90 (0.22 to 1.58)	**0.01**	0.21 (−0.25 to 0.66)	0.37
MRI erosion	−0.09 (−0.20 to 0.02)	0.11			3.10 (−4.69 to 10.90)	0.44		
MRI JSN	−0.03 (−0.16 to 0.10)	0.65			−3.33 (−12.06 to 5.40)	0.46		
MRI combined inflammation*	0.02 (0.01 to 0.03)	**<0.001**	0.00 (0.00 to 0.01)	0.10	0.96 (0.48 to 1.44)	**<0.001**	0.42 (0.06 to 0.78)	**0.02**
MRI combined damage*	−0.09 (−0.20 to 0.02)	0.12			1.62 (−5.92 to 9.16)	0.67		
VAS-pain†								
MRI synovitis	0.09 (0.05 to 0.13)	**<0.001**	0.02 (−0.01 to 0.05)	0.14	4.64 (3.20 to 6.08)	**<0.001**	2.20 (0.87 to 3.53)	**0.001**
MRI tenosynovitis	0.05 (0.03 to 0.06)	**<0.001**	0.01 (−0.01 to 0.05)	0.19	2.19 (1.30 to 3.09)	**<0.001**	−0.02 (−0.91 to 0.87)	0.96
MRI osteitis	0.01 (0.00 to 0.02)	0.20			0.89 (0.21 to 1.57)	**0.01**	0.21 (−0.27 to 0.68)	0.39
MRI erosion	−0.06 (−0.17 to 0.05)	0.28			0.13 (−6.85 to 7.10)	0.97		
MRI JSN	0.01 (−0.10 to 0.12)	0.83			−1.24 (−9.09 to 6.61)	0.76		
MRI combined inflammation*	0.02 (0.01 to 0.02)	**<0.001**	0.00 (0.00 to 0.01)	0.16	1.02 (0.55 to 1.49)	**<0.001**	0.48 (0.11 to 0.85)	**0.01**
MRI combined damage*	−0.08 (−0.19 to 0.03)	0.15			−3.74 (−10.12 to 2.65)	0.25		

*If sum scores in univariate GEE had a p-value≤0.10, this was included in a separate multivariable GEE model with CRP and SJC.
†Log-transformed for status scores.
Association between MRI parameters and patient-reported outcomes for status scores and change scores. All generalised estimating equations (GEE) were adjusted for age and sex. MRI parameters with a univariate p≤0.10 were included in multivariable GEE analysis where CRP and SJC were incorporated.
HAQ, health assessment questionnaire; JSN, joint space narrowing; PtGlobal, patient global; VAS, visual analogue scale.

tenosynovitis at baseline. Incorporating MCP joints to the analyses did not improve the associations between MRI pathology and PROs.

This study is the first to document statistically significant associations between MRI inflammation and different PROs, and changes therein, in a longitudinal setting for patients with early RA. The association between MRI features and PROs has previously been described in different settings.[17] Ranganath *et al*[10] found a trend but non-significant association between MRI inflammation and HAQ in patients with RA in remission. Benton *et al*[18] reported that osteitis was the only single MRI parameter correlating with HAQ at baseline in patients with early RA. After 6 years, a statistically significant and borderline significant correlation with HAQ was found for bone erosion and tendinitis, respectively. The study was limited by small sample size (n=42). In a cross-sectional study, Burgers *et al*[9] showed that MRI-assessed synovitis, tenosynovitis and osteitis were associated with HAQ in univariate linear regression models in 514 patients with early, clinically confirmed arthritis. In multivariable regression analyses, tenosynovitis was associated

Table 4 Univariate and multivariable linear regression analyses for wrist scores

	Δ Baseline–1-year follow-up				Δ Baseline–5 years follow-up			
	Univariate		Multivariable		Univariate		Multivariable	
	β (95% CI)	p Value	β (95% CI)	p Value	β (95% CI)	p Value	β (95% CI)	p Value
HAQ								
ΔRI synovitis	0.06 (0.02 to 0.10)	**0.01**			0.09 (0.03 to 0.15)	**0.003**	0.07 (0.01 to 0.13)	**0.02**
ΔMRI tenosynovitis	0.04 (0.02 to 0.07)	**0.001**			0.04 (0.00 to 0.07)	**0.04**		
ΔMRI osteitis	0.02 (0.00 to 0.03)	**0.05**			0.02 (0.00 to 0.05)	0.10		
ΔMRI erosion	0.01 (–0.07 to 0.08)	0.85			–0.03 (–0.06 to 0.01)	0.11		
ΔJSN	–0.01 (–0.13;0.11)	0.84			–0.05 (–0.11 to 0.02)	0.16		
ΔSJC	0.03 (0.02 to 0.05)	**<0.00001**	0.02 (0.01 to 0.04)	**0.01**	0.04 (0.02 to 0.06)	**0.001**		
ΔCRP	0.01 (0.01 to 0.01)	**0.00002**	0.01 (0.01 to 0.01)	**<0.00001**	0.01 (0.00 to 0.01)	**0.001**	0.01 (0.00 to 0.01)	**0.02**
ΔMRI combined inflammation*	0.01 (0.00 to 0.02)	**0.01**			0.02 (0.01 to 0.04)	**0.01**	0.02 (0.00 to 0.04)	**0.02**
ΔMRI combined damage*	0.00 (–0.05;0.06)	0.93			–0.02 (–0.04 to 0.00)	0.11		
VAS-PtGlobal								
ΔMRI synovitis	3.08 (1.18 to 4.98)	**0.002**	2.53 (0.65 to 4.41)	**0.01**	4.56 (2.33 to 6.79)	**0.0001**	3.37 (0.95 to 5.79)	**0.01**
ΔMRI tenosynovitis	1.11 (0.02 to 2.21)	**0.05**			1.33 (0.01 to 2.65)	**0.05**		
ΔMRI osteitis	0.70 (0.02 to 1.39)	**0.05**			1.33 (0.43 to 2.23)	**0.004**		
ΔMRI erosion	–1.22 (–4.63;2.20)	0.50			–0.84 (–2.07 to 0.40)	0.18		
ΔMRI JSN	0.40 (–5.01;5.81)	0.89			–1.82 (–4.26 to 0.62)	0.14		
ΔSJC	0.83 (0.13 to 1.53)	**0.02**			1.94 (1.16 to 2.72)	**<0.00001**	1.29 (0.31 to 2.27)	**0.01**
ΔCRP	0.28 (0.15 to 0.38)	**0.00002**	0.22 (0.10 to 0.35)	**0.001**	0.22 (0.06 to 0.38)	**0.01**		
ΔMRI combined inflammation*	0.50 (0.04 to 0.96)	**0.03**			1.11 (0.54 to 1.67)	**0.0002**	0.78 (0.14 to 1.42)	**0.02**
ΔMRI combined damage*	–0.58 (–3.15;2.00)	0.66			–0.62 (–1.46 to 0.23)	0.15		
VAS-pain								
ΔMRI synovitis	3.54 (1.82 to 5.27)	**0.00008**	2.90 (1.20 to 4.61)	**0.001**	5.57 (3.52 to 7.61)	**<0.00001**	2.53 (0.22 to 4.84)	**0.03**
ΔMRI tenosynovitis	1.18 (0.18 to 2.18)	**0.02**			1.83 (0.58 to 3.09)	**0.01**		
ΔMRI osteitis	0.60 (–0.03;1.23)	0.06			1.49 (0.63 to 2.35)	**0.001**		
ΔMRI erosion	–1.94 (–5.03;1.15)	0.22			–1.12 (–2.31 to 0.07)	0.07		
ΔMRI JSN	1.92 (–3.32;7.17)	0.47			–1.51 (–3.89 to 0.87)	0.21		
ΔSJC	1.01 (0.38 to 1.63)	**0.001**			2.20 (1.52 to 2.89)	**<0.00001**	0.86 (–0.01;1.74)	**0.05**
ΔCRP	0.27 (0.16 to 0.37)	**<0.00001**	0.22 (0.10 to 0.33)	**0.0002**	0.40 (0.26 to 0.53)	**<0.00001**	0.26 (0.10 to 0.41)	**0.00**
ΔMRI combined inflammation*	0.52 (0.10 to 0.94)	**0.02**			1.33 (0.80 to 1.85)	**<0.00001**	0.96 (0.37 to 1.56)	**0.00**
ΔMRI combined damage*	–0.71 (–3.06;1.65)	0.55			–0.71 (–1.53 to 0.10)	0.09		

*If combined inflammation or damage scores in univariate regression analyses had a p value ≤0.10, this was included in a separate multivariable regression model with CRP and SJC.

Analyses for associations between the change of MRI features and change in patient-reported outcomes. All multivariable analyses were adjusted for sex and age. Statistically significant or borderline significant p values (≤0.05) are written in bold.

Δ, change; CRP, C reactive protein; HAQ, health assessment questionnaire; JSN, joint space narrowing; PtGlobal, patient global; SJC, swollen joint count; VAS, visual analogue scale.

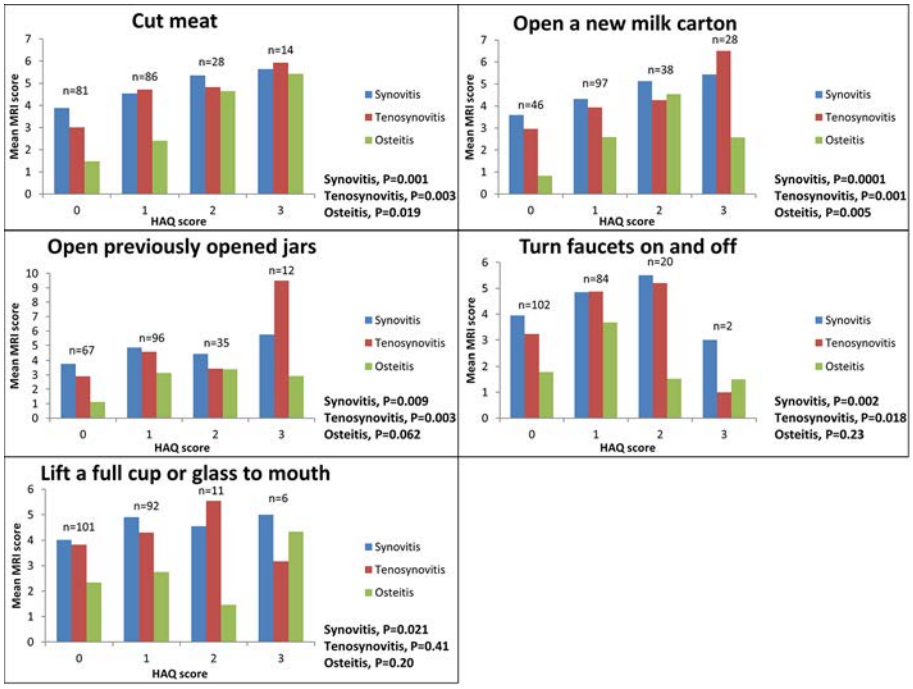

Figure 1 Mean inflammatory involvement distributed on specific hand-related HAQ items. The columns refer to the mean RAMRIS scores for the inflammatory features for each level of the hand-related HAQ item (x-axis). The HAQ scores are interpreted as follows: 0: no difficulty, 1: some difficulty, 2: much difficulty, 3: unable to perform the activity. n, number of patients; HAQ, health assessment questionnaire; RAMRIS, rheumatoid arthritis MRI score.

with HAQ, independently of clinical and biochemical measures. Bone erosion was not independently associated with HAQ. This pattern was also present in a subanalysis of 206 patients that fulfilled the European League Against Rheumatism/ACR 2010 criteria for RA at baseline. Furthermore, the group showed increasing amounts of synovitis and tenosynovitis with each score of hand-related HAQ items. The study did not assess JSN and the associations between MRI and HAQ were not studied in longitudinal settings. Baker *et al*[8] reported that MRI-assessed synovitis, osteitis and bone erosion were associated with HAQ, VAS-PtGlobal and VAS-pain at all time points between baseline and 1-year follow-up in a subset of 291 erosive methotrexate and biologic-naïve patients with RA from the GO-BEFORE study. Tenosynovitis and JSN were not assessed. Thus, previous and present data consistently support the importance of MRI-assessed inflammation for the patient experience in RA.

In our study, the multivariable linear regression analyses and GEE for change scores showed that change in MRI-assessed synovitis was consistently associated with changes in VAS-pain and VAS-PtGlobal. This association was also observed in the study by Baker *et al*.[8] These findings suggest that patients are prone to rate their global disease activity and pain higher with increasing amount of MRI-assessed synovitis in their wrists. Our analyses showed some associations between HAQ and synovitis, but also with tenosynovitis and osteitis. While Burgers *et al* found that MRI-assessed tenosynovitis had the strongest baseline associations with HAQ, our analyses showed a more variable pattern. The median combined wrist inflammation score was markedly lower in the cohort studied by Burgers *et al* (median 3.0 vs 11.2 in our cohort). A plausible cause to the different associations may be that lower amount of inflammation may contribute to variable relationships of synovitis, tenosynovitis, osteitis with HAQ. In agreement with our findings, Burgers *et al* found that the amount of tenosynovitis and synovitis in the wrist increased with higher level of disability in hand-related HAQ items at baseline.[9] We believe the results from our study support a connection between the local degree of inflammation in the wrist and impaired physical function of the hand.

Statistically significant association between radiographic damage and PROs was only seen between bone erosion and VAS-pain at baseline and between changes in JSN and VAS-PtGlobal between baseline and follow-up. These associations were, however, inverse and we therefore consider this a spurious finding.

The fact that the current study did not find an association between PROs and MRI/radiographic damage may be explained by the lower level of damage in the patients with early RA participating in the CIMESTRA and OPERA studies as compared with the GO-BEFORE subanalysis, where patients with RA were more erosive on radiographs and had longer disease duration (mean 1.2 years).[19] Indeed, the mean baseline total MRI bone erosion score was 14.5 in the study by Baker *et al* as compared with 2.5 in the CIMESTRA/OPERA cohort. Several studies have previously documented an association between radiographic damage and physical impairment.[2 20–22] However, in early RA the lack of substantial structural damage may result in a lack of influence of this disease feature on PROs compared with the inflammatory load. Interestingly, our results suggest that the influence of structural damage on PROs is minimal throughout at least 5 years of follow-up. In the current study, the PROs remained stable between 1 and 5 years follow-up. In the CIMESTRA and OPERA studies, a persistent treat-to-target strategy was applied to inflamed joints by aggressive use of intra-articular glucocorticoids and simultaneous escalation of disease-modifying treatment throughout the study.[11 12] This may explain why the change in structural damage was limited and not associated with change in PROs, after neither 1 nor 5 years follow-up.

In this post hoc analysis, we chose to use the wrist score as our primary analysis to achieve the largest sample size. Additional analyses showed that the assessment of the MCP joints provided little additional information. In general, comparing analyses of total scores (wrist+MCP scores) and wrist scores in the subset of patients with both wrist+MCP scores available gave similar results. Hence, MRI-assessed inflammation in the wrist rather than in the MCP joints seems to be most important for the physical function, pain and global assessment of disease activity in the patients with early RA. The MRIs were scored according to the RAMRIS, which includes the first carpometacarpal joint, but not the first MCP and interphalangeal joint. Including these joints could have provided further information on the association of thumb inflammation with the function of the hand. Hand osteoarthritis may coexist with RA and may influence PROs. However, bone damage did not show any significant associations with the PROs, suggesting that osteoarthritis had no major influence on our results.

Strengths of this post hoc analysis include the longitudinal setting and the large sample size, which allowed comprehensive analyses using linear regressions and GEE. Limitations include missing MRI data at different time points, although the range of missing MRIs was only 6%–8%, 3%–8% and 6%–9% for baseline, 1 year and 5 years, respectively. Furthermore, the results from 5 years follow-up are limited by the lower sample size. This study assessed associations between MRI features and PROs longitudinally in patients with early RA. Future studies should focus on assessing longitudinal associations in other cohorts, such as more advanced disease and RA in remission. Furthermore, the association between MRI features and designated measures of hand function should be investigated.[23]

In conclusion, MRI-assessed inflammation, but not damage, in early RA wrists is associated with patient-reported physical impairment, global assessment of disease activity and pain and the amount of wrist inflammation influences the level of physical function in the hand.

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EXTENDED REPORT

Long-term outcomes after disease activity-guided dose reduction of TNF inhibition in rheumatoid arthritis: 3-year data of the DRESS study - a randomised controlled pragmatic non-inferiority strategy trial

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ABSTRACT

Objective Tumour necrosis factor inhibitors (TNFi) are effective in rheumatoid arthritis (RA), but disadvantages include adverse events (AEs) and high costs. This can be improved by disease activity-guided dose reduction (DR). We aimed to assess long-term outcomes of TNFi DR in RA by using 3-year data from the DRESS study (Dose REduction Strategy of Subcutaneous TNF inhibitors study).

Methods In the intervention phase (month 0–18) of the DRESS study (Dutch trial register, NTR 3216), patients were randomised to DR or usual care (UC). In the extension phase (month 18–36), treatment strategies in both groups converged to continuation of protocolised tight control and allowed dose optimisation. Intention-to-treat analyses were done on flare, disease activity (28 joint count-based disease activity score with C reactive protein (DAS28-CRP)), functioning (health assessment questionnaire-disability index (HAQ-DI)), quality of life (Euroqol 5 dimensions 5 levels questionnaire (EQ5D–5L)), medication use, radiographic progression (Sharp van der Heijde score (SvdH)) and AE.

Results 172/180 patients included in the DRESS study were included in the extension phase. Cumulative incidences of major flare were 10% and 12% (−2%, 95% CI −8 to 15) in DR and UC groups in the extension phase, and 17% and 14% (3%, 95% CI −9 to 13) from 0 to 36 months. Cumulative incidences of short-lived flares were 43% (33 to 52%)%) and 35% (23 to 49%)%) in DR and UC groups in the extension phase, and 83% (75 to 90%)%) and 44% (31 to 58%)%) from 0 to 36 months. Mean DAS28-CRP, HAQ-DI, EQ5D-5L and SvdH remained stable and not significantly different between groups. TNFi use remained low in the DR group and decreased in the UC group. Cumulative incidences of AE were not significantly different between groups.

Conclusions Safety and efficacy of disease activity guided TNFi DR in RA are maintained up to 3 years, with a large reduction in TNFi use, but no other benefits. Implementation of DR would vastly improve the cost-effective use of TNFi.

INTRODUCTION

The treatment of rheumatoid arthritis (RA) has improved in the last two decades, due to, among other things, the introduction of the first widely used class of anticytokine drugs: tumour necrosis factor inhibitors (TNFi). These drugs are effective and safe in the treatment of RA, providing benefits for symptoms, functioning, quality of life and inhibition of joint damage.[1 2]

However, TNFis are not without their drawbacks. These include (dose dependent) increased risk of infection,[3] skin cancer[4] and idiosyncratic adverse reactions like induction of multiple sclerosis, lupus or heart failure.[5–7] Furthermore, the need for regular self-injection poses a burden for patients. Lastly, the costs of these drugs are significant, both per patient per year (Europe: $17 000; USA: $26 000), as well as on a macroeconomic scale.[8 9]

These disadvantages might be ameliorated by dose reduction (DR) or discontinuation of TNFi after disease control has been achieved, and this indeed has been shown to be possible in a relevant proportion of patients.[10–12] A disease activity-guided TNFi DR strategy has been tested previously in the DRESS study (Dose REduction Strategy of Subcutaneous TNF inhibitors) and has been shown to be feasible and non-inferior to usual care (UC) with regard to prolonged flaring.[13] The strategy also did not result in differences in disease activity, functioning, quality of life or relevant radiographic progression after 18 months. However, short-lived flares and minimal radiographic progression occurred more frequently in the DR arm, probably due to the temporary effects of unsuccessful DR attempts on disease activity.[14] Although no benefits were seen with regard to side effects, the cost effectiveness was very high, reaching $440 000 saved per lost quality-adjusted life year.[15]

Some important questions remain, especially with regard to long-term risks and benefits of this strategy. Does the occurrence of major flare remain comparable between groups after longer follow-up? Is the small difference in radiographic joint damage between groups only due to a temporary difference in disease activity, or should we expect more damage in subsequent years? And finally, can a lower risk of adverse events (AEs) (eg, infections) be demonstrated?

In an attempt to answer these questions, we performed an extension study of the original DRESS study, exploring long-term effects of this DR strategy on disease activity, functioning, quality of life, radiographic progression and (serious) adverse events ((S)AE).

METHODS

Study design and participants

This is a long-term extension study of the DRESS study, an 18-month, pragmatic, open label, randomised controlled, non-inferiority strategy trial in patients with RA, in which a disease activity-guided DR strategy of adalimumab or etanercept was compared with UC. For inclusion criteria, we refer to van Herwaarden *et al*.[13] Disease activity was measured using DAS28-CRP (28 joint count-based disease activity score with C reactive protein (CRP)).

The DRESS study was registered at the Dutch trial register (www.trialregister.nl, NTR 3216), and its design and results have been reported previously.[13–17] The extension study was performed from May 2014 to January 2016 in the Sint Maartenskliniek Nijmegen and Woerden, The Netherlands, and was approved by the local ethics committee (CMO region Arnhem-Nijmegen, NL37704.091.11).

Randomisation and masking

In the intervention phase (month 0–18) of the DRESS study, patients were randomised to the DR or UC group in a ratio of 2:1, stratified for adalimumab and etanercept. In the extension phase (month 18–36), the original group allocation was maintained. Both the intervention and extension phase were non-blinded.

Procedures

In the intervention phase, patients allocated to UC continued a standardised tight control treatment protocol (maintaining DAS28-CRP <3.2).

In the DR group, patients received identical care, with addition of a specific DR advice given to the treating rheumatologist for that particular patient. The DR strategy consisted of 3-monthly stepwise increase of injection time interval until flare or discontinuation. For details we refer to van Herwaarden *et al*.[13] If the flare persisted after 4 weeks despite bridging with intramuscular or intra-articular steroids or non-steroidal anti-inflammatory drugs, TNFi was increased stepwise, if needed, to the shortest registered interval. If a flare persisted thereafter, treatment was switched. Only one DR attempt was advised.

As flare criterion, a DAS28-CRP increase >1.2, or a DAS28-CRP increase >0.6 compared with baseline and current DAS28-CRP ≥3.2 was used (short-lived flares).[18] Major flare was defined as a flare persisting >12 weeks.

In the extension phase, the treatment strategies in both groups converged to the same strategy: treatment choices were left to the discretion of the treating rheumatologist and were based on local treatment protocols that included (1) disease activity measurement every 3–6 months and using treat-to-target to achieve at least low disease activity, (2) a preferential order of biological Disease Modifying Anti-Rheumatic Drugs (bDMARDS) (see online supplementary appendix 1) and (3) a bDMARD dose optimisation protocol (see online supplementary appendix 2). Patients originally allocated to UC were therefore also able to initiate TNFi DR (see online supplementary appendix figure 1) but without specific DR advices. After March 2015, DAS28-CRP cut-off levels for low disease activity and remission were slightly lowered to 2.9 and 2.4, as it was shown that DAS28-CRP thresholds should be slightly lower in comparison with DAS28-ESR.[19]

Outcomes

For the extension phase, the same endpoints were used as in the original DRESS study, although in an explorative, non-hypothesis testing manner. The primary endpoint was the difference in cumulative incidence of major flare between DR and UC group, during the extension phase and during the entire study (0–36 months).

Secondary endpoints were cumulative incidence of patients with short-lived flares, difference in mean time-weighted (MTW) DAS28-CRP score, MTW functioning, measured with the health assessment questionnaire-disability index (HAQ-DI) and quality of life at 36 months measured with EQ5D-5L (EUROQOL 5 dimensions 5 levels questionnaire), proportions of patients in whom DR or discontinuation was successful, bDMARD use, mean change (Δ) in Sharp van der Heijde score (SvdH) and cumulative incidence and incidence density (ID) of (S)AE.

Successful DR was defined as being on a lower dose than at baseline with concomitant low disease activity, measured both at 18 and 36 months. Successful discontinuation was defined as complete withdrawal of adalimumab or etanercept with concomitant low disease activity, measured both at 18 and 36 months.

In the extension phase, DAS28-CRP and HAQ-DI were measured at least every 6 months, and an EQ5D-5L was repeated at 36 months. For bDMARD use, the normalised proportion of the defined daily dose (DDD) was calculated with IQR with 1.0 being the full dose equivalent.

Radiographs of hands and feet were obtained at 36 months and assessed using the modified SvdH score, by the same two blinded, trained readers that assessed the original DRESS radiographs. Scoring was done pairwise with radiographs from months 18 and 36 in known sequential order, but without rescoring baseline and 18 months, for efficiency reasons, as suggested for long-term studies.[20] Mean Δ in SvdH and proportion of patients with ΔSvdH exceeding three different cut-off levels were compared between groups: (1) minimal clinical important change (MCIC) of eight points in 18 months,[21] (2) smallest detectable change (SDC)[22 23] and (3) 0.5 SvdH units for minimal radiographic progression.

Statistical analysis

Stata IC V.13.1 was used. In the DRESS study, per protocol (PP) analyses were used for the primary outcomes because of the non-inferiority nature of the analyses. Because of (1) the more exploratory analyses in this extension phase, (2) difficulty defining 'per protocol' when treatment decisions are left to the treating physician and (3) minor differences in PP and intention-to-treat (ITT) analyses in the original study, an ITT approach was chosen. Patients who did not give informed consent or were lost to follow-up before 24 months were excluded. All analyses were done for the extension phase and when appropriate for the entire study. Since previously no differences between TNFi (adalimumab or etanercept) were found, stratified analyses were deemed unnecessary for the extension phase.

For the primary analysis, we kept the original non-inferiority margin of 20% difference in major flare between DR and UC groups, based on the same reasoning as mentioned before.[16] Point estimates with CI (95% CI) of the difference in cumulative incidence of major flare between groups were calculated and the upper limits of the CI were compared with the non-inferiority margin. A t-test compared mean/median medication use (MTW)

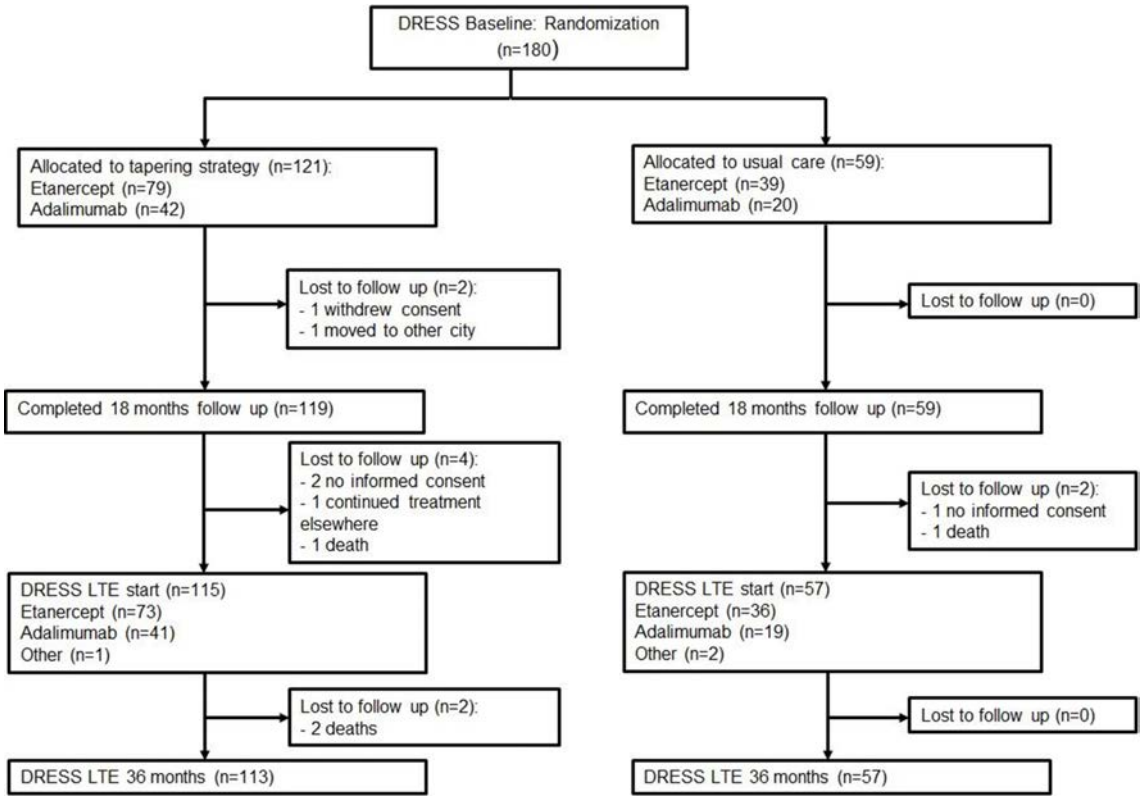

Figure 1 Flow chart. DRESS, Dose REduction Strategy of Subcutaneous TNF inhibitors.

DAS28-CRP, HAQ-DI and EQ5D-5L. Differences in cumulative incidence of flares and levels of disease activity at 24, 30 and 36 months were compared by χ^2 testing. Different time points were tested separately, with no repeated measure analyses performed.

Role of the funding source

This study was funded by the department of rheumatology at the Sint Maartenskliniek Nijmegen, The Netherlands.

RESULTS

Of 180 patients included in the DRESS study, 172 patients were enrolled in the extension phase: 115 patients in the DR group and 57 in the UC group (figure 1). Baseline characteristics at start of the extension phase were similar between groups, except for higher prevalence of conventional synthetic DMARD (csDMARD) comedication in the UC group (table 1). The percentage of missing data was low: 2% of planned visits and 2%–8% missing per variable, therefore multiple imputation was deemed unnecessary and simple imputation using last observation carried forward in case the last observation was missing or mean of previous and next were calculated for in-between missings.

Flaring

The cumulative incidences of major flare during the extension phase were 12/115 (10%) in the DR and 7/57 (12%) in the UC group (difference −2%, 95% CI −8% to 15%). The upper limit of the 95% CI around the difference was <20%, compatible with non-inferiority of DR to UC group. The cumulative incidence from month 0–36 was 20/115 (17%) in the DR and 8/57 (14%) in the UC group (difference 3%, −10 to 15). There was no significant difference in cumulative incidence of short-lived flares during the extension phase: 49/115 (43%, 33% to 52%) in the DR and 20/57 (35%, 23% to 49%) in the UC group. From month 0–36, the cumulative incidence of flare remained different between groups: 96/115 (83%, 75% to 90%) in the DR and 25/57 (44%, 31% to 58%) in the UC groups. Additional analyses within the DR and UC groups on the occurrence of major and short-lived flares comparing adalimumab with etanercept, showed no significant differences in both the extension phase (18–36 months) as well as the whole study duration (0–36 months).

Disease activity, function and quality of life

In the extension phase, MTW-DAS28-CRP was 2.2 (SD 0.7) in the DR group and 2.1 (SD 0.7) in the UC group (difference 0.08, –0.15 to 0.30). MTW–DAS28-CRP from 0 to 36 months was 2.3 (SD 0.6) in the DR group and 2.1 (SD 0.7) in the UC group (difference 0.16, –0.03 to 0.35). DAS28-CRP, HAQ-DI and EQ5D-5L remained stable during the extension phase and complete follow-up and were not significantly different between groups at any time point (figure 2). Disease activity states were not significantly different between groups at any time point in the extension phase (see online supplementary appendix table 1).

TNFi tapering and medication use

In the intervention phase, 23/115 (20%, 13% to 28%) patients in the DR group had successfully discontinued their bDMARD, 52/115 (45%, 36% to 55%) successfully reduced their bDMARD and in 40/115 (35%, 26% to 44%) no DR was possible. Nineteen out of 115 (17%, 10% to 25%) patients in the DR group persisted being biological free with maintenance of low disease activity from the intervention phase until 36 months, and 33/115 (29%, 21% to 38%) of patients in the DR group persisted being successfully dose reduced from the intervention phase until 36 months.

Table 1 Patient characteristics at DRESS baseline and at start of DRESS extension phase

	DRESS baseline		DRESS extension	
	DR (n=115)	UC (n=57)	DR (n=115)	UC (n=57)
General characteristics				
Age, years (SD)*	59 (10.0)	58 (9.2)	60.9 (10.0)	59.7 (9.2)
Disease duration (years)†	10 (5–16)	10 (6–16)	11 (7–17)	12 (7–18)
SvdH score‡	21 (5.5–59)	19 (8.5–46.5)		
Female sex	71 (62)	39 (68)		
Diagnosis according to 2010 and/or 1987 ACR criteria, n (%)	109 (95)	57 (100)		
Rheumatoid factor positive	90 (78)	47 (82)		
Anticitrullinated peptide antibodies positive	82 (71)	44 (77)		
Erosive disease	94/112 (84)	52 (91)		
Disease activity characteristics				
No. of swollen joints†	0 (0–0)	0 (0–1)	0 (0–1)	0 (0–0)
No. of tender joints†	0 (0–1)	0 (0–0)	0 (0–1)	0 (0–1)
Erythrocyte sedimentation rate (mm/h)*	17 (14)	16 (10)	20 (15)	16 (10)
C reactive protein (mg/L)*	4 (4)	4 (4)	7 (16)	4 (12)
DAS28-CRP score*	2.2 (0.7)	2.1 (0.8)	2.2 (0.9)	2.0 (0.9)
DAS28-ESR score*	2.5 (0.7)	2.5 (0.8)	2.7 (1.0)	2.5 (1.0)
2011 ACR/EULAR Boolean based remission	30 (26)	21 (37)	28 (23)	23 (39)
Treatment characteristics				
Etanercept/adalimumab/other	76/39 (66/34)	37/20 (65/35)	73/41/1 (63/36/1)	36/19/2 (63/33/4)
Duration of TNFi use at inclusion (years)*	3.5 (2.5)	3.5 (2.2)	5 (2.5)	5 (2.2)
Previous number of csDMARD treatments†	2 (1–3)	2 (1–3)	2 (1–3)	2 (1–3)
Previous number of TNFi treatments†	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Concomitant treatment				
csDMARD	68 (59)	45 (79)	69 (60)	40 (70)
Methotrexate	55 (48)	39 (68)	54 (47)	35 (61)
Methotrexate dose (mg)*	15.9 (5.7)	16.3 (5.6)	17.0 (6.5)	15.3 (5.0)
Glucocorticoids	3 (3)	3 (5)	6 (5)	6 (11)
NSAIDs	63 (55)	34 (60)	70 (61)	35 (61)

Data are number (%) of patients unless stated otherwise.
*Mean (SD).
†Median (IQR).
‡n=101 in the DR group, n=55 in the UC group.
ACR/EULAR, American College of Rheumatology/European League Against Rheumatism; csDMARD, conventional synthetic disease modifying antirheumatic drug; DAS28-CRP, 28 joint count-based disease activity score with C reactive protein; DAS28-ESR, 28 joint count-based disease activity score with erythrocyte sedimentation rate; DR, dose reduction; DRESS, Dose REduction Strategy of Subcutaneous TNF inhibitors; NSAIDs, non-steroidal anti-inflammatory drugs; SvdH, Sharp van der Heijde score; TNFi, tumour necrosis factor inhibitor; UC, usual care.

During the intervention phase, 49/57 (86%, 74% to 94%) patients in the UC group did not attempt DR (eight patients tapered or discontinued their bDMARD, mostly due to AEs). In the extension phase, in 32/49 (65%, 50% to 78%) patients, a DR attempt was made. Of these, 19/49 (39%, 25% to 54%) were successfully dose reduced and 7/49 (14%, 1% to 27%) were successfully discontinued at 36 months. In 12/32 (38%, 21% to 56%) patients in the UC group in whom a DR attempt was made, a short-lived flare occurred. In patients in the UC group in whom a DR attempt was made, four experienced a major flare. Two of these patients reached low disease activity at the next visit after re-escalation or reinstallment. The remaining two patients had a major flare that was due to a high VAS score and high tender joint count. Re-escalation was thus deemed unnecessary by both the treating rheumatologist as well as the patient.

At study end, between group differences in numbers of patients successfully tapered or stopped were smaller but still existent (figure 3).

During the intervention phase, the proportion of the DDD of TNFi use was 0.50 (IQR 0.48 to 0.51) in the DR group and 0.92 (0.90 to 0.94) in the UC group (difference −0.42, −0.45 to −0.39). During the extension phase, this difference decreased but remained significant: 0.54 (0.51 to 0.58) in the DR and 0.67 (0.64 to 0.71) in the UC group (difference −0.13, −0.18 to 0.08). From 0 to 36 months, DDD was 0.53 (0.51 to 0.54) in the DR and 0.80 (0.78 to 0.82) in the UC group (difference −0.27, −0.30 to −0.25).

In the extension phase, no significant between-group differences in csDMARD use were observed. At 36 months, <10% of patients in both groups used oral steroids (difference −1%, −10% to 8%). During the extension phase, intramuscular or intra-articular glucocorticoid injections were given to 48/115 (42%, 33% to 51%) in the DR and 21/57 (37%, 24% to 51%) in the UC group (difference 5%, −11% to 21%).

Radiological outcomes

One hundred and fifty-six patients (101 DR; 55 UC) had radiographs available at 18 and 36 months. No significant difference in mean progression score between groups was observed for the extension phase (table 2). Two out of 101 (2%) patients in the

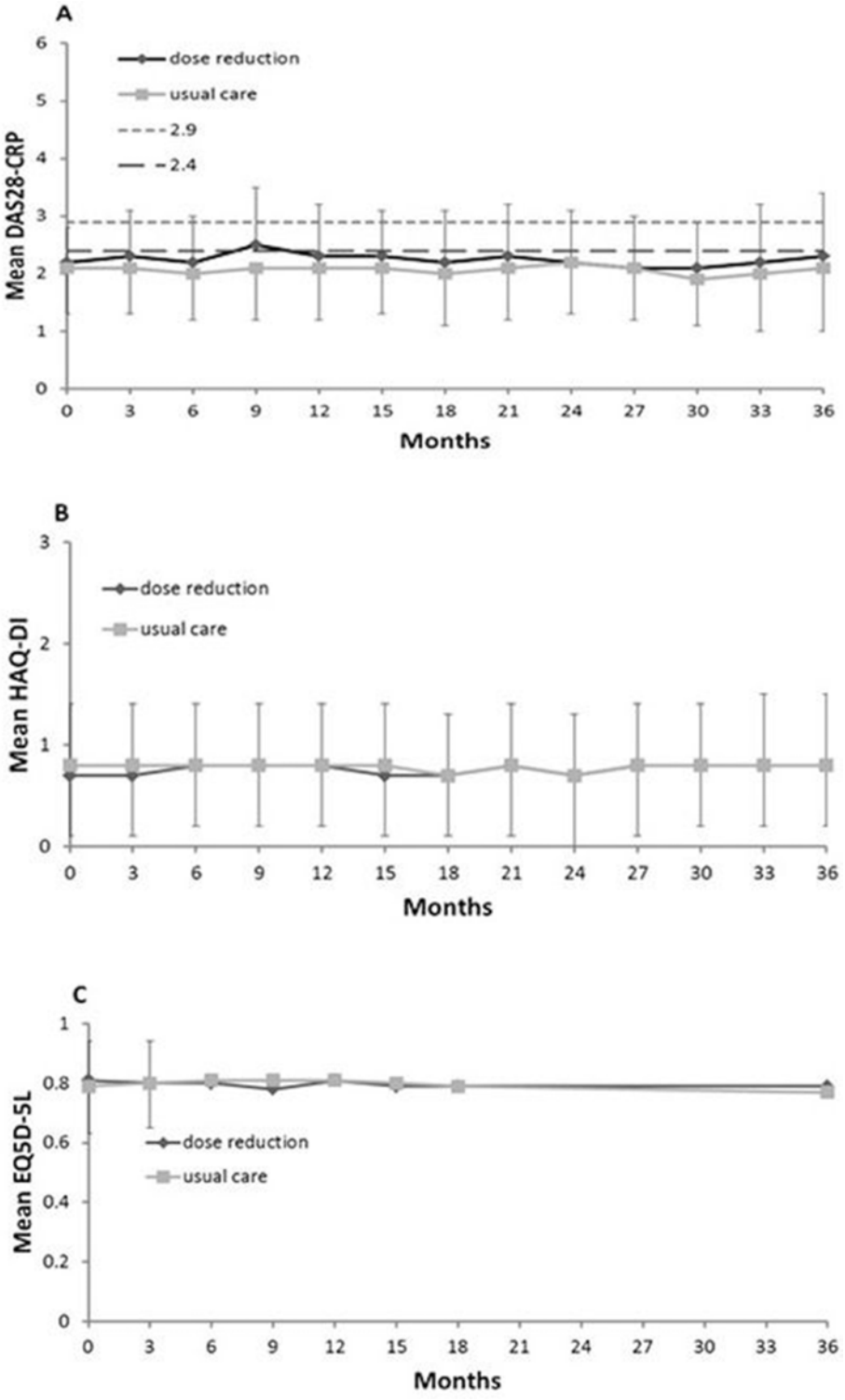

Figure 2 Mean (A) disease activity (measured with DAS28-CRP), (B) functioning (measured with HAQ-DI) and (C) quality of life (measured with EQ5D-5L). DAS28-CRP, 28 joint count-based disease activity score with C reactive protein; HAQ-DI, health assessment questionnaire-disability index. EQ-5D-5L, Euroqol 5 dimensions 5 levels questionnaire.

DR group and no patients in the UC group exceeded the MCIC. No significant between-group differences were seen for the SDC (calculated as 5.1 points) or minimal radiographic progression as cut-off values.

Safety

The cumulative incidence of AEs during the extension phase was equal in both groups: 39/115 (34%, 25% to 43%) in the DR and 22/57 (39%, 26% to 52%) in the UC group (difference −5%, −11 to 21), and the number of patients with SAEs was also not different between groups (difference 3%, −11 to 15). From month 0–36, 103/115 (90%, 82% to 94%) patients had an AE in the DR group and 54/57 (95%, 85% to 99%) in the UC group (difference −5%, −14% to 4%) and the number of patients with SAEs was different (difference 17%, 8% to 31%), caused by a higher incidence of elective surgery in the DR group.

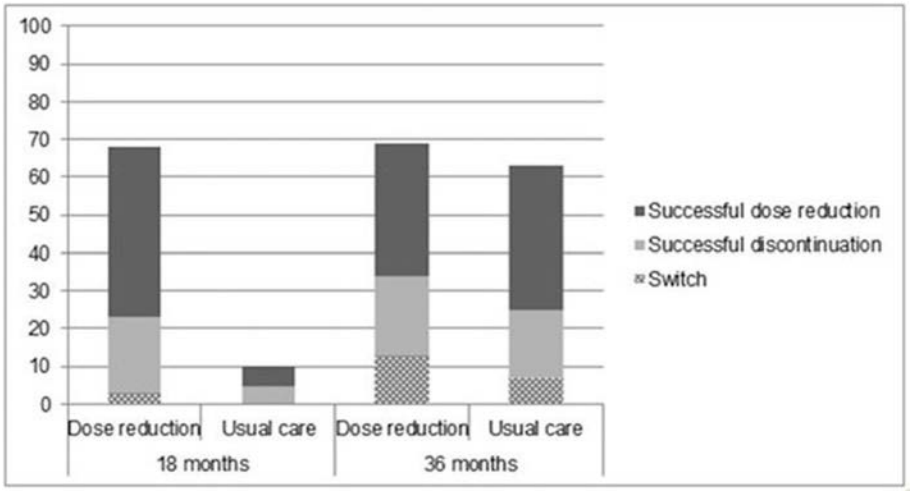

Figure 3 Dose optimisation in dose reduction and usual care group (percentages) at 18 and 36 months.

Overall, low IDs per 100 patient-years were observed for other SAE categories with no significant between-group differences (see online supplementary appendix table 2).

DISCUSSION

To our knowledge, this is the first study investigating long-term effects of disease activity-guided DR of adalimumab and etanercept in patients with RA. Results show that the initial efficacy and safety of this strategy are maintained. No relevant difference in the number of major flares could be demonstrated between DR and UC group, and disease activity, functioning and quality of life were also very similar. Furthermore, no significant difference in radiographic progression was found, although this might be caused by less contrast between groups, due to the converging treatment strategies. However, other benefits of tapering, including less AEs (eg, infections), were not observed.

There are some factors to take into account when interpreting our data. The design choices that were made for the extension study have advantages but also some drawbacks. Considering the latter, the convergence of strategies between groups and subsequent loss of contrast should lead to caution when interpreting the lack of differences between groups. The similarity in outcomes may be caused by the former DR group doing well, but also in part by the original UC group doing worse than before. However, the latter seems less likely, considering the very stable 3-year course in disease activity, functioning and quality of life. The outcomes are also highly comparable between the DR group from 18 to 36 months and the UC group from 0 to 18 months. Furthermore, in the extension phase of this study, flare criteria were altered since it was shown that cut-off values of DAS28-CRP for low disease activity and remission should be slightly lower than the validated flare criterion cut-offs using DAS28-ESR.[19] It is unclear, however, how this would have altered our results. Tight control would have become even more tight, but this would have occurred in both DR and UC groups. Future studies should investigate how using different flare criteria influences treatment strategy outcomes.

Nevertheless, the design of the study did allow to assess—in the former UC group—what level of TNFi DR can be achieved when no specific DR advice is given. Interestingly, in the majority of those patients an attempt to dose reduce was observed, and subsequent results were also comparable with those in the original DR group. This further supports generalisability of the results to clinical practice.

Although there was some drop-out during the extension phase, for the primary outcome, our study seems well powered. A type II error might be present for analyses we did not power for. In the design of the original DRESS study, a sample size calculation showed that to be able to reject the null hypothesis in this study (ie, the intervention is inferior compared with the control arm by more than the non-inferiority margin) with a power of 80%, and accounting for a 10% drop-out, 180 patients in total were included. At the end of the long-term extension phase (month 36), 113 and 57 patients were still included. Thus, this is only slightly below the numbers as calculated above and total drop-out is still below 10%.

Comparison of our findings with other long-term studies on disease activity-guided DR or discontinuation is difficult, due to paucity and heterogeneity of these studies, with different DR strategies (eg, interval lengthening vs dose tapering, gradual vs fixed DR, or using different tapering schemes) and different definitions for relapse or flare.[10] A recently published paper of

Table 2 Radiographic outcomes

	Baseline to 18 months			18–36 months		
	DR (n=101)	UC (n=55)	Difference (95% CI)	DR (n=101)	UC (n=55)	Difference (95% CI)
Progression total SvdH	0.68 (1.5)	0.17 (1.1)	0.51 (0.06 to 0.97)	1.29 (2.4)	1.45 (2.2)	−0.16 (−0.93 to 0.62)
Progression erosion	0.26 (0.8)	0.13 (0.7)	0.13 (−0.13 to 0.39)	0.56 (1.3)	0.81 (1.6)	−0.25 (−0.71 to 0.21)
Progression JSN	0.43 (1.2)	0.05 (0.9)	0.38 (0.15 to 0.75)	0.73 (1.5)	0.64 (1.9)	0.09 (−0.46 to 0.64)
Progression > MCIC	0 (0%)	0 (0%)	0% (−8 to 4)	2 (2%)	0 (0%)	2% (−2 to 6)
Progression > SDC	5 (4%)	0 (0%)	4% (−4 to 10)	3 (3%)	3 (5%)	−2% (−9 to 4)
Progression >0.5	37 (32%)	9 (15%)	17% (2 to 29)	50 (50%)	29 (53%)	3% (−20 to 13)

Data are mean (SD) or n (%).
DR, dose reduction; JSN, joint space narrowing; MCIC, minimal clinical important change (eight units); Progression, in units per 18 months; SDC, smallest detectable change (5.1 units); SvdH, modified Sharp van der Heijde score.

Raffeiner *et al* is the only other study to show long-term (median follow-up 3.6 years) data from a prospective semirandomised tapering trial.[24] However, only etanercept was studied, and fixed dose halving was used instead of disease activity-guided DR versus continuation of etanercept. Reassuringly, the outcomes of this study were very similar to ours, with no significant differences in clinical outcomes and radiographic progression, although with much higher absolute baseline radiographic damage.

Two points are of interest with regard to AEs, SAEs being more frequent in the DR group and no observed benefits of TNFi tapering on risk for infections. First, the higher incidence of SAEs seems an artefact, because it is almost exclusively caused by more elective surgery and SAEs that arose from study-related PET/CT scanning, that was only done in the DR group, resulting in information bias. Second, we were not able to demonstrate lower infection risks in the DR group, which is in contrast to Raffeiner *et al* and to what might be pharmacologically expected.[3] This difference in outcome might be caused by lower patient numbers and follow-up time and less contrast between the treatment arms in the extension phase. Furthermore, duration of TNFi use before study start was much longer in our study and as patients susceptible to infections would have been more likely to have discontinued their bDMARD before inclusion, this could have led to selection bias (healthy survivor bias).

In conclusion, a disease activity-guided DR strategy of TNFi in patients with RA doing well seems a reasonable long-term approach in RA treatment. Further optimisation of this strategy could consist of identification of predictors for successful DR or discontinuation, as this might prevent short-lived flaring.

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Contributors CAMB, NvH, FHJvdH, JF, RFvV, HB, AvdM and AAdB were involved in the study design. CAMB, NvH, AvdM, FHJvdH and AAdB were involved in the data collection. CAMB, NvH, JF, AvdM and AAdB performed the data analyses. All authors were involved in writing and revision of the manuscript. CAMB declares she had full access to all the data in this study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Competing interests HB received grants and personal fees from Pfizer and AbbVie during the conduct of the study; grants and personal fees from Roche, BMS, MSD, UCB, all outside the submitted work. RFvV received grants from AbbVie, Amgen, BMS, GSK, Pfizer, Roche, UCB and personal fees from AbbVie, Biotest, BMS, Celgene, Crescendo, GSK, Janssen, Lilly, Merck, Novartis, Pfizer, Roche, UCB, Vertex, outside the submitted work. JF received a research grant from BMS. AAdB received congress invitations from Roche and Abvie and an expert witness fee from Amgen. The other authors declare that they have no conflicts of interest.

Ethics approval CMO region Arnhem Nijmegen.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The authors commit to making the relevant anonymized patient level data available on reasonable request.

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EXTENDED REPORT

Pattern of risks of systemic lupus erythematosus among statin users: a population-based cohort study

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ABSTRACT

Objectives To examine the association between the use of statins and the risk of systemic lupus erythematosus (SLE) with focus on describing the patterns of risks over time.

Setting A population-based cohort study using the UK Clinical Practice Research Datalink.

Participants All patients aged 40 years or older who had at least one prescription of statins during the period 1995–2009 were selected and matched by age, sex, practice and date of first prescription to non-users. The follow-up period of statin users was divided into periods of current, recent and past exposure, with patients moving among these three exposure categories over time. Current statin users were also stratified into ≤1 year or >1 year of use.

Main outcome measures Time-dependent Cox models were used to calculate HRs of SLE, adjusted for disease history and previous drug exposure.

Results We included 1 039 694 patients, of whom 519 847 were statin users. Current statin users did not have an increased risk of developing SLE among patients aged ≥40 years ($HR_{adjusted}$ 0.75, 95% CI 0.53 to 1.07). Current statin users who continued the therapy for >1 year had a 38% lower risk of developing SLE ($HR_{adjusted}$ 0.62, 95% CI 0.42 to 0.93). When more specific definitions for SLE were used, this latter finding, however, was not observed.

Conclusions Our findings showed no effect of statins on the risk of developing SLE among patients aged ≥40 years. Further research is needed to study the long-term effects of statins on SLE.

INTRODUCTION

Statins are effective in reducing the risk of cardiovascular morbidity and mortality in patients with hyperlipidaemia, hypertension or diabetes.[1–3] Besides their cholesterol-lowering activity, several studies have shown that statins have anti-inflammatory and immunomodulatory properties and may suppress the expression of ongoing autoimmune responses. Specifically, several studies have shown that statins decrease the proinflammatory biomarkers and/or disease activity scores in patients with SLE.[4–7] Alternatively, we previously suggested that statins may facilitate the development of autoimmunity.[8–10] In these studies, however, we used different study designs, study populations, study outcomes and definitions of the exposure to statins.[8–10] Four studies that included analyses of reports of adverse drug reactions suggested that statins could trigger the development of lupus-like syndrome.[10–13] The mean time from statin exposure to the onset of SLE has been described as 12.8±18 months (range 1 month–6 years).[12] However, one study showed that statin use was associated with a decreased risk of connective tissue disease (CTD), including SLE[14] To date, there is no robust evidence of whether statins have an effect on the development of SLE. We examined the association between the use of statins and the risk of SLE with focus on describing the pattern of risk of SLE over time.

METHODS

Data source

Data were derived from the Clinical Practice Research Datalink (CPRD), an ongoing primary care database of anonymous medical records from general practitioners. CPRD contains the computerised medical records of 625 general practices, representing approximately 8% of the population in the UK and has been described in detail elsewhere.[15] The data recorded in the database include demographic information, diagnoses, prescription details, preventive care provided, referrals to specialist care, hospital admissions and related major outcomes.[15] Several independent validation studies have shown that the CPRD database has a high level of completeness and validity.[16] The current study was approved by the Independent Scientific Advisory Committee for Medicines and Healthcare Products Regulatory Agency Database Research.

Study population

We conducted a matched cohort study with prospectively collected data among patients who had at least one prescription of statins during the period 1995–2009. The date of the first prescription of statins was defined as the index date. Statin users were matched to a single control (non-users of statins) randomly selected from patients of the same age (±5 years) and sex at index date, with the index date of the control being the same as that of the statin user (ie, matching on calendar time). Statin users and non-users were also matched on practice as they had to be registered at the same general practice as the statin user to control for differences in prescribing regimens per practice.

Statin users and non-users had to have at least 1 year of data collection before the index date. After this matching, statin users and non-users who had ever been diagnosed with SLE, had used disease-modifying anti-rheumatic drugs (DMARDs) and/or were younger than 40 years

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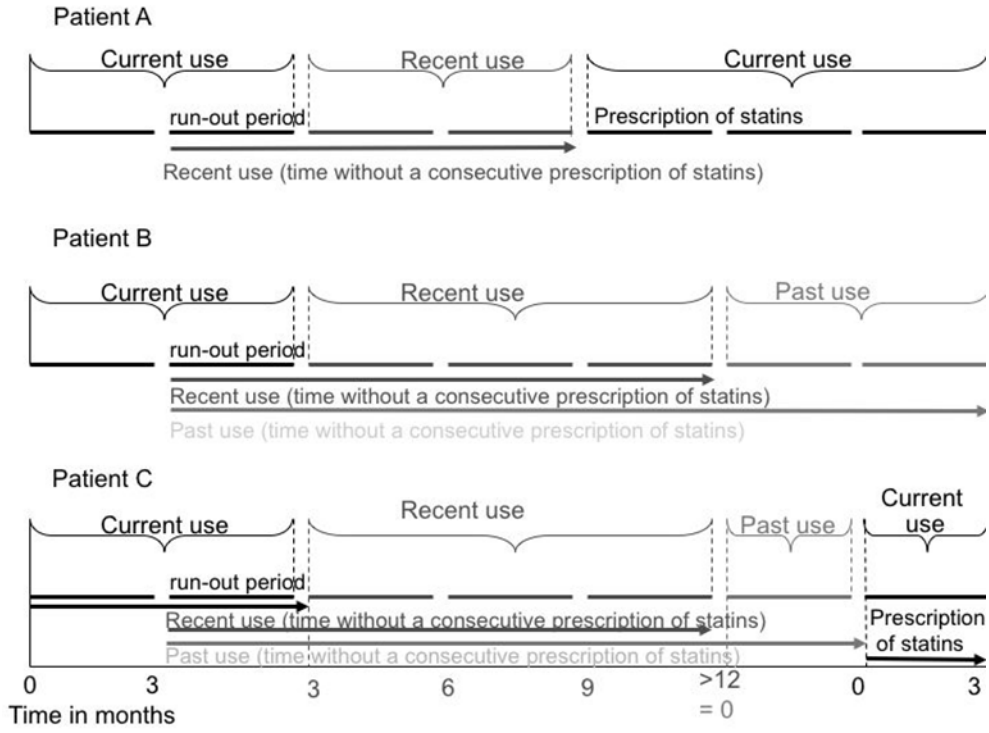


Figure 1 Three examples of time-dependent exposure to statins in patients A, B and C. During follow-up of the patients who initiated statin therapy, time was divided into periods of current, recent and past exposure to statins, with patients moving between these three exposure categories over time. We illustrated this pattern of statin exposure by three examples. Patient A: the follow-up of statin use of patient A was divided into periods of current, recent and current exposure to statins. Patient B: the follow-up of statin use of patient B was divided into periods of current, recent and past exposure to statins. Patient C: the follow-up of statin use of patient C was divided into periods of current, recent, past and current exposure to statins. Black arrow: current exposure, time from the start date of a prescription until 3 months after its expected duration of use. The expected duration of use was defined as 3 months (run-out period). When the consecutive prescription of statins was prescribed within these 3 months, the exposure to statins was defined as current exposure. Dark grey arrow: recent exposure, time from 3 to 12 months after the end date of the most recent prescription of statins. Light grey arrow: past exposure, time from 12 months or longer after the end date of the most recent prescription of statins.

before or at the index date were excluded. Patients aged ≥40 years were considered more likely to receive a statin than patients <40 years.

Exposure to statins

All prescriptions for statins were identified. Each prescription length was calculated by dividing the number of prescribed tablets by the prescribed daily dose. Since statin therapy compliance declines substantially over time,[17] the time of follow-up was divided into periods of current, recent and past exposure to statins, with patients moving between these three exposure categories over time.[18] Current exposure was defined as the time from the date of a prescription until 3 months after its expected duration of use. The expected duration of statin use was defined as 3 months. When the consecutive prescription of statins was prescribed within these 3 months, patients continued to be 'current users'. Since patients can move between different categories of exposure to statins over time, patients can be defined more than once as 'current users'. Current statin users were also classified as ≤1 year or >1 year of use. Recent exposure was defined as the period of time from 3 to 12 months after the end date of the most recent prescription, and past exposure was the period of time from 12 months or longer after the end date of the most recent prescription of statins (figure 1).

Clinical outcome

Each patient was followed from the index date up to the date of the first record, diagnosis, of SLE (identified from CPRD's Read coded data)[19] or the date when the patient left the general practice, died or the end date of data collection, whichever date came first. When a patient was referred to a rheumatologist before the date of the first SLE code, the date of the first referral was defined as the event date.

Risk factors

Potential risk factors for SLE were derived from the literature, including studies investigating the effects of statins on SLE, comorbidities in patients with early SLE and comedication with anti-inflammatory and immunomodulating effects which may potentially result in SLE.[20–22] The risk factors in the year before the index date included body mass index (BMI), smoking and alcohol status (currently smoking or drinking, ex-smoker or ex-drinker, or never smoked or drank) and a history of hypertension, diabetes mellitus, hyperlipidaemia, cardiovascular disease, asthma, inflammatory bowel and thyroid disease.[23] Diabetes mellitus was defined as having a diagnosis of diabetes mellitus or using antidiabetic therapy. Patients were classified as hypertensive if they received a prescription for antihypertensive drugs or had a diagnosis of hypertension. Comedications with anti-inflammatory and immunomodulating properties within 6 months before the index date were non-steroidal

anti-inflammatory drugs, aspirin, proton pump inhibitors (PPIs), antibiotics, hormone replacement therapy, antidepressants, anticonvulsants, antipsychotics, antiarrhythmic and other lipid-lowering agents.[24]

Statistical analysis

The incidence rate was estimated by dividing the number of patients with incident SLE by the total follow-up time. We estimated the HRs and 95% CIs for the risk of developing SLE among statin users. A multivariate time-dependent Cox proportional hazards model was used to assess the risk of SLE in current, recent and past users compared with non-users of statins. Potential risk factors were only included in the final model if they independently changed the estimated effect for statin use by at least 5%. Multiple imputation was used to address missing data for BMI (missing: 12.9%), smoking (9.2%) and alcohol status (17.9%). Missing data were imputed by the multiple imputation method using the fully conditional specification method.[25] All original exposure, outcome and co-variables as presented in tables 1 and 2 were included in the imputation model. Twenty imputations were created, analysed and pooled. Results from the complete and multiple imputation analyses were compared, and multiple imputation analyses are presented.

Prespecified subgroup analyses of patients with cardiovascular diseases or risk factors were performed. A previous study suggested different associations between statin use and the risk of developing SLE in patients with cardiovascular diseases, hypertension or diabetes.[26] Despite the increased lipid levels, statins could also have been prescribed to patients with only diabetes mellitus or a low socioeconomic status or a family history of cardiovascular disease or a high-risk ethnicity, as has been described in the National Institute for Health and Care Excellence clinical guideline lipid modification.[27] A subgroup analysis in patients with or without a medical history of hyperlipidaemia was conducted. In previous studies, it was found that older women were more likely to experience an adverse effect of statins.[28 29] Therefore, the analyses were also stratified by age and sex. Data analyses were performed using SAS V.9.2 (SAS Institute, Cary, North Carolina, USA).

Sensitivity analyses

Five sensitivity analyses were carried out of which the first three were evaluating the impact of potential case misclassification by changing the definition of incident SLE into:

1. having at least two medical records of which the first record was used as the event date;
2. the first-time diagnosis of SLE with a referral to a rheumatologist or at least one prescription of the frequently prescribed drugs for SLE (azathioprine, cyclophosphamide, cyclosporine or methotrexate) and/or received at least two prescriptions of corticosteroids or (hydroxyl)chloroquine after the first medical record for SLE;
3. the required minimum of two physicians' claims for SLE at least 2 months apart within a 2-year span, an algorithm which has been proposed by Bernatsky and colleagues.[30]
4. It is likely that there is a lag time between the onset of symptoms and the diagnosis of SLE, and therefore, we excluded the 2 years following initiation of statin treatment.[31]
5. We considered the date of SLE exactly 2 years before the first-time diagnosis of SLE because of the potential late manifestation of the clinically apparent symptoms of SLE.[31]

Table 1 Baseline characteristics of statin users and non-statin users

Baseline characteristics	Statin users (n=519 847)	Non-users (n=519 847)
Duration of follow-up (years)		
Mean (SD)	4.5 (3.4)	4.1 (2.6)
Sex, n (%)		
Female	250 608 (48.2)	250 608 (48.2)
Age (years)		
Mean (SD)	63.1 (12.1)	62.9 (12.5)
Age by category, years (%)		
40–49	70 047 (13.5)	74 647 (14.4)
50–59	148 461 (28.6)	158 441 (30.5)
60–79	242 331 (46.6)	221 013 (42.5)
80+	59 008 (11.3)	65 746 (12.6)
BMI (kg/m^2)		
Mean (SD)	26.9 (8.4)	21.0 (11.6)
Unknown BMI	28 284 (5.4)	105 970 (20.4)
Smoking status, n (%)		
Non-smoker	213 123 (41.7)	234 762 (45.1)
Ex-smoker	216 786 (31.6)	111 623 (21.5)
Smoker	164 492 (22.3)	100 837 (19.4)
Unknown smoking status	22 935 (4.4)	72 625 (14.0)
Drinking status, n (%)		
Non-drinker	65 250 (12.6)	54 528 (10.5)
Ex-drinker	32 799 (6.3)	20 856 (4.0)
Drinker	358 004 (68.8)	321 916 (61.9)
Unknown drinking status	63 794 (12.3)	122 547 (23.6)
Drug use within previous 6 months, n (%)		
Antihypertensive agents	323 170 (62.2)	124 169 (23.9)
Fibrates	8565 (1.6)	900 (0.2)
Ezetimibe	1969 (0.4)	133 (0.03)
Antidiabetic agents	122 185 (23.5)	18 603 (3.6)
Aspirin	145 039 (27.9)	36 945 (7.1)
Antiarrhythmic agents	20 625 (4.0)	11 301 (2.2)
NSAIDs	202 011 (38.9)	88 625 (17.0)
Proton pump inhibitors	84 995 (16.4)	48 211 (9.3)
Hormone replacement therapy or oral contraceptives	21 629 (4.2)	21 005 (4.0)
Oral corticosteroids	17 673 (3.4)	15 574 (3.0)
Antibiotics	47 321 (9.1)	36 493 (7.0)
Anticonvulsants	10 850 (2.1)	8126 (1.6)
Antipsychotics	5444 (1.0)	6190 (1.2)
Antidepressants	115 564 (22.2)	95 293 (18.3)
History of disease ever before, n (%)		
Hypertension*	323 170 (62.2)	124 169 (23.9)
Hyperlipidaemia	153 758 (29.6)	12 734 (2.4)
Diabetes†	122 515 (23.6)	18 762 (3.6)
Cardiovascular diseases	174 982 (33.7)	47 675 (9.2)
Cerebrovascular disease	59 891 (11.5)	17 077 (3.3)
Cancer	35 099 (6.8)	40 046 (7.7)
Psoriasis	20 182 (3.9)	16 544 (3.2)
Inflammatory bowel disease	5185 (1.0)	5155 (1.0)
COPD	21 113 (4.1)	20 849 (4.0)
Asthma	61 503 (11.8)	53 183 (10.2)
Dementia	5075 (1.0)	8610 (1.7)
Depression	72 446 (13.9)	49 371 (9.5)

*Diagnosis of hypertension or use of antihypertensive agents.
†Diagnosis of diabetes mellitus or use of antidiabetic therapy.
COPD, chronic obstructive pulmonary disease; NSAIDs, non-steroidal anti-inflammatory drugs.

Table 2 Risk of systemic lupus erythematosus in statin users compared with non-statin users

	SLE (n)	IR*	Age- and sex-adjusted HR (95% CI)	Fully adjusted HR (95% CI)†
No statin use	98	0.6	1.00	1.00
Past statin use	22	1.0	1.61 (1.01 to 2.56)	1.30 (0.79 to 2.13)
Recent statin use	20	1.1	1.67 (0.98 to 2.84)	1.31 (0.75 to 2.29)
Current statin use	117	0.6	0.98 (0.73 to 1.30)	0.75 (0.53 to 1.07)
≤1 year	64	1.9	1.31 (0.88 to 1.93)	1.01 (0.65 to 1.56)
>1 year	53	0.3	0.83 (0.59 to 1.16)	0.62 (0.42 to 0.93)

*Incidence rate is calculated for each recency of statin use by dividing the number of events by the person time within each given recency of use.
†Adjusted for age, sex, practice, smoking, cardiovascular diseases, hyperlipidaemia, hypertension, diabetes and use of non-steroid anti-inflammatory drugs.
IR, incidence rate (per 10 000 person-years); SLE, systemic lupus erythematosus.

RESULTS

A total number of 1 107 988 statin users and controls were identified in the CPRD: 40 320 patients who were younger than 40 years, 3346 patients with a medical history of SLE and 24 628 patients with a prescription of DMARD before the index date were excluded. Of the remaining 1 039 694 patients, 519 847 were statin users and 519 847 were non-users (figure 2). Due to matching, statin users and non-users had similar distributions of age (statin users: mean age, 63.1 years and non-users: 62.9 years) and sex (statin users and non-users: 48.2% women). Compared with non-users, statin users were more frequently previous smokers and diagnosed with cardiovascular disease, hyperlipidaemia, hypertension and diabetes. Statin users were more likely to have a history of exposure to aspirin, antihypertensive, antidiabetic agents and PPIs compared with non-users (table 1).

In our study population, the incidence rate for SLE was 0.7 cases per 10 000 person-years. Current statin users had a risk of developing SLE among patients aged ≥40 years which was comparable to that of non-users ($HR_{adjusted}$ 0.75; 95% CI 0.53 to 1.07) (table 2). Current statin users who continued the therapy for >1 year had a 38% decreased risk of developing SLE ($HR_{adjusted}$ 0.62, 95% CI 0.42 to 0.93). Recent and past statin users had no increased risk of developing SLE. The $HR_{adjusted}$ for recent and past statin users were 1.31 (95% CI 0.75 to 2.29) and 1.30 (95% CI 0.79 to 2.13), respectively.

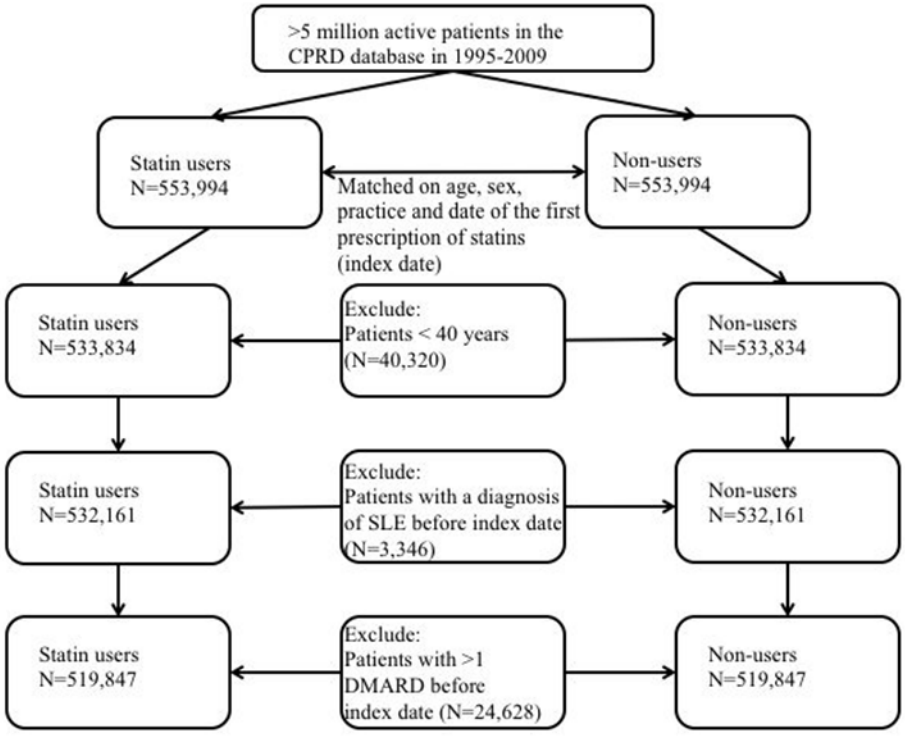
Table 3 shows several potential factors that may have influenced the risk of developing SLE after statin exposure. No clear effect modifiers for the association among current, recent and past statin exposures and the risk of developing SLE were found. It seems that patients with a history of cardiovascular disease or diabetes who currently used statins, irrespective of the duration of use, had a decreased risk of developing SLE.

We observed also a tendency towards a decreased risk of developing SLE in patients aged 61–80 year and women who currently continued statin therapy for >1 year.

Table 4 shows the results of five different sensitivity analyses. Since our definition of SLE[19] was rather unspecific, we subsequently used three more specific definitions. These analyses showed similar results. The decreased risk of SLE for current users who continued therapy for >1 year, however, was not found anymore. In addition, the sensitivity analysis where we excluded the first 2 years after initiation of statin treatment showed that current statin use, irrespective of the duration of the therapy, was associated with a decreased risk of SLE.

DISCUSSION

Our study demonstrated no association between current statin use and the risk of developing SLE among patients aged ≥40



CPRD, Clinical Practice Research Datalink; SLE, systemic lupus erythematosus; DMARD, disease modifying anti-rheumatic drug

Figure 2 Flow diagram showing the selection of the study population from the Clinical Practice Research Datalink.

Table 3 Confounding and modifying effects of systemic lupus erythematosus risk in statin users versus non-statin users

			Adjusted HR (95% CI)*				
	SLE (n)	IR†	Past statin use	Recent statin use	Current statin use	Current statin use≤1 year	Current statin use>1 year
By age, years							
40–60	109	0.7	1.44 (0.65 to 3.18)	2.23 (0.98 to 5.03)	1.07 (0.61 to 1.88)	1.35 (0.68 to 2.69)	0.92 (0.49 to 1.73)
61–80	137	0.7	1.12 (0.58 to 2.16)	0.69 (0.29 to 1.60)	0.51 (0.31 to 0.82)	0.75 (0.41 to 1.38)	0.40 (0.23 to 0.69)
>80	11	0.3	2.37 (0.22 to 24.73)	4.22 (0.56 to 31.80)	1.46 (0.29 to 7.53)	1.48 (0.23 to 9.31)	1.46 (0.21 to 9.99)
By sex							
Women	202	1.0	1.36 (0.79 to 2.33)	1.52 (0.82 to 2.82)	0.73 (0.49 to 1.10)	1.02 (0.62 to 1.68)	0.59 (0.38 to 0.94)
Men	55	0.3	1.01 (0.28 to 3.62)	0.73 (0.19 to 2.86)	0.79 (0.37 to 1.73)	0.94 (0.37 to 2.37)	0.71 (0.30 to 1.66)
By any previous history of disease							
No previous cardiovascular disease	191	0.7	1.62 (0.93 to 3.32)	1.75 (0.92 to 3.32)	0.96 (0.64 to 1.45)	1.32 (0.79 to 2.21)	0.77 (0.48 to 1.24)
Previous cardiovascular disease	66	0.6	0.45 (0.16 to 1.27)	0.39 (0.13 to 1.21)	0.27 (0.14 to 0.52)	0.32 (0.14 to 0.72)	0.24 (0.12 to 0.50)
No previous cardiovascular risk factor‡	102	0.6	1.69 (0.77 to 3.74)	0.60 (0.14 to 2.62)	0.63 (0.33 to 1.20)	0.71 (0.31 to 1.65)	0.56 (0.24 to 1.29)
Previous cardiovascular risk factor	155	0.7	1.49 (0.76 to 2.94)	1.87 (0.93 to 3.76)	0.95 (0.57 to 1.58)	1.37 (0.74 to 2.53)	0.80 (0.46 to 1.37)
No previous hyperlipidaemia	189	0.6	0.93 (0.47 to 1.82)	1.47 (0.75 to 2.85)	0.73 (0.49 to 1.10)	0.97 (0.58 to 1.61)	0.62 (0.38 to 1.00)
Previous hyperlipidaemia	68	0.8	5.33 (0.69 to 41.14)	2.73 (0.32 to 23.14)	2.02 (0.27 to 14.82)	2.69 (0.35 to 20.89)	1.71 (0.23 to 12.95)
No previous hypertension	139	0.7	1.61 (0.86 to 3.01)	0.56 (0.19 to 1.68)	0.71 (0.42 to 1.18)	0.85 (0.45 to 1.61)	0.61 (0.33 to 1.11)
Previous hypertension	118	0.7	1.01 (0.43 to 2.36)	1.99 (0.93 to 4.28)	0.80 (0.45 to 1.41)	1.16 (0.58 to 2.32)	0.66 (0.36 to 1.22)
No previous diabetes	218	0.6	1.21 (0.69 to 2.12)	1.42 (0.76 to 2.61)	0.88 (0.60 to 1.28)	1.26 (0.79 to 2.03)	0.68 (0.44 to 1.06)
Previous diabetes	39	0.7	1.10 (0.33 to 3.70)	0.71 (0.17 to 2.99)	0.29 (0.10 to 0.81)	0.29 (0.09 to 0.97)	0.29 (0.10 to 0.85)

*Adjusted for confounders as shown in table 2.
†Incidence rate is calculated for each recency of statin use by dividing the number of events by the person time within each given recency of use.
‡Cardiovascular risk factor included previous hyperlipidaemia, hypertension and diabetes.
IR, incidence rate (per 10 000 person-years); SLE, systemic lupus erythematosus.

years. However, we did find a 38% decreased risk of developing SLE in current users who continued their therapy for >1 year, although this finding of a decreased SLE risk disappeared in the sensitivity analyses.

We were unable to find any previous studies examining the association between statin use and the risk of developing SLE. However, a propensity score matched cohort study of 6956 pairs of statin users and non-users showed an association between statin use and a lower risk of CTD (approximately 13% of the CTD patients were patients with SLE) during a 1-year study period.[14] In the first year of statin exposure, we found no decrease in the development of SLE which became only significant after 1 year of statin use. Differences between the study by Schmidt and colleagues and our study may be partially explained by the inclusion of other rheumatic diseases and defining statin exposure. In our study, statin exposure was defined by the recency of use and duration (≤1 year and >1 year) within the current statin users, whereas Schmidt and colleagues defined statin use as receiving at least a 90-day supply during a 1-year study period.[14]

Several clinical trials and open-label studies investigating the effects of statins in patients with SLE have found beneficial effects of statin therapy on lipid levels, proinflammatory biomarkers and the endothelial markers.[4 6 32–36] It has been hypothesised that atherosclerosis often develops prematurely among patients with SLE in the setting of chronic inflammation in conjugation with the traditional cardiovascular risk factors.[26] Recently, a US population-based lupus cohort study demonstrated an increased number of cardiovascular events in the 2 years before the diagnosis of SLE, suggesting accelerated atherosclerosis before the onset or diagnosis of SLE.[37] Consistent with our finding of no association between current statin use and the risk of developing SLE, several clinical trials and open-label studies evaluating the effects of statins in patients with SLE found no association between statin use and a change in disease activity score as measured by the Systemic Lupus Erythematosus Disease Activity Index.[4 6 32–36]

Our findings did not show an increased risk of SLE after statin use. In a previous study conducted by our research group, it was found that statin use was more often reported in patients with lupus-like syndrome than in patients who experienced other adverse drug events.[10] The findings of this study were consistent with the results of a study that used the French PharmacoVigilance database.[13] Furthermore, two reviews, including studies of case reports of adverse drug reactions, found an increased risk of developing SLE in statin users.[11 12] A major limitation of these studies was the use of data that were not population based but based on pharmacovigilance databases with selective reporting of adverse drug reactions.

The underlying mechanisms by which statins may interfere the risk of developing rheumatic autoimmune diseases[4–10] are unknown and could not be investigated in our study. Statins are suggested to have anti-inflammatory and immunomodulating properties beyond their lipid-lowering effects.[38 39] Importantly, statins may skew T cell differentiation toward regulatory T cells (Treg) and away from proinflammatory T helper (Th) 17 cells via geranylgeranylation of proteins, resulting in promoting Treg differentiation in the periphery, while blocking Th17 cell differentiation which may be protective against SLE.[39 40] However, it has been suggested that statins may promote a shift in Th1/Th2 balance[12 38] or lead to unstable peripheral Tregs[41 42] and thus

Table 4 Several sensitivity analyses to test the robustness of our findings

Sensitivity analyses	SLE (n)	IR†	Adjusted HR (95% CI)*			
			Past statin use	Recent statin use	Current statin use	Current statin use
					≤1 year	>1 year
1. Restrict to SLE patients with at least two medical records for SLE	83	0.2	2.11 (0.97 to 4.63)	1.83 (0.75 to 4.46)	0.80 (0.42 to 1.52)	1.22 (0.58 to 2.56)	0.53 (0.24 to 1.16)
2. Restrict to SLE patients who were referred to a rheumatologist or received at last one prescription of azathioprine, cyclophosphamide, cyclosporine or methotrexate and/or received at least two prescriptions of corticosteroids or (hydroxy)chloroquine after the first-time diagnosis of SLE	121	0.3	2.37 (0.98 to 4.77)	1.74 (0.76 to 4.01)	1.19 (0.71 to 2.01)	1.54 (0.81 to 2.93)	1.02 (0.57 to 1.82)
3. Restrict to SLE patients with a minimum of two medical records for diagnosis of SLE at least 2 months apart but within a 2-year span.	44	0.1	3.85 (0.94 to 10.58)	2.72 (0.79 to 9.42)	1.13 (0.46 to 2.78)	1.87 (0.66 to 5.34)	0.69 (0.23 to 2.08)
4. Exclude 2 years after initiation of statin treatment	139	0.5	0.90 (0.50 to 1.63)	0.81 (0.37 to 1.82)	0.38 (0.23 to 0.63)	0.26 (0.13 to 0.53)	0.43 (0.26 to 0.72)
5. Shift the event (SLE) date exactly 2 years before the date of the first-time diagnosis of SLE	140	0.4	1.07 (0.54 to 2.13)	0.61 (0.23 to 1.65)	0.74 (0.46 to 1.20)	0.58 (0.31 to 1.06)	0.84 (0.51 to 1.39)

*Adjusted for age, sex, practice, smoking, cardiovascular diseases, hyperlipidaemia, hypertension, diabetes and use of non-steroid anti-inflammatory drugs.
†Incidence rate is calculated for each sensitivity analysis by dividing the number of events by the person time within each given recency of use.
IR, incidence rate (per 10 000 person-years); SLE, systemic lupus erythematosus.

may promote autoimmunity. Statins may not cause autoimmunity by themselves, but they may promote a pre-existing autoimmune-prone condition to progress toward a clinical manifest disease.

Our study has several strengths including the large sample size, representativeness of the population, completeness of follow-up and information on matched non-users, and detailed information on confounders (eg, smoking status) was available. Furthermore, data are prospectively collected in the CPRD and thus not subjected to recall bias.

Our study has also some drawbacks. We used prescription data on statin exposure rather than on actual drug use, which could have resulted in an overestimation of statin use. Furthermore, we used a definition of incident SLE as has been previously used by Somers and colleagues.[19] Although this definition was previously used in the CPRD database, it is rather unspecific for the diagnostic outcome (SLE). Therefore, we performed a series of sensitivity analyses regarding different more specific definitions of SLE. All analyses consistently showed no association between current statin use and the risk of developing SLE. The association between current statin use for >1 year and the decreased risk of developing SLE, however, disappeared when more specific definitions of SLE were used.

Since patients aged ≥40 years should be screened for cardiovascular risk,[43] we investigated the risk of SLE in patients aged ≥40 years using statins. SLE is typically a disease of young women, and we cannot conclude that there is no effect of statins on the risk of developing SLE in young women (ie, <40 years).

We had no information on dietary intake, physical activity and race/ethnicity. Since our study was performed in the UK with a predominantly Caucasian population, knowledge of race/ethnicity may be relevant in other studies as SLE occurs more frequently in blacks.[44] Also, we had limited data on lipid, blood pressure and glucose levels, and inflammatory markers (eg, C reactive protein) which all could be potentially confounders. Our subgroup analyses were limited by limited number of patients and statistical power, and it is likely that some patients with hyperlipidaemia, hyperglycaemia or high blood pressure levels were misclassified. This misclassification typically leads to an underestimate of the treatment effect. Also, ascertainment bias may have occurred as patients starting statin therapy may have had more visits to the general practitioner and blood tests than non-users, thereby increasing the likelihood of detecting more abnormalities (eg, SLE).[45 46] Nonetheless, our study did not show an increased risk of developing SLE in current statin users who continued the therapy for ≤1 year. Another limitation was that SLE may have been present and was not well documented before the start of statin use. We defined the onset date of SLE by the first medical record for SLE, but the onset date of symptoms is unknown in our study. The median time between onset of symptoms to diagnosis of SLE of may be as long as 2 years.[31] Our sensitivity analysis where we excluded the 2 years following the initiation of statin treatment showed similar results with regard to long-term statin use and the risk of developing SLE.

In summary, this is the first observational study assessing the risk of developing SLE with changes in statin exposure over time. We found that current statin use is not associated with an increased risk of developing SLE among patients aged ≥40 years. We observed a decreased SLE risk among current statin users who continued their therapy for >1 year, but further research is needed to substantiate this signal of a long-term effect of statin on SLE risk.

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Contributors HJIDJ contributed to the concept and design of the study, performed the data analysis, contributed to the interpretation of the results and drafted the manuscript. TVS initiated and obtained the funding for the project to which the study presented belongs, contributed to the concept and design of the study and interpretation of the results, provided background information for the study and reviewed the manuscript. AL and FDV contributed to the concept and design of the study, performed the data analysis and reviewed the manuscript. RJV provided background information for the study and reviewed the manuscript. HVL initiated and obtained the funding for the project to which the study presented belongs, provided background information for the study and reviewed the manuscript. OHK contributed to the concept and design of the study and interpretation of the results, provided background information for the study and reviewed the manuscript. JWCT contributed to the concept and design of the study and interpretation of the results, provided background information for the study and drafted and reviewed the manuscript.

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Patient consent For the present study, a separate ethical approval was not required, since the patients were not directly involved in formulating the research question nor were patients actively involved in the design and/or conduct of the research. The CPRD Group has obtained ethical approval from a National Research Ethics Service Committee for all purely observational research using anonymised CPRD data, namely, studies which do not include patient involvement (which is the vast majority of CPRD studies).

Ethics approval National Research Ethics Service Committee (NRES); CPRD Independent Scientific Advisory Committee (ISAC)

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EXTENDED REPORT

The yield of a positive MRI of the spine as imaging criterion in the ASAS classification criteria for axial spondyloarthritis: results from the SPACE and DESIR cohorts

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ABSTRACT

Objectives To assess the prevalence of spinal inflammation on MRI in patients with chronic back pain (CBP) of maximally 3 years duration and to evaluate the yield of adding a positive MRI-spine as imaging criterion to the Assessment of SpondyloArthritis international Society (ASAS) classification criteria for axial spondyloarthritis (axSpA).

Methods Baseline imaging of the sacroiliac joints (X-SI), MRI of the sacroiliac joints (MRI-SI) and MRI-spine were scored by ≥2 experienced central readers per modality in the SPondyloArthritis Caught Early (SPACE) and DEvenir des Spondylarthropathies Indifférenciées Récentes (DESIR) cohorts. Inflammation suggestive of axSpA was assessed in the entire spine. A positive MRI-spine was defined by the presence of ≥5 inflammatory lesions. Alternative less strict definitions were also tested.

Results In this study, 541 and 650 patients with CBP from the SPACE and DESIR cohorts were included. Sacroiliitis on X-SI and MRI-SI was found in 40/541 (7%) and 76/541 (14%) patients in SPACE, and in DESIR in 134/650 (21%) and 231/650 (36%) patients, respectively. In SPACE and DESIR, a positive MRI-spine was seen in 4/541 (1%) and 48/650 (7%) patients. Of the patients without sacroiliitis on imaging, 3/447 (1%) (SPACE) and 8/382 (2%) (DESIR) patients had a positive MRI-spine. Adding positive MRI-spine as imaging criterion led to new classification in only one patient in each cohort, as the other patients already fulfilled the clinical arm. Other definitions of a positive MRI-spine yielded similar results.

Conclusion In two cohorts of patients with CBP with a maximum symptom duration of 3 years, a positive MRI-spine was rare in patients without sacroiliitis on MRI-SI and X-SI. Addition of MRI-spine as imaging criterion to the ASAS axSpA criteria had a low yield of newly classified patients and is therefore not recommended.

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INTRODUCTION

MRI has become of great interest as a diagnostic tool in the evaluation of patients suffering from chronic back pain (CBP) suspected of axial spondyloarthritis (axSpA).[1–8] For years, clinicians (and researchers) relied on conventional imaging of the sacroiliac joints (X-SI) to detect abnormalities suggestive of axSpA.[9] However, X-SI only captures structural damage, which generally takes months to years to develop, leaving aside that not all patients will develop structural bone damage in the axial skeleton. This jeopardises the early recognition of patients with axSpA. MRI, however, can visualise both inflammation and structural damage, and therefore may help in recognising patients with axSpA who do not (yet) have radiographic sacroiliitis.

The Assessment of SpondyloArthritis international Society (ASAS) classification criteria for axSpA comprise a criteria set combining the information obtained from patient history taking, physical examination, laboratory testing and imaging (X-SI and MRI of the sacroiliac joints).[10] A pivotal aspect of these criteria is the 'two arms' concept, commonly referred to as the 'imaging arm' and the 'clinical arm'. According to the ASAS classification criteria, patients with CBP with the onset of back pain at <45 years of age are classified via the imaging arm when (1) radiographic sacroiliitis plus ≥1 spondyloarthritis (SpA)-feature is present or (2) sacroiliitis on MRI plus ≥1 additional SpA-feature is present also defined as 'positive MRI'. Currently, a positive MRI in the ASAS classification criteria is solely based on the presence of inflammatory lesions in the sacroiliac joints and positive findings are defined according to the ASAS definition: one bone marrow oedema (BME) lesion highly suggestive of axSpA present on ≥2 consecutive slices or ≥2 BME lesions highly suggestive of axSpA on a single slice.[11 12]

Several studies have shown that besides inflammation on MRI of the sacroiliac joints (MRI-SI), inflammatory lesions in the spine on MRI (MRI-spine) may also occur.[8 13] Remarkably, in a phase III, multicentre, randomized, controlled trial of adalimumab versus placebo (ABILITY-1) spinal inflammation—in the absence of sacroiliitis on MRI—was observed in about half of the patients with longer disease duration and clinically active disease.[14] A consensus definition for a positive MRI-spine was developed by the ASAS/Outcome

Measures in Rheumatology (OMERACT) MRI working group.[8] In this consensus definition a positive MRI-spine is described as the presence of ≥3 inflammatory lesions in the vertebrae, whereas each lesion needs to be present on ≥2 consecutive slices. De Hooge *et al* recently proposed a cut-off value of ≥5 inflammatory lesions that defines a positive MRI-spine with higher specificity of ≥95% (ie, <5% patients without axSpA with a positive MRI-spine).[15]

The main objectives of this study were to evaluate the presence of spinal inflammatory lesions on MRI in patients with a maximum CBP duration of 3 years in two different cohorts, the SPondyloArthritis Caught Early (SPACE) cohort and the DEvenir des Spondylarthropathies Indifférenciées Récentes (DESIR) cohort. In addition, we assessed the added value of spinal inflammatory lesions on MRI, represented in various definitions of a positive MRI-spine, as imaging criterion in the ASAS classification criteria for axSpA.

METHODS

Cohorts and clinical assessments

For this analysis, baseline data from the SPACE and the DESIR cohorts were used which have been described in detail before.[16 17]

In summary, SPACE is an ongoing observational cohort in which patients with a minimum age of 16 years with short-term CBP (≥3 months, ≤2 years and an onset <45 years) are included. Patients are recruited from multiple Rheumatology centres in Europe: the Netherlands, Norway, Italy and Sweden. The clinical database used for the current study was locked on 30 September 2016.

DESIR is a longitudinal cohort for which the inclusion period was from December 2007 until April 2010 in 25 centres in France. Patients aged 18–50 years with inflammatory back pain (IBP) according to the Calin[18] or Berlin[19] criteria, persisting ≥3 months but <3 years, suggestive of axSpA according to the treating rheumatologist, were included. The clinical database used for the current study was locked on 30 April 2015.

As part of both study protocols and to determine fulfilment of the ASAS-criteria, presence of all SpA-features was assessed. These include human leucocyte antigen B27 (HLA-B27), positive family history of SpA, IBP, psoriasis, peripheral arthritis, dactylitis, heel enthesitis, acute anterior uveitis, inflammatory bowel disease (IBD), good response to non-steroidal anti-inflammatory drugs (NSAIDs), elevated C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) and sacroiliitis on X-SI and MRI-SI, all according to the published definitions.

In both cohorts, informed consent forms from all study participants as well as approval from all local medical ethical committees were obtained beforehand.

IMAGING

Detailed descriptions of the applied scoring methods in both cohorts have been published previously. In brief, all available baseline imaging modalities were scored by experienced central readers. In the SPACE cohort, each imaging modality was scored by three central readers. In the DESIR cohort, each imaging modality was scored by two central readers and an adjudicator in case of disagreement. The readers were blinded for all clinical and laboratory data as well as the other imaging modalities, and the various modalities were scored separately.

Imaging assessments

Sacroiliac joints were evaluated on pelvic radiographs using the modified New York criteria in which sacroiliitis is defined as grade ≥2 bilaterally or grade 3–4 unilaterally.[9] MRI-SI and MRI-spine were performed on a 1T–1.5T scanner. For both modalities, Short Tau Inversion Recovery (TR2500-4000/TE600) and T1-weighted Turbo Spin-Echo (TR500-700/TE10-55) sequences were acquired. MRI-SI were performed in coronal oblique plane and MRI-spine in sagittal plane with a slice thickness of 4 mm. Readers provided an overall judgement on a positive MRI-SI (yes/no) according to the ASAS definition.[11] X-SI and MRI-SI were considered positive if ≥2 readers agreed.

MRI-SPINE

In the SPACE cohort, a positive MRI-spine was defined by the presence of ≥5 corner BME lesions highly suggestive of axSpA each visible on ≥2 consecutive slices and if ≥2 readers agreed.[15] A positive MRI-spine was also defined by the ASAS consensus definition (≥3 corner BME lesions on ≥2 consecutive slices) and if ≥2 readers agreed.[8]

In addition, spinal inflammation suggestive of axSpA was scored according to the Spondyloarthritis Research Consortium of Canada (SPARCC) method. Presence of BME was marked on three consecutive sagittal slices per vertebral unit (VU).[20] In case of BME lesions on more than three slices, the three most affected consecutive slices were selected. The 23 VUs each are divided into four quadrants and assessed for the presence (score of 1) or absence (score of 0) of inflammatory lesions (maximum possible score 276). In addition, for each VU, a score of 1 could be assigned to the presence of an 'intense' signal (maximum possible score of 69), defined as a signal as intense as a blood vessel and a 'deep' lesion (maximum possible score of 69), defined as a homogeneous, unequivocal increase in STIR signal extending at least 1 cm from the vertebral end plate. In total, the maximum possible total SPARCC-score is 414. We tested two additional definitions of a positive MRI-spine using the SPARCC-score (including intensity and depth of a lesion): first, in which a positive MRI-spine was defined by a SPARCC-score ≥5 by≥2 readers and second, in which a positive MRI-spine was defined by a mean SPARCC-score ≥5 of all 3 readers. All four of the described definitions of a positive MRI-spine (ie, ≥5 BME lesions on ≥2 consecutive slices; ≥3 BME lesions on ≥2 consecutive slices (ASAS definition); SPARCC score ≥5 by ≥2 readers and mean SPARCC score ≥5 of 3 readers) were applied to assess the added value of a positive MRI-spine in the ASAS classification criteria.

In the DESIR cohort, spinal inflammation suggestive of axSpA was scored according to the Berlin method.[21] In total, the maximum possible total Berlin-score is 69. Additionally, the 23 VUs were each divided into four quadrants and assessed for the presence (score of 1) or absence (score of 0) of inflammatory lesions. Inflammatory lesions were scored when present on ≥2 consecutive slices. When central readers disagreed on the presence of inflammation, an adjudicator provided scores for all 23 VUs. A positive MRI-spine was defined by the presence of ≥5 corner inflammatory lesions and if ≥2 readers agreed. A positive MRI-spine was also defined by the ASAS consensus definition (ie, ≥3 inflammatory lesions) and when ≥2 readers agreed. Only these two definitions were used to assess the additional value of a positive MRI-spine in the ASAS classification criteria, as the SPARCC method was not applied.

DATA ANALYSIS

Baseline demographic and clinical characteristics are presented using descriptive statistics for both the SPACE and DESIR cohort (reported in this order). Results are presented as mean and SD

Table 1 Baseline clinical and demographic characteristics of patients with CBP suspected of axSpA in the SPACE and DESIR cohorts

	SPACE (n=541)	DESIR (n=650)
Male	186 (34)	301 (46)
Age (years) at onset of back pain	29.1 (8.4)	33.7 (8.7)
Symptom duration, months	13.0 (7.1)	18.2 (10.5)
HLA-B27 positive	211 (39)	381 (59)
Number of SpA-features, mean (SD)	2.4 (1.6)	4.0 (1.4)
IBP	358 (66)	650 (100)*
Good response to NSAIDs†	217/521 (42)	518 (80)
Positive family history of SpA‡	235 (43)	248 (38)
Peripheral arthritis	76 (14)	366 (56)
Dactylitis	28 (5)	82 (13)
Enthesitis	105 (19)	322 (50)
Acute anterior uveitis	42 (8)	55 (8)
IBD	35 (6)	29 (4)
Psoriasis	57 (11)	103 (16)
Elevated CRP/ESR	138 (26)	257 (40)
ASAS axSpA classification	207 (38)	411 (63)
Imaging arm (with/without clinical arm)		
Sacroiliitis on MRI (mNY-MRI+)	53 (10)	115 (21)
Sacroiliitis on radiograph (mNY+MRI+/MRI-)	39 (7)	121 (21)
Clinical arm only	115 (21)	175 (31)

Results are presented as mean (±SD) or number (%).
*Inclusion criterion.
†Back pain not present anymore or is much better 24–48 hours after a full dose of NSAID.
‡Patient reported presence in first-degree or second-degree relatives of any of the following: ankylosing spondylitis, acute anterior uveitis, reactive arthritis, IBD, or psoriasis.
ASAS, Assessment of SpondyloArthritis international Society; axSpA, axial spondyloarthritis; CBP, chronic back pain; CRP, C reactive protein; DESIR, DEvenir des Spondylarthropathies Indifférenciées Récentes; ESR, erythrocyte sedimentation rate; HLA-B27, human leucocyte antigen B27; IBP, inflammatory back pain; IBD, inflammatory bowel disease; mNY, modified New York; NSAIDs, non-steroidal anti-inflammatory drugs; SpA; spondyloarthritis; SPACE, SPondyloArthritis Caught Early.

or numbers and percentages (%). Agreement (Cohen's kappa, ƙ) between readers regarding a positive MRI-SI and MRI-spine was calculated.

Stata 14 (StataCorp) was used for data analysis.

RESULTS

For 541/639 and 650/708 patients from the SPACE and DESIR cohorts, complete scores of MRI-SI, MRI-spine, X-SI and clinical data were available for analyses. Of these, 34% and 46% were male, mean symptom duration (SD) was 13.0 (7.1) and 18.2 (10.5) months and mean age was 29.1 (8.4) and 33.7 (8.7) years (table 1), respectively.

Inter-reader reliability

The agreement between the readers in the SPACE and DESIR cohorts regarding a positive MRI-spine according to the ASAS definition was ƙ=0.66 and ƙ=0.58, respectively. Inter-reader agreement for a positive MRI-spine according to the cut-off of ≥5 BME lesions in SPACE was ƙ=0.60 and in DESIR ƙ=0.49. For MRI-SI, the reliability between the readers in the SPACE cohort was ƙ=0.76 and in the DESIR cohort ƙ=0.73.

In both cohorts, the majority of patients did not show abnormalities on MRI-SI, MRI-spine and X-SI (444/541 (82%) and 374/650 (58%), SPACE and DESIR, respectively) (table 2). In total, 40/541 (7%) and 134/650 (21%) patients in the SPACE and DESIR cohorts had radiographic sacroiliitis (figure 1). Of the remaining patients without radiographic sacroiliitis, 447/501 (89%) and 382/516 (74%) in SPACE and DESIR had no evidence of inflammation on MRI-SI. In both cohorts, an isolated positive MRI-spine (without sacroiliitis on either MRI-SI or X-SI), applying the cut-off of ≥5 BME lesions, was found in 3/447 (1%) and 8/382 (2%) patients (table 2).

Table 2 Cross-tabulations of baseline sacroiliac imaging (MRI-SI and X-SI) and MRI-spine of patients with CBP suspected of axSpA in the SPACE (n=541) and DESIR cohorts (n=650)

SPACE

	Any SI-imaging (MRI-SI/X-SI)		
MRI of the spine, cut-off≥5 BME lesions	**Positive**	**Negative**	**Total**
Positive	1	3	4
Negative	93	444	537
Total	94	447	541

DESIR

	Any SI-imaging (MRI-SI/X-SI)		
MRI of the spine, cut-off ≥5 BME lesions	**Positive**	**Negative**	**Total**
Positive	40	8	48
Negative	228	374	602
Total	268	382	650

axSpA, axial spondyloarthritis; BME, bone marrow oedema; CBP, chronic back pain; DESIR, DEvenir des Spondylarthropathies Indifférenciées Récentes; SI, sacroiliac joints; SPACE, SPondyloArthritis Caught Early; X, radiography.

Two of the three patients in the SPACE cohort with a positive MRI-spine (definition of ≥5 BME lesions) but without sacroiliitis on MRI-SI and X-SI, already fulfilled the clinical arm of the ASAS-criteria, were male and their mean (SD) number of SpA-features was 3.5 (0.7) (table 3). When hypothetically adding a positive MRI-spine as imaging criterion to the ASAS-criteria for axSpA, the remaining patient could be additionally classified via the imaging arm. Therefore, 447 MRIs of the spine have to be performed to additionally classify this single patient. This patient was a male with one SpA-feature (ie, good response to NSAIDs).

In the DESIR cohort, eight patients had a positive MRI-spine (definition of ≥5 BME lesions) without having any signs of inflammation or structural damage suggestive of axSpA on MRI-SI and X-SI (figure 1). As patients within the DESIR cohort are included until the age of 50 and the ASAS-criteria are, in principle, to be applied to patients≤45 years of age with CBP, we report this separately. In total, seven out of eight patients already fulfilled the clinical arm of the ASAS-criteria: 6/8 patients≤45 years and 1/8>45 years (table 3). Of these seven patients, one was male and the mean (SD) number of SpA-features was 3.7 (2.0). When hypothetically adding a positive MRI-spine as imaging criterion to the ASAS-criteria for axSpA, the remaining patient could be additionally classified via the imaging arm. Therefore, 382 MRI of the spine have to be performed to additionally classify this single patient. This was a HLA-B27 negative male patient with the following six SpA-features: positive family history of SpA, IBP, good response to NSAIDs, heel enthesitis, IBD and peripheral arthritis.

Alternative definitions of a positive MRI-spine

SPARCC-score

Two other definitions of a positive MRI-spine were tested in the SPACE cohort using the SPARCC-score (table 4). When

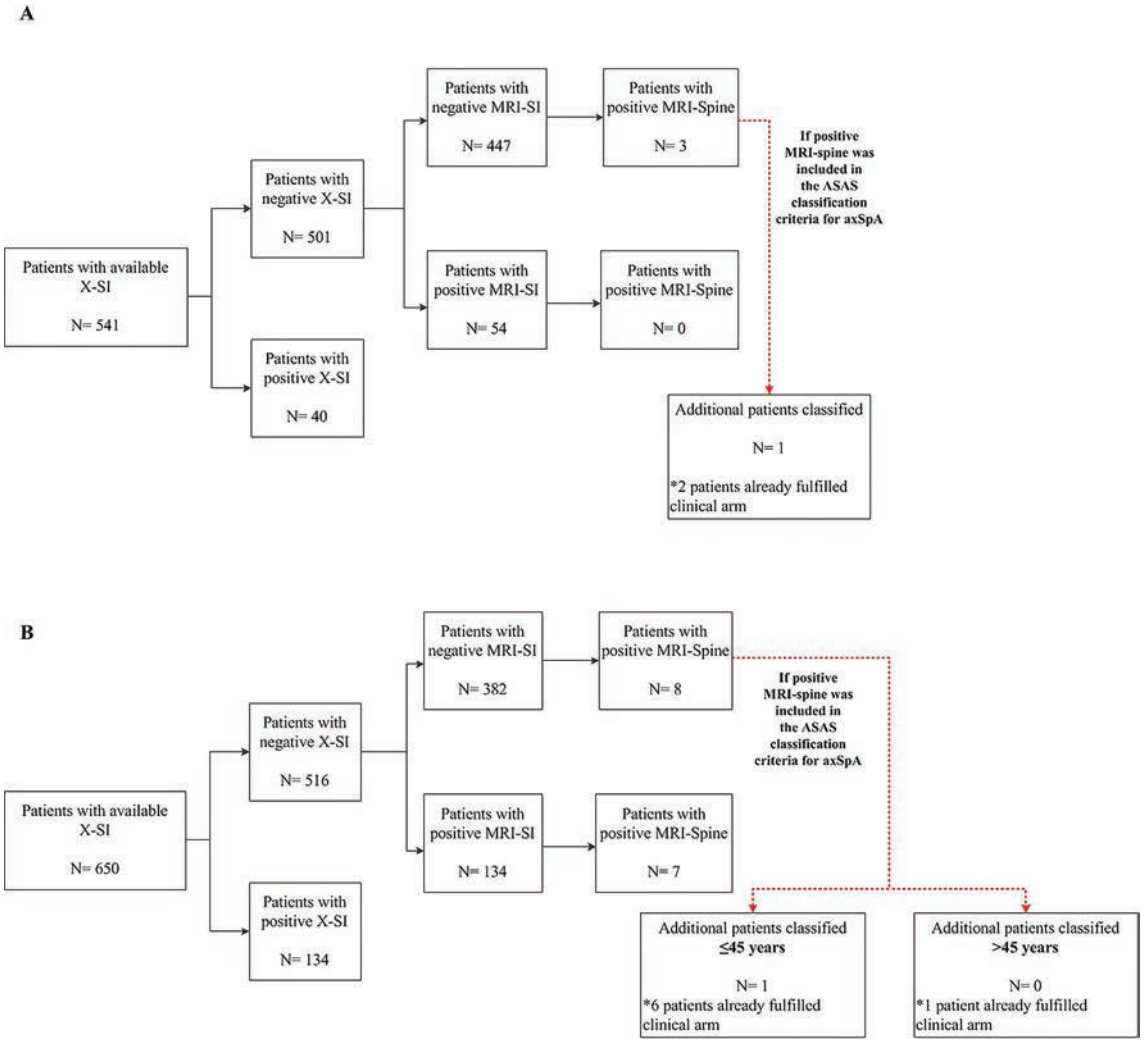

Figure 1 ASAS classification of patients with CBP with negative MRI-SI and positive MRI-spine defined by ≥5 inflammatory lesions and the effect of adding positive MRI-spine as an imaging criterion to the ASAS axSpA criteria on classification of patients. (A) Additional classification of patients with CBP in the SPACE cohort. (B) Additional classification of patients with CBP in the DESIR cohort. ASAS, Assessment of SpondyloArthritis international Society; axSpA, axial spondyloarthritis; CBP, chronic back pain; SPACE, SPondyloArthritis Caught Early.

a positive MRI-spine was defined as a SPARCC-score of ≥5 by ≥2 central readers, a total of 21/447 (5%) patients without sacroiliitis on MRI-SI and X-SI had a positive MRI-spine (see online supplementary figure S1). Eight patients (38%) already fulfilled the clinical arm and when adding MRI-spine to the ASAS-criteria, 13/21 (62%) patients could be additionally classified via the imaging arm. Of these 13 patients almost half had ≥2 SpA-features. Most frequently reported SpA-features were good response to NSAIDs, positive family history of SpA and IBP (data not shown). When a positive MRI-spine was defined as a mean SPARCC-score ≥5 of all readers, a total of 13/447 (3%) patients had a positive MRI-spine (see online supplementary figure S2). Of these, 7/13 (54%) already fulfilled the clinical arm and the remaining six (46%) patients could be additionally classified via the imaging arm when adding positive MRI-spine to the ASAS-criteria. Half of these patients had ≥2 SpA-features and positive family history of SpA and IBP were among the frequent features (data not shown).

ASAS consensus definition

According to the ASAS consensus definition (≥3 spinal inflammatory lesions on ≥2 consecutive slices), 5/447 (1%) patients from the SPACE cohort and 25/382 (7%) patients from the DESIR cohort had a positive MRI-spine without sacroiliitis on MRI-SI and X-SI (table 4). Of these, 3/5 (60%) patients and 18/25 (72%) patients (16 patients≤45 years and two patients>45 years) already fulfilled the clinical arm (see online supplementary figure S3). The addition of a positive MRI-spine as a criterion in the ASAS-criteria would have resulted in the additional classification of 2/5 (40%) and 7/25 (28%) patients (six patients≤45 years and one patient >45 years) for SPACE and DESIR, respectively.

DISCUSSION

In patients with CBP of a maximum duration of 3 years and suspected of axSpA from two different cohorts (SPACE and DESIR), the overall prevalence of spinal inflammation is low. In addition, spinal inflammation in the absence of sacroiliitis on MRI-SI and X-SI occurred in a very small proportion of patients. Consequently, adding a positive MRI-spine (represented in various definitions) as an imaging criterion to the ASAS-criteria resulted in a very low percentage of newly classified patients. Considering the number of MRI-spine needed to additionally classify a few patients, the longer scanning time for the patient and higher costs, we conclude that the yield of adding

Table 3 Baseline clinical characteristics and disease activity of patients with CBP additionally classified according to the ASAS axSpA criteria by baseline presence of sacroiliitis on MRI and spinal inflammation defined by ≥5 inflammatory lesions

SPACE	Imaging arm (n=1)	Clinical arm (n=2)
Male, n (%)	1 (100)	2 (100)
HLA-B27 positive, n (%)	0	2 (100)
Number of SpA features	1	3.5 (0.7)
SPARCC spine score, 0–414	13.3	14 (2.4)
Disease activity		
BASDAI†	3.4	2.9 (0)
CRP≥5 mg/L, n (%)	0	1 (50)
CRP, mg/L	3	30 (41)
ASDAS-CRP†	2.4	4.1 (0)
DESIR	**Imaging arm (n=1)**	**Clinical arm* (n=7)**
Male, n (%)	1 (100)	1 (14)
HLA-B27 positive, n (%)	0	7 (100)
Number of SpA features	6	3.7 (2)
Berlin spine score, 0–69	9.5	8.5 (2)
Disease activity		
BASDAI	2.9	5 (1.4)
CRP≥5 mg/L, n (%)	0	2 (29)
CRP, mg/L	4	5.3 (4.2)
ASDAS-CRP	1.8	2.8 (0.8)

Unless stated otherwise, results are presented as mean ± SD (range).
*Patients already fulfilling the clinical arm of the ASAS-criteria, 1/7 patients was >45 years of age.
†n=1 for patients already fulfilling the clinical arm.
ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial spondyloarthritis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CBP, chronic back pain; CRP, C reactive protein; DESIR, DEvenir des Spondylarthropathies Indifférenciées Récentes; HLA-B27, human leucocyte antigen B27; MRI-SI, MRI sacroiliac joints; SPARCC, Spondyloarthritis Research Consortium of Canada.

MRI-spine to the ASAS-criteria is unacceptably low in relation to the number of MRI-spine needed to be performed in patients with early disease.

Multiple studies assessed the presence of spinal inflammation in different patient groups (eg, non-radiographic axSpA); however, to the best of our knowledge, this is the first study to report on the value of a positive MRI-spine in classifying patients according to the ASAS-criteria using different definitions in patients with recent onset CBP. Our findings are in line with a recent study by Weber *et al* in which they state that the combination of MRI-spine and MRI-SI has little incremental value compared with MRI-SI alone.[22] Using global assessment in patients with non-radiographic axSpA and normal MRI-SI, approximately 20% of MRI-spine were judged as being consistent with axSpA. However, reading of MRI-spine in non-specific back pain patients and healthy controls yielded similar percentages.

In the SPACE and DESIR cohorts, the majority of patients with only spinal inflammation (≥5 BME lesions and normal MRI-SI and X-SI) already fulfilled the clinical arm of the ASAS-criteria (2/3 and 6/7, respectively). The remaining patients, hypothetically classified via the imaging arm solely because of a positive MRI-spine, can either be misclassified or truly 'missed' cases.

We did not formally investigate the role of MRI-spine in diagnosis making in routine clinical practice and this may still be considered as an imaging tool in the differential diagnosis of patients with CBP or in the confirmation of the diagnosis in specific patients.

This study has several strengths. Two independent early axSpA cohorts were examined in which patients with CBP have been assessed according to a similar standardised protocol. Both cohorts were rather complete in terms of images being present and scored by multiple readers and the findings in both cohorts were similar providing credit to the robustness of our data. Furthermore, the observation that in both cohorts several definitions of a positive MRI-spine in the ASAS-criteria for axSpA yielded comparable misclassifications strengthens the validity of the findings.

It can be seen as a limitation that we only used patients with short symptom duration. Consequently, the results can only be extrapolated to this group of patients. However, we feel that this is the most applicable group to test the usefulness of MRI-spine as part of the ASAS classification criteria as abnormalities on imaging of the sacroiliac joints are more likely to become positive with longer symptom duration. Moreover, our data are very much in line with the data from Weber *et al* in a study with more established disease.

In summary, in both the SPACE and the DESIR cohorts, a positive MRI-spine in patients with CBP suspected of early axSpA was infrequent. Furthermore, spinal inflammation in the absence

Table 4 Patients additionally classified through positive MRI-spine according to four different definitions and diagnosis of axSpA in the SPACE and DESIR cohorts

SPACE	SPARCC-score≥5, ≥2 readers n=21	SPARCC-score≥5, mean of 3 readers (n=13)	≥3 BME lesions, ≥2 consecutive slices (n=5)	≥5 BME lesions, ≥2 consecutive slices (n=3)
Already classified by clinical arm	8 (38%)	7 (54%)	3 (60%)	2 (67%)
axSpA diagnosis	7/8	7/7	3/3	2/2
Additionally classified by imaging arm	13 (62%)	6 (46%)	2 (40%)	1 (33%)
axSpA diagnosis	5/13	2/6	1/2	0
DESIR	**SPARCC-score≥5, ≥2 readers n/a**	**SPARCC-score≥5, mean of 3 readers n/a**	**≥3 BME lesions, ≥2 consecutive slices (n=25)**	**≥5 BME lesions, ≥2 consecutive slices (n=8)**
Already classified by clinical arm*	–	–	18 (72%)	7 (88%)
axSpA diagnosis†	–	–	13/18	5/7
Additionally classified by imaging arm*	–	–	7 (28%)	1 (12%)
axSpA diagnosis†	–	–	3/7	1/1

*Classification for all patients<50 years.
†Diagnosis based on rheumatologist's level of confidence regarding axSpA diagnosis ≥8.
axSpA, axial spondyloarthritis; BME, bone marrow oedema; DESIR, DEvenir des Spondylarthropathies Indifférenciées Récentes; HLA-B27, human leucocyte antigen B27; n/a, not assessed; SPACE, SPondyloArthritis Caught Early; SPARCC, Spondyloarthritis Research Consortium of Canada.

of sacroiliitis was observed in very few patients. In this early disease stage, the addition of a positive MRI-spine as imaging criterion to the ASAS classification criteria for axSpA yielded a very low number of newly classified patients in both cohorts. Therefore, the use of MRI-spine is not recommended in the classification of patients with CBP suspected of axSpA.

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Contributors ZEZ designed the study, performed the statistical analyses, interpreted the data, drafted and revised the manuscript. DvdH and FvG designed the study, interpreted the data and revised the manuscript. All authors contributed to the acquisition of data, revised the manuscript and approved the final manuscript.

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EXTENDED REPORT

Survival benefit of statin use in ankylosing spondylitis: a general population-based cohort study

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ABSTRACT

Objectives Recent studies have shown an increase in both cardiovascular and all-cause mortality in ankylosing spondylitis (AS). We examined the potential survival benefit of statin use in AS within a general population context.

Methods We performed an incident user cohort study with time-stratified propensity score matching using a UK general population database between 1 January 2000 and 31 December 2014. To account for potential confounders, we compared propensity score-matched cohorts of statin initiators and non-initiators using 1-year cohort accrual blocks. The variables used to create the propensity score model included disease duration, body mass index, lifestyle factors, comorbidities and medication use.

Results Using unmatched AS cohorts, statin initiators (n=1430) showed a 43% higher risk of mortality than non-initiators (n=1430) (HR=1.43; 95% CI 1.12 to 1.84). After propensity score matching, patients with AS who initiated statins (n=1108) had 96 deaths, and matched non-initiators (n=1108) had 134 deaths over a mean follow-up of 5.3 and 5.1 years, respectively. This corresponded to mortality rates of 16.5 and 23.8 per 1000 person-years (PY), respectively, resulting in an HR of 0.63 (95% CI 0.46 to 0.85) and an absolute mortality rate difference of 7.3 deaths per 1000 PY (95% CI 2.1 to 12.5).

Conclusion This general population-based cohort study suggests that statin initiation is associated with a substantially lower risk of mortality among patients with AS. The magnitude of the inverse association appears to be larger than that observed in randomised trials of the general population and in population-based cohort studies of patients with rheumatoid arthritis.

INTRODUCTION

Ankylosing spondylitis (AS) is a chronic inflammatory spondyloarthropathy associated with elevated systemic inflammation and substantial disability unless treated appropriately. With systemic inflammation linked to accelerated atherosclerosis, patients with AS, similar to those with rheumatoid arthritis (RA), are at an increased risk for cardiovascular disease (CVD) and mortality.[1–5] A recent large population-based cohort study showed that patients with AS are at a 60% higher risk for premature mortality compared with the general population.[6] However, little is known about the effect of CVD risk modification and treatment in these patients and the relative benefit compared with the general population.

The mortality benefits of statins, hydroxymethylglutaryl coenzyme A reductase inhibitors, have been well described in the general population, with studies demonstrating a 9%–14% reduction in mortality when statins are used for primary prevention.[7 8] Furthermore, the Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin trial evaluated patients without known CVD but with an elevated C reactive protein and found a 44% reduction (HR 0.56; 95% CI 0.77 to 1.36) in major cardiac events and a 20% reduction in all-cause mortality (HR 0.80; 95% CI 0.67 to 0.97) when patients were treated with rosuvastatin.[9] These studies highlight the potential dual cholesterol-lowering and anti-inflammatory effects of statins.

To that end, in the Trial of Atorvastatin in Rheumatoid Arthritis, the use of statins in patients with RA resulted in improvement in both the Disease Activity Score and swollen joint count as well as inflammatory markers and low-density lipoprotein levels.[10] The RORA-AS study (ROsuvastatin in RA, AS and other inflammatory joint diseases) evaluated the effect of intensive statin treatment with regard to change in carotid plaque height in patients with RA, AS and psoriatic arthritis and found that rosuvastatin induced significant atherosclerotic regression.[11] The pleiotropic effects of statins, independent of their lipid-lowering effects, have been hypothesised to be significant in these inflammatory conditions, but little data are available for AS.

In a recent large general population cohort study, we found that statin initiation was associated with a 20% lower risk of mortality among patients with RA.[12 13] However, it is unknown whether statin use is also associated with a survival benefit in patients with AS and, if so, to what degree. To address this important knowledge gap, we evaluated the relationship between statin initiation and the risk of all-cause mortality in patients with AS in a general population context.

METHODS

Data source

Our source population was The Health Improvement Network (THIN), a computerised medical record database created by general practitioners in the UK. This database currently includes the records of approximately 11.1 million patients (approximately 6.2% of the UK population). The clinical information stored in THIN includes demographic information, diagnoses from specialists' visits and

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hospital admissions, laboratory tests and additional anthropometric and lifestyle information, including height, weight, alcohol use and smoking status. Specific physician diagnoses are coded using the Read classification system.[14] Prescriptions written by physicians are coded using the Multilex classification system, a UK standard drug terminology library that includes data on formulation and strength. THIN data reflect a routine medical practice environment and have been shown to be valid for use in clinical and epidemiological research studies.[15]

Study population

The population studied included patients over 20 years of age who carried a recorded diagnosis of AS between 1 January 2000 and 31 December 2014.[16 17] Patients who were included in the cohort were required to have at least 1 year of enrolment within the general practice prior to the index date of statin initiation. Individuals were excluded if they previously or currently used statin medication or if their covariates (ie, cholesterol level, body mass index (BMI), alcohol use and smoking status) were incomplete.

Propensity score-matched sequential cohort study

To adjust for potential imbalances regarding the baseline characteristics between statin initiators and comparators (non-initiators), we used a propensity score-matched sequential cohort study. To adjust for potential secular trends in statin use and mortality risk, we matched cohorts within 1-year cohort accrual blocks of calendar time (15 blocks from January 2000 to December 2014). Propensity scores (ie, the predicted probability of statin initiation) were estimated using logistic regression within each annual accrual block. Each statin initiator was compared with a propensity score-matched patient who did not start a statin during the same annual accrual block using a 5-digit to 1-digit 'greedy matching' algorithm.[18 19] The statin initiation date was used as the index date for statin initiators, and a random date within the 1-year accrual block was assigned as the index date for non-initiators.

The variables used to create the propensity score model included AS disease duration prior to the index date, sociodemographic factors, lifestyle factors, BMI, medication use, comorbidities, total cholesterol levels and healthcare utilisation. A complete list of variables is displayed in table 1.

Statistical analysis

The outcome of interest was all-cause mortality, defined by the death date recorded in THIN. Statin initiators and non-initiators were followed from the index date until death, the subject's disenrolment from THIN or the end of the study period, whichever came first. Statin initiators and non-initiators both retained their original exposure status throughout the follow-up, mirroring an intention-to-treat approach used in clinical trials, which maintains the comparability of these two exposure groups for the baseline characteristics and provides conservative estimates.

Cox proportional hazard models were used to estimate the association of statin initiation with the risk of mortality, stratified by 1-year accrual blocks. Cumulative mortality curves were generated to display trends in the occurrence of death over time. For all HRs, we calculated 95% CIs, and all p values were two-sided. We also calculated the corresponding mortality rate difference with 95% CIs.[20] All statistical analyses were conducted using SAS V.9.2.

Sensitivity analyses

To examine the potential impact of statin discontinuation among the initiators over time, we performed sensitivity analyses with the follow-up truncated at Year 1, Year 2, Year 3, Year 4 and Year 5. Furthermore, to explore the potential impact of misclassification of AS diagnosis, we also performed sensitivity analyses for the extreme scenario that up to 30% of our AS cohort comprised non-AS individuals (who therefore had the same protective association with statin use as the general population (ie, a 9% and 14%[7 8] relative risk reduction)). Our definition of AS has been previously shown to have a positive predictive value ranging from 72% to 86%.[6 16 21]

RESULTS

Unmatched analysis

Without the use of propensity score matching, 1430 patients with AS who initiated a statin were compared with non-initiators using randomly chosen 1:1 matching. Statin initiators had a higher prevalence of CVD and comorbidities, coronary heart disease risk factors (including higher cholesterol levels) and use of cardiovascular medications than non-initiators, suggesting the expected presence of confounding by indication (table 1). There were 149 deaths over a mean follow-up period of 5.5 years in the statin initiator group and 107 deaths over 5.6 years in the non-initiator group. The corresponding mortality rates were 19.1 (95% CI 16.1 to 22.4) per 1000 person-years (PY) versus 13.3 (95% CI 10.9 to 16.1) per 1000 PY, respectively, with an HR of 1.43 (95% CI 1.12 to 1.84) (see online supplementary figure S1).

Propensity score-matched analysis

With the use of propensity score matching, the baseline characteristics for these patients were well balanced (n=1108 in each group) (all p values for the difference between groups >0.15) (table 1). There were 96 deaths in the statin initiator group and 134 deaths in the non-initiator group over a mean follow-up period of 5.3 and 5.1 years, respectively (table 2 and figure 1). This corresponded to mortality rates of 16.5 and 23.8 per 1000 PYs, respectively, and the HR for mortality associated with statin initiation was 0.63 (95% CI 0.46 to 0.85). The corresponding absolute mortality rate difference was 7.3 deaths per 1000 PYs (95% CI 2.1 to 12.5).

Sensitivity analyses

When we truncated the follow-up duration, the HRs for 1, 2, 3, 4 and 5 years of follow-up were 0.64 (95% CI 0.33 to 1.24), 0.49 (95% CI 0.29 to 0.82), 0.55 (95% CI 0.35 to 0.85), 0.56 (95% CI 0.38 to 0.83) and 0.60 (95% CI 0.42 to 0.85), respectively (table 3). The HRs for mortality associated with statin initiation during the entire follow-up were 0.58 (95% CI 0.40 to 0.83) among men and 0.60 (95% CI 0.14 to 2.51) among women. Similarly, when we stratified the study population according to various age groups, there was no apparent difference in the effect estimates. Finally, for the extreme scenario that up to 30% of our AS cohort comprised non-AS individuals (who therefore had the same protective association with statin use as the general population (ie, a 9% and 14%[7 8] relative risk reduction)), our sensitivity analysis showed a propensity score-matched HR of 0.56 (95% CI 0.41 to 0.77) and 0.58 (95% CI 0.42 to 0.78), respectively.

DISCUSSION

In this large general population-based cohort study of patients with AS, statin initiation was associated with a 37% reduction

Table 1 Baseline characteristics in the unmatched and propensity score-matched cohorts

	Unmatched* cohort		Propensity score-matched cohort	
Baseline characteristics	**Statin initiators (n=1430)**	**Non-initiators (n=1430)**	**Statin initiators (n=1108)**	**Non-initiators (n=1108)**
Demographics				
Age, years	61.7	57.3	61.4	61.8
Male, %	80.0	72.9	79.5	78.5
Socioeconomic deprivation index	2.5	2.3	2.4	2.4
Score†				
AS characteristics				
Disease duration, years	20.2	18.6	20.0	19.6
Lifestyle factors				
BMI, kg/m^2	28.0	26.8	27.8	27.9
Smoking status				
Current, %	21.9	20.6	21.9	20.0
Past, %	34.2	30.0	33.9	34.8
None, %	43.9	49.4	44.1	45.3
Alcohol use				
Current, %	79.2	81.6	79.8	78.2
Past, %	3.9	2.2	3.3	4.5
None, %	16.9	16.2	17.0	17.3
Measures of comorbidity				
Myocardial infarction,%	7.9	1.3	4.6	3.9
Atrial fibrillation, %	4.3	2.1	3.9	3.9
Ischaemic heart disease, %	17.8	3.3	10.9	10.8
Peripheral vascular disease, %	2.0	0.1	1.4	0.8
Congestive heart failure, %	3.1	1.3	2.3	3.1
Valvular heart disease, %	2.4	1.6	2.3	2.3
Stroke, %	4.5	0.5	3.2	2.9
Hypertension, %	54.3	35.0	53.7	55.9
Other circulatory disease, %	1.7	0.4	1.0	1.1
Angina,%	9.0	2.8	5.8	6.0
Chronic obstructive pulmonary disease, %	4.6	2.8	4.7	4.9
Chronic kidney disease (stage >3), %	5.5	3.4	5.6	5.9
Liver disease, %	2.6	2.2	2.3	2.6
Diabetes, %	20.1	7.1	17.8	18.2
Cancer, %	9.2	7.9	8.8	9.0
Pneumonia or infection, %	8.0	7.5	7.7	8.4
Dementia, %	0.2	0.5	0.2	0.3
Depression, %	10.6	11.2	10.6	10.6
Venous thromboembolism, %	2.8	1.5	3.0	2.3
Varicose veins, %	6.8	6.9	6.2	7.0
Hyperlipidaemia, %	19.9	2.7	16.2	16.1
Medications				
Nitrates, %	11.2	2.0	7.0	6.3
Antihypertensive medicine, %	66.9	36.6	62.9	64.5
ACE inhibitors, %	35.0	14.4	31.2	31.5
Beta-blockers, %	27.6	12.5	23.8	22.8
Calcium channel blockers, %	25.9	14.4	24.7	27.2
Aspirin, %	35.4	7.4	27.8	28.4
NSAIDs, %	53.8	47.4	53.5	55.1
Loop diuretics, %	8.0	4.1	6.5	6.3
HCTZ, %	20.3	12.1	19.9	20.1
Thiazide-like diuretic, %	2.9	1.7	3.0	2.9
Potassium-sparing diuretics, %	3.0	1.5	2.2	2.0
Insulin, %	3.6	1.7	3.0	2.6
Glucocorticoids, %	7.8	7.6	7.1	7.1
Anticoagulants, %	3.7	2.0	3.1	3.2

Continued

Table 1 Continued

Baseline characteristics	Unmatched* cohort		Propensity score-matched cohort	
	Statin initiators (n=1430)	Non-initiators (n=1430)	Statin initiators (n=1108)	Non-initiators (n=1108)
Laboratory measurements				
Cholesterol, mg/dL	231.2	201.0	228.3	226.6
Healthcare utilisation				
General practice visits‡	11.9	9.7	11.5	11.5
Hospitalisations‡	0.7	0.5	0.6	0.6
Specialist referrals‡	0.9	0.8	0.9	0.8

*A non-initiator was randomly selected to match to each initiator within 1-year cohort accrual blocks.
†The Socio-Economic Deprivation Index was measured by the Townsend Deprivation Index, which was grouped into quintiles from 1 (least deprived) to 5 (most deprived).
‡Frequency during the past 2 years.
AS, ankylosing spondylitis; BMI, body mass index; HCTZ, hydrochlorothiazide; NSAID, non-steroidal anti-inflammatory disease.

in all-cause mortality. This association was independent of age, sex, BMI socioeconomic status, relevant comorbidities, cardiovascular medication use, total cholesterol levels and healthcare utilisation. The magnitude of the inverse association between statin initiation and mortality was higher than that observed in multiple meta-analyses of statin use for primary prevention in the general population (ie, 9% and 14%).[7 8] As patients with AS are affected by systemic inflammation and are at a higher risk for CVD,[22] the dual anti-inflammatory and lipid-lowering effects of statins may be more pronounced than in the general population. While these pleiotropic effects may lead to mortality benefits in this patient population, this has not been previously studied. To our knowledge, this is the first study to find a potentially substantial mortality benefit of statin use in patients with AS.

Previous studies have shown that patients with AS have an increased risk of premature mortality.[6 23–26] For example, a recent nationwide population-based study found a higher risk of mortality in patients with AS compared with age and sex-matched controls, with an HR of 1.60 (95% CI 1.44 to 1.77).[6] The risk was evident among both men and women (HRs=1.53 and 1.83).[6] These findings echo a recent Canadian population-based study of 21 473 patients with AS that found an adjusted HR of 1.36 (95% CI 1.13 to 1.87) for cardiovascular mortality compared with the general population.[26]

Prior data about the potential benefit of statins among patients with AS are limited to those with intermediate end points and a small sample size. The RORA-AS study measured carotid plaque height in serial ultrasound measurements over 18 months in patients with RA, AS and psoriatic arthritis who were treated with rosuvastatin, and found that statin use induced significant atherosclerotic regression.[11] This study included only 21 patients with AS without a comparison group. The cardiovascular benefit of statin use in patients with inflammatory arthritis was suggested in post hoc analyses of two prospective trials (the Treating to New Targets and Incremental Decrease in End Points Through Aggressive Lipid Lowering studies) (HR 0.80; 95% CI 0.76 to 0.85) with statin therapy in patients with and without inflammatory joint disease (n=280 and 18 609, respectively).[27] Only 46 patients with AS were included in this analysis, limiting the relevant power and validity of these findings to patients with AS.

The substantial inverse association between statin use and mortality risk seen in patients with AS in our study may be due to the dual anti-inflammatory and lipid-lowering roles of statins. A recent prospective study of 32 patients with active AS suggested that treatment with rosuvastatin leads to an improvement in disease activity and a reduction in acute phase reactants (including tumour necrosis factor-alpha, interleukin-6 and (intercellular adhesion molecule 1) independent of immunosuppression.[28 29] The pleiotropic effect of statins has been more extensively studied in RA, where two randomised, placebo-controlled trials have shown an anti-inflammatory effect of atorvastatin on joint count end points and inflammatory markers.[10 30] Moreover, a recent meta-analysis with 15 studies and 992 patients also demonstrated the pleiotropic effects of statins in decreasing RA disease activity.[31] While a possible reason for the larger magnitude of inverse association in AS as

Table 2 Association between statin initiation and all-cause mortality (propensity score-matched cohort)

	Statin initiator (n=1108)	Non-initiator (n=1108)
Mean follow-up (PY)	5.3	5.1
No of deaths	96	134
Mortality rate/1000 PY (95% CI)	16.51 (13.37 to 20.16)	23.79 (19.93 to 28.17)
HR (95% CI)	0.63 (0.46 to 0.85)	1.00 (Ref)
Rate difference/1000 PY (95% CI)	−7.3 (−12.5 to −2.1)	0.0 (Ref)

PY, person-years.

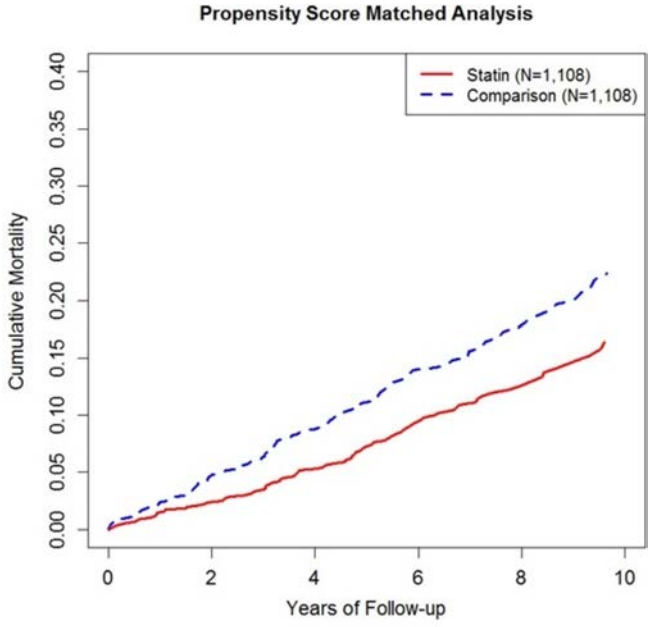

Figure 1 Time to death for the propensity score-matched cohort of patients with ankylosing spondylitis.

Table 3 Association between statin initiation and all-cause mortality according to follow-up period (propensity score-matched cohort)

	Statin initiator (n=1108)		Non-initiator (n=1108)		
Follow-up period	Deaths (n)	Mortality rate/1000 PY (95% CI)	Deaths (n)	Mortality rate/1000 PY (95% CI)	HR (95% CI)
1 year	15	15.86	25	26.59	0.64 (0.33 to 1.24)
2 years	24	13.43	47	26.61	0.49 (0.29 to 0.82)
3 years	33	13.04	60	24.09	0.55 (0.35 to 0.85)
4 years	46	13.09	79	22.97	0.56 (0.38 to 0.83)
5 years	59	14.37	94	23.42	0.60 (0.42 to 0.85)
Total follow-up	96	16.51	134	23.79	0.63 (0.46 to 0.85)

PY, person-years.

compared with RA could be the relative sexual predilection of these conditions, effect estimates were similar between sexes in RA as well as in AS.[13] Furthermore, the potential pleiotropic effects of statins in cardiac pathologies other than atherosclerotic disease, which are more common in AS than in RA, might play a role in AS more so than in RA. It would be valuable to further delineate these potential pleotropic effects of statins in patients with AS in future studies.

Our study has several strengths and limitations. This was a large-scale study of patients with AS, derived from the general population and with a substantial number of statin initiators and non-initiators to provide meaningful estimates. Use of a propensity score-matched cohort helped to address the issue of confounding by indication, which can cause significant bias in epidemiological studies of medication use for the indicated outcomes. The unmatched analysis showed markedly different baseline characteristics between the statin initiators and non-initiators, with higher risks of baseline CVD and other comorbidities. As such, the patients who were started on statins had higher mortality rates than their unmatched comparators. However, after the use of propensity score matching, which balanced the baseline covariates, our analysis found an inverse association between statin initiation and mortality in patients with AS. Furthermore, by matching patients within 1-year accrual blocks, we addressed potential changes in the relative importance of potential confounding variables over different calendar times. Our definition of AS has been previously shown to have positive predictive values ranging from 72% to 86%.[6 16 21] To that end, our results became more protective when we considered potential misclassification of the AS cohort. Finally, the sensitivity analyses truncating the follow-up at 1, 2, 3, 4 and 5 years addressed the potential effect of variable statin adherence over the follow-up period.

Although our study found that statin use was associated with an overall all-cause mortality risk reduction, we were unable to examine cause-specific mortality due to incomplete data within THIN. Nevertheless, knowledge of the overall mortality impact of statin use among patients with AS is critically important in its own right, as overall mortality represents the overall net health outcome of various benefits and risks associated with statin use.[32] We were also unable to assess disease activity given the absence of this information in the THIN database, similar to other recent population-based studies.[6 26] However, disease activity is relatively unlikely to influence a physician's decision to initiate a statin, making it also unlikely that disease activity would be a confounder in our analysis. Similarly, disease-modifying antirheumatic drug and biological use, which tend to be incomplete in this data set, are unlikely to influence a physician's decision to start a statin, making it unlikely that there was a meaningful imbalance in the use of these drugs between our matched cohorts. This is reflected in the well-balanced use of glucocorticoids and non-steroidal anti-inflammatory diseases between the two groups. Nevertheless, confirming our findings in a data set that can fully incorporate disease activity and DMARD information would be valuable. Lastly, although many variables were adjusted for by propensity score matching, our study was observational and thus subject to confounding by unknown variables.

In conclusion, our study found that the use of statins in patients with AS was associated with a 37% reduced all-cause mortality risk. This magnitude of association is larger than that seen in both RA and in the general population, suggesting that statin therapy may lower the risk of mortality substantially in patients with AS. As current guidelines for the management of cardiovascular risk in AS lack strong evidence-based recommendations for cardiovascular screening, our promising findings call for further studies to generate the high-level evidence needed to define the role of statin use in AS care.[33 34]

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CONCISE REPORT

Obesity and rates of clinical remission and low MRI inflammation in rheumatoid arthritis

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ABSTRACT

Objectives Obesity has been proposed as a risk factor for refractory rheumatoid arthritis (RA). We evaluated the impact of obesity on achieving clinical and imaging definitions of low disease activity.

Methods This study evaluated 470 patients with RA from GO-BEFORE and GO-FORWARD randomised clinical trials. Included patients had blinded clinical disease activity measures and MRI at baseline, 24 and 52 weeks. Synovitis, osteitis and total inflammation scores were determined using the RA MRI scoring system. Multivariable logistic regression analyses compared odds of achieving Disease Activity Score using 28 joints and C-reactive protein (DAS28-CRP) remission, low component measures, or low MRI inflammation measures at 24 weeks in patients with obesity versus no obesity.

Results At 24 weeks, patients with obesity were significantly less likely to achieve DAS28(CRP) remission (OR 0.47; 95% CI 0.24 to 0.92, p=0.03). In contrast, patients with obesity had similar odds of achieving low synovitis (OR 0.94; 95% CI 0.51 to 1.72, p=0.84) and inflammation scores (OR 1.16; 95% CI 0.61 to 2.22, p=0.64) and greater odds of achieving low osteitis scores (OR 2.06; 95% CI 1.10 to 3.84, p=0.02) versus normal weight patients.

Conclusions Patients with RA and obesity have lower rates of DAS28 remission but similar rates of low MRI activity compared with patients without obesity, suggesting that obesity and its associated comorbidities can bias clinical disease activity measures.

Trial registration number NCT00361335 and NCT00264550; Post-results.

INTRODUCTION

Obesity is one of the most common comorbid conditions among patients with rheumatoid arthritis (RA). Numerous studies have suggested that patients with obesity have a poorer response to treatment and lower likelihood of achieving RA disease remission.[1–6] While some have concluded that obesity is associated with more refractory RA, an alternative explanation is that obesity and its related symptoms and comorbidities directly influence and bias specific components of disease activity measures.[7]

MRI can be used to assess both damage and inflammatory activity in RA. MRI measured synovitis and osteitis (bone oedema) are sensitive to change and have been used as outcome measures in clinical trials. These measures are also predictive of progressive joint damage independent of clinical disease activity.[8–10] Recently, thresholds for low MRI activity have been defined and validated using RA MRI scores (RAMRIS). These thresholds identify patients unlikely to have structural progression, even if definitions of clinical remission are not met.[10]

The objective of this study was to compare the impact of obesity on attaining different clinical and imaging definitions of low activity and remission. We hypothesised that patients with obesity would be less likely to attain clinical remission but equally likely to meet MRI definitions of low activity versus patients without obesity.

METHODS

The study population comes from secondary analysis of the GO-BEFORE (Golimumab Before Employing Methotrexate as the First-Line Option in the Treatment of Rheumatoid Arthritis of Early Onset; Clinicaltrials.gov identifier NCT00361335) and GO-FORWARD (Golimumab in Active Rheumatoid Arthritis Despite Methotrexate Therapy; NCT00264550) randomised, multicentre, double-blind, placebo-controlled trials, which evaluated the efficacy of tumour necrosis factor-α antagonist golimumab for the treatment of RA. Both studies compared golimumab in combination with methotrexate with methotrexate or golimumab monotherapy. GO-BEFORE studied methotrexate-naïve patients and GO-FORWARD studied patients with inadequate methotrexate response. Detailed methods and results of both studies have previously been published.[11 12] The trials were conducted according to the Declaration of Helsinki. The secondary analysis of deidentified trial data was considered exempt by the Internal Review Board at the University of Pennsylvania.

This analysis includes the subset of patients in both studies who had MRIs scored for synovitis, osteitis, and/or bone erosion at baseline and during follow-up. Patients aged ≥18 years who met the American College of Rheumatology (ACR) 1987 criteria for RA and had active disease were recruited into the MRI substudy at participating sites. Data collection at each 4-week visit through 52 weeks included blinded assessments of disease activity score in 28 joints (DAS28(C reactive protein (CRP))) and Health Assessment Questionnaire (HAQ). MRI was performed at baseline, week 24 and week 52. Body mass index (BMI) at baseline was calculated as weight in kilograms divided by height in metres squared, and categorised as

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Table 1 Baseline characteristics of the study population by BMI group

	BMI <20, n=51	BMI 20 to <25, n=164	BMI 25 to <30, n=152	BMI ≥30, n=103	p Value
Female	43 (84%)	136 (83%)	126 (83%)	87 (84%)	0.98
Age, years	44±14	47±12	51±11	52±11	<0.001
Race					
White	15 (29%)	92 (56%)	106 (70%)	87 (84%)	<0.001
Black	0 (0%)	0 (0%)	1 (1%)	1 (1%)	0.61
Asian	35 (69%)	61 (37%)	25 (16%)	7 (7%)	<0.001
Other	1 (2%)	11 (7%)	20 (13%)	8 (8%)	0.05
GO-BEFORE	26 (51%)	94 (57%)	89 (59%)	61 (59%)	0.78
GO-FORWARD	25 (49%)	70 (43%)	63 (41%)	42 (41%)	0.78
CCP positive	39 (76%)	136 (83%)	121 (80%)	75 (73%)	0.25
DAS28(CRP)	5.5±1.2	5.3±1.1	5.6±1.1	5.5±1.0	0.06
Swollen joint count	8.9±6.0	8.7±5.1	9.9±5.8	9.7±5.8	0.19
Tender joint count	12.2±7.4	11.5±6.9	14.4±7.5	13.8±7.0	0.002
Patient global	6.3±2.4	5.7±2.4	6.0±2.4	6.0±2.2	0.41
HAQ	1.3±0.7	1.3±0.7	1.6±0.7	1.7±0.7	<0.001
CRP, mg/dL	1.2 (0.4–3.6)	1.0 (0.3–2.4)	0.9 (0.4–2.3)	0.9 (0.4–2.0)	0.59
RAMRIS scores at baseline					
Synovitis	7.9 (4.5–13.5)	8.0 (4.3–12.0)	9.0 (5.0–12.5)	7.5 (4.0–10.8)	0.07
Osteitis	8.0 (1.0–19.5)	8.0 (1.5–18.4)	4.5 (1.4–10.1)	3.0 (0.5–9.0)	<0.001
Inflammation	26.2 (10.1–46.6)	28.8 (8.6–50.2)	21.0 (11.0–32.8)	14.5 (7.4–30.0)	0.003
Bone erosion	14.5 (8.7–37.0)	16.4 (9.0–40.0)	14.0 (9.5–22.5)	13.5 (8.3–20.0)	0.02

Mean±SD compared with ANOVA, median (IQR) compared with Kruskal-Wallis, proportions compared with χ^2.
BMI, body mass index; DAS28(CRP), disease activity score using 28 joints and CRP; CCP, citrullinated peptide; CRP, C reactive protein; RAMRIS, rheumatoid arthritis MRI scores.

BMI <20 (underweight), BMI 20 to <25 (normal weight), BMI 25 to <30 (overweight) and BMI ≥30 (obese).

MRIs of the dominant wrist and second to fifth metacarpophalangeal joints were obtained using a 1.5 T MRI with contrast enhancement as previously described and scored by two independent blinded readers using the RAMRIS scoring system.[9] Low synovitis and low osteitis scores were defined as ≤3 based on recently defined thresholds.[10] Inflammation scores were calculated by adding the synovitis score to twice the osteitis score as previously described, with a low score defined as ≤9.[10]

Clinical remission was defined as a DAS28(CRP) score <2.6. Thresholds for a low swollen joint count, tender joint count, patient global score and CRP in mg/dL were all defined as ≤1 and low HAQ as ≤0.5 as defined in the 2011 ACR/European League Against Rheumatism Boolean definitions of remission.[13]

Data were analysed with STATA V.13.1 software (StataCorp, College Station, Texas, USA). Differences in demographics, disease activity and MRI measures at baseline across BMI categories were evaluated with χ^2, ANOVA and Kruskal-Wallis tests. In the primary analysis, multivariable logistic regression models evaluated the association between BMI category (normal BMI as the reference) and each of the 24-week clinical disease activity or imaging outcomes, adjusting for age, sex, race, anticyclic citrullinated peptide (CCP) antibody status, study and treatment assignment. The probability of reaching low activity thresholds was determined from these models for each BMI category at the means of all covariates and displayed graphically. Secondary analysis evaluated the same outcomes at 52 weeks.

RESULTS

Baseline characteristics of the 470 patients in the cohort are shown in table 1. Overweight and patients with obesity were older and more often white. Overweight and patients with obesity had higher tender joint counts and worse HAQ scores at baseline, although DAS28(CRP) scores were similar across BMI categories. As has been previously published from this cohort, overweight and patients with obesity had substantially lower osteitis (bone oedema) scores and fewer erosions at baseline (table 1).[14]

At 24 weeks, DAS28(CRP) remission was present in 28% of underweight, 28% of normal weight, 27% of overweight, but only 17% of patients with obesity. After adjustment, patients with obesity were less likely to achieve DAS28(CRP) remission (OR 0.47; 95%CI 0.24 to 0.92, p=0.03) or a low HAQ (OR 0.49;95%CI 0.28 to 0.89, p=0.02) compared with normal weight patients (figure 1) (see online supplementary table 1). Results using Simplified Disease Activity Index (SDAI), Clinical Disease Activity Index (CDAI) or Boolean remission were similar, although not statistically significant (see online supplementary figure 1). Patients with obesity were also less likely to have a favourable patient global score ≤1 (OR 0.47; 95% CI 0.24 to 0.92, p=0.03) and less likely to have a CRP ≤1 mg/dL (OR 0.44 ; 95% CI 0.23 to 0.84, p=0.01) at 24 weeks. Results were similar with adjustment for baseline DAS28(CRP) (not shown).

In contrast, low synovitis scores ≤3 and low inflammation scores ≤9 on MRI occurred at similar rates across BMI groups, while low osteitis (bone oedema) scores were more common in patients with obesity (69% of patients with obesity vs 50% of normal weight patients, p=0.02). In multivariable models, patients with obesity were not less likely to have low synovitis (OR 0.94; 95% CI 0.51 to 1.72, p=0.84) or low inflammation scores (OR 1.02; 95% CI 0.53 to 1.96, p=0.95) at 24 weeks versus normal weight patients (figure 2) (see online supplementary table 2). Patients with obesity were more likely to achieve a low osteitis score compared with normal weight patients (OR 2.06; 95% CI 1.10 to 3.84, p=0.02). The odds of a low osteitis score was similar across BMI categories after adjusting for baseline osteitis (obese vs normal weight OR 1.01; 95% CI 0.40 to 2.51, p=0.99).

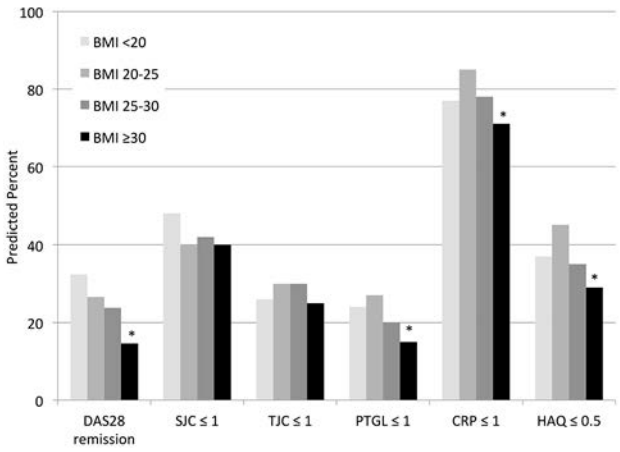

Figure 1 Rates of low disease activity measures at 24 weeks among different BMI groups. Predicted probabilities were obtained from multivariable logistic regression models at the means of age, sex, race, cyclic citrullinated peptide antibody status, study, treatment assignment. *p<0.05. BMI, body mass index; DAS28, disease activity score in 28 joints using CRP; SJC, swollen joint count; TJC, tender joint count; PTGL, patient global visual analogue scale score; CRP, C reactive protein; HAQ, Health Assessment Questionnaire.

Analyses at 52 weeks were similar except that patients with obesity were significantly less likely to have a low tender joint count versus normal weight patients (OR 0.47; 95% CI 0.27 to 0.82, p=0.01) and differences in achieving low HAQ were not significant (see online supplementary table 2).

DISCUSSION

Obesity was associated with a lower likelihood of achieving DAS28 remission among patients with RA enrolled in these clinical trials. In contrast, these same patients with obesity achieved low MRI activity at a similar rate compared with patients without obesity. These results suggest that obesity is not associated with more severe or refractory RA, but rather that obesity may bias clinical disease activity measures and thereby reduce the likelihood of achieving remission based on clinical assessments.

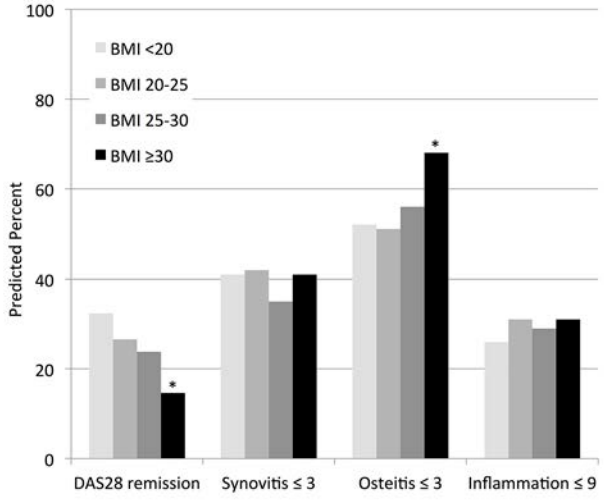

Figure 2 Rates of low clinical disease activity or low MRI scores at 24 weeks among different BMI groups. Predicted probabilities were obtained from multivariable logistic regression models at the means of age, sex, race, cyclic citrullinated peptide antibody status, study, treatment assignment. *p<0.05. BMI, body mass index; DAS28, disease activity score in 28 joints using C reactive protein.

Patients with obesity were less likely to have low DAS28 scores at 24 and 52 weeks. Patients with obesity were also less likely to achieve a low patient global score, tender joint count, CRP level and HAQ. These results support previous studies demonstrating that patients with RA and obesity have worse subjective disease activity measures at baseline and poorer response of these subjective measures to treatment.[3 4 15 16] Inflammatory markers such as CRP, although considered more objective, may also be elevated in patients with obesity independent of RA disease activity.[17]

In contrast, patients with obesity had similar rates of achieving a low MRI synovitis or total inflammation score and higher rates of achieving a low osteitis score at 24 and 52 weeks (similar rates when controlling for baseline osteitis). These observations are supported by previous studies showing that obesity is associated with a lower risk of radiographic and MRI joint damage progression.[14 18 19] This study provides new evidence that obesity is not associated with more severe or refractory disease by showing that patients with obesity achieve similar rates of low MRI disease activity despite apparent differences in clinical responses.

This study uses clinical trial data that include rigorous assessment of clinical disease activity measures and blinded MRI scoring at regular intervals. A 'gold standard' assessment of disease activity does not exist and MRI may not capture all aspects of RA disease activity. MRI does, however, provide an objective measure of inflammatory joint disease, a key and defining feature of RA. While very low levels of synovitis or osteitis may be common and non-specific,[20] our use of validated cut-off scores that identify an informative degree of inflammatory disease is an advance over previous literature. Residual confounding by unmeasured factors is also possible in this observational study, although adjustment for baseline demographics, race, CCP antibody positivity and baseline disease activity did not substantially impact the results.

In conclusion, although patients with RA and obesity are less likely to achieve DAS28 remission, these patients have similar rates of achieving low MRI activity. This study addresses an ongoing controversy about the impact of obesity on RA disease activity and suggests that obesity is not associated with more refractory RA. These results highlight the critical role of the clinician, whose challenge is to recognise the importance and limitations of disease activity measures and to consider the impact of comorbidities on disease activity scores and symptoms.

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Contributors All authors were involved in the study design, data collection and interpretation and approved the final version of the manuscript. MG and JB performed the statistical analyses and wrote the manuscript with input from the coauthors.

Competing interests PGC has done speakers bureaus or consultancies for AbbVie, BMS, Janssen, Lilly, Novartis, Pfizer and Roche. PE has received consulting fees, speaking fees and/or honoraria from Pfizer, Merck, AbbVie, UCB, Roche, BMS, Lilly and Novartis (less than US$10,000 each). DGB is an employee of Janssen Biotech. M Østergaard has received fees for consultancy or speaker fees and/or research support from Abbott, AbbVie, BMS, Boehringer-Ingelheim, Celgene, Centocor, Eli-Lilly, GlaxoSmithKline, Hospira, Janssen, Merck, Mundipharma, Novartis,

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CONCISE REPORT

Rheumatoid arthritis and polymyalgia rheumatica occurring after immune checkpoint inhibitor treatment

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ABSTRACT

Objectives Immune checkpoint inhibitors (ICIs) targeting cytotoxic T-lymphocyte-associated protein 4 and programmed cell death protein 1 (PD-1) have demonstrated improved survival for multiple cancers. However, these new drug classes have led to increased immune-related adverse events (IrAE). Rheumatic IrAEs have not been well described in clinical trials. We report here cases of rheumatoid arthritis (RA) and polymyalgia rheumatica (PMR) occurring after ICI treatment.

Methods This was a retrospective study of patients receiving an ICI in whom symptoms of arthritis or arthralgia developed and revealed a diagnosis of RA or PMR.

Results In 10 patients who received ICI therapy (all anti-PD-1 or anti-PDL1 antibodies), RA or PMR developed at a median of 1 month (1 to 9) after exposure. No patient had pre-existing rheumatic or autoimmune disease. RA developed in six patients; all six were positive for anti-cyclic citrullinated peptide (anti-CCP) antibodies and four for rheumatoid factor. Anti-CCP antibodies were detected in two out of three patients tested before immunotherapy. Disease-modifying antirheumatic drugs were needed for three patients; the three others received corticosteroids or non-steroid anti-inflammatory drugs. PMR was diagnosed in four patients, all responded to corticosteroids. Despite these IrAEs, immunotherapy was pursued for all but one patient until cancer progression.

Conclusions This is the first description of RA occurring after ICI therapy for cancer. PMR can also occur after ICI, particularly after anti-PD-1 therapy. All cases responded to corticosteroids or with immunosuppressive therapy. Collaboration between rheumatologists and oncologists is crucial and could lead to better recognition and care of these patients.

In recent years, immunotherapy has dramatically transformed the prognosis of several cancers, including principally metastatic melanoma and non-small cell lung cancer (NSCLC). Ipilimumab, an immune checkpoint inhibitor (ICI) targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), has been approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) for treating metastatic melanoma. The ICIs nivolumab and pembrolizumab, targeting programmed cell death protein 1 (PD-1), or atezolizumab, targeting its ligand PDL1, have been FDA and EMA approved for treating metastatic melanoma and NSCLC.[1 2]

Adverse events (AEs) observed with ICI therapy are related to their mechanisms of action, although the precise pathophysiology needs to be better understood. Immune-related AEs (IrAEs), such as colitis, autoimmune thyroid disease and vitiligo, have been described with ipilimumab and anti-PD-1 therapy. Others seem to be more specific, such as hypophysitis with ipilimumab and pneumonitis with anti-PD-1 antibodies. Some can be life threatening, such as pneumonitis.[3–5] Relapse or flare of pre-existing autoimmune diseases has been reported,[6] but the occurrence of new autoimmune diseases seems to be less frequent. However, a series of 21 patients with psoriasis induced by anti-PD-1 therapy was recently published.[7]

Little is known about rheumatic AEs with ICI therapy.[8] Indeed, in phase III studies, arthralgia, which includes all musculoskeletal disorders, was present in about 5% of patients receiving ipilimumab for melanoma, 9%–20% with pembrolizumab and 5%–16% with nivolumab for melanoma or NSCLC versus <1% with placebo.[4] However, these AEs may be underestimated, and no clinical description was provided. Recently, a series of 13 patients with non-classified rheumatic IrAEs was published[9]: non-specific inflammatory arthritis developed in 9 patients without autoantibodies and 4 presented sicca symptoms but did not fulfil criteria for Sjögren syndrome.

In this article, we report a series of 10 patients in whom seropositive rheumatoid arthritis (RA) or polymyalgia rheumatica (PMR) developed after ICI treatment.

METHODS

The Gustave Roussy Cancer Center (Villejuif, France) has established a national pharmacovigilance registry called Registre des Effets Indésirables Sévères des Anticorps Monoclonaux Immunomodulateurs en Cancérologie dedicated to collecting immunotherapy AEs. We also performed a retrospective multicentre collection of observations through the Club Rhumatismes et Inflammation (CRI) network, a section of the French Society of

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Rheumatology: between September 2016 and January 2017, all rheumatologists and internal medicine practitioners registered on the CRI website (http://www.CRI-net.com), almost 2400 physicians all over France, were contacted by successive newsletters over 6 months to report cases.

Patients were included if they had received ipilimumab, nivolumab, pembrolizumab or another ICI in development and thereafter had a diagnosis of RA according to the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria[9] or PMR according to EULAR/ACR criteria 2012.[10] All patients underwent a rheumatological assessment by a trained rheumatologist or internal medicine specialist with biological and radiological evaluations. Patients had no pre-existing rheumatic disease or arthritis based on the rheumatologist's questioning.

RESULTS

Ten cases of rheumatic inflammatory diseases following ICI therapy were collected: four from Gustave Roussy and six from other French centres (tables 1 and 2 and supplementary file). All patients received an anti-PD-1 antibody (nivolumab or pembrolizumab) or anti-PDL1 antibody (one patient in an open phase I clinical study). One received ipilimumab in addition to nivolumab for four cycles and then nivolumab alone. The cancers treated were metastatic melanoma, endometrial adenocarcinoma, mesothelioma, lung adenocarcinoma, squamous cell carcinoma of the vagina, gastric adenocarcinoma and colon adenocarcinoma.

In six patients, seropositive RA developed and in four, PMR developed. The mean age of patients was 65 years and 60% were male. The median time to IrAE after ICI exposure was 1 month (range 1–9 months). Only one patient showed other types of IrAEs before rheumatic IrAE: a rash with anti-PD-1 antibody (patient 2). Disease-modifying anti-rheumatic drugs (DMARDs) were needed for three patients with RA (hydroxychloroquine for two and methotrexate for one), the three others patients with RA received only corticosteroids or non-steroid anti-inflammatory drugs (NSAIDs). The four patients with PMR responded to corticosteroids. The ICI therapy was continued for all patients but one until cancer progression. Nivolumab was stopped in only one patient (patient 6) who showed no improvement with corticosteroids and because cancer was stable, methotrexate being introduced at the same time for RA.

DISCUSSION

To our knowledge, this is the first series describing seropositive RA occurring in patients receiving ICI therapy. Only one published series described non-specific rheumatic IrAEs but no case of seropositive RA.[11]

In our series, the six patients with RA fulfilled the 2010 ACR/EULAR criteria[9] and were seropositive (anti-cyclic citrullinated peptide (CCP) positivity in all six and rheumatoid factor (RF) in four). Anti-CCP antibodies could be detected in 2/3 patients (patients 1, 4 and 5) with available serum samples before immunotherapy. Three patients were negative for RF before ICI therapy and one showed weak positivity (18 U/mL) afterwards.

The short time between ICI treatment and the development of joint symptoms and anti-CCP positivity before ICI therapy in 2/3 patients suggested that some of these patients had a pre-RA status and that the treatment with ICI may have triggered the clinical disease. Indeed, studies have shown that antibodies (anti-CCP and RF) may be present several years before RA onset.[12] Nevertheless, no patient presented arthralgia

Table 1 Characteristics of patients with RA after ICI treatment for cancer

Patients	Sex/age, years	Type of cancer	ICI	Date of first ICI exposure	Date of IrAE	Type of rheumatic IrAE	IrAE response to treatment	Autoantibody results before ICI	Autoantibody results	Tumour response
1	F 55	Squamous cell carcinoma of the vagina	Nivolumab	October 2015	October 2015	RA	Resolution with NSAIDs	CCP: 61 U/mL RF:negative	CCP:671 U/mL RF:18 UI/mL	Progression death
2	F 66	Endometrial adenocarcinoma	Pembrolizumab	March 2016	April 2016	RA	Resolution with prednisone 10 mg/day	Not available	CCP:233 U/mL RF:180 UI/mL	Stable disease
3	M 59	Lung adenocarcinoma	Nivolumab	May 2016	July 2016	RA	Resolution with prednisone 10 mg/day	Not available	CCP:61 U/mL RF:47 UI/mL	Good response
4	F 56	Metastatic melanoma	Pembrolizumab	August 2015	September 2015	RA	NSAIDS and HCQ 400 mg/day:good response	CCP:22 U/mL RF:negative	CCP:18 U/mL RF<15 UI/mL	Stable disease
5	M 80	Metastatic melanoma	Nivolumab	April 2016	April 2016	RA	Prednisone 15 mg/day and HCQ 200 mg/day:good response	CCP:negative RF:not available	CCP:42 U/mL RF<15 UI/mL	Stable disease
6	M 68	Lung adenocarcinoma	Nivolumab	June 2015	July 2015	RA	NSAID: no effect stopping nivolumab and MTX 10 mg/week:good response	Not available	CCP:>300 U/mL RF:246 UI/mL	Stable disease

CCP, cyclic citrullinated peptide; F, female; HCQ, hydroxychloroquine; ICI, immune checkpoint inhibitor; IrAE, immune-related adverse event; M, male; MTX, methotrexate; NSAIDs, non-steroidal anti-Inflammatory drugs; RA, rheumatoid arthritis; RF, rheumatoid factor.

Table 2 Characteristics of patients with PMR after ICI treatment for cancer

Patients	Sex/age, years	Type of cancer	ICI	Date of first ICI exposure	Date of IrAE	Type of rheumatic IrAE	IrAE response to treatment	Autoantibody results	Tumour response
7	F 76	Mesothelioma	Anti-PDL1	June 2014	March 2015	PMR	Resolution with prednisone 20 mg/day then tapered	ANA, RF, CCP negative	Progression switch for pemetrexed
8	M 69	Gastric adenocarcinoma	Pembrolizumab	September 2016	October 2016	PMR	Resolution with prednisone 20 mg/day then tapered	ANA, RF, CCP negative	Progression
9	M 62	Colon adenocarcinoma	Nivolumab+ipilimumab (four cycles) then nivolumab alone	June 2015	October 2015	PMR	Resolution with prednisone 60 mg/day then tapered	ANA 1:320 with anti-ENA negative, RF, CCP negative	Stable disease
10	M 68	Metastatic melanoma	Nivolumab	August 2016	August 2016	PMR	Resolution with prednisone 40 mg/day then tapered	RF, CCP negative	Stable disease

ANA, antinuclear antibodies; anti-ENA, anti-extractable antibodies; CCP, cyclic citrullinated peptide; F, female; ICI, immune checkpoint inhibitor; IrAE, immune-related adverse event; M, male; PDL1, programmed cell death ligand protein 1; PMR, polymyalgia rheumatica; RF, rheumatoid factor.

before ICI treatment. All cases of RA occurred after anti-PD-1 treatment.

The observation that PD-1 inhibition can trigger RA may suggest an important role of the PD-1/PDL1 pathway in RA pathogenesis. PD-1 has been found important for self-tolerance, because *PD1*$^{-/-}$ mice showed spontaneous autoimmune disease development.[13] PD-1 polymorphisms have been associated with increased susceptibility to RA, and membrane and soluble PD-1 expression is decreased in patients with RA.[14] A recent case report showed RA recurrence in a patient with pre-existing RA in remission after receiving nivolumab for NSCLC.[15]

Two cases of PMR with ipilimumab have been reported,[16] but the PD-1/PDL1-2 system could also be important in the pathophysiology of PMR. Indeed, two cases were previously described[17] and we now report four additional cases. Interestingly, giant cell arteritis was recently found to involve a deficiency in the PD-1 immune checkpoint: vessel-wall dendritic cells fail to express PDL1, which leaves lesional T cells unchecked.[18]

The fact that our 10 cases occurred only after anti-PD-1 and not anti-CTLA-4 antibody treatment (except one patient who received both during four cycles) suggests a contrast between the IrAE occurring after blockade with CTLA-4 (colitis, endocrine disorders, skin rashes) and PD-1/PDL1 like non-specific arthritis, sicca syndrome[11] RA, PMR (this report) and connective tissue diseases.[19]

Pre-existing autoimmune disease (AID) was an exclusion criterion in clinical trials of ICI. In real life, these patients are frequently not excluded from ICI treatment because the priority is obviously the treatment of cancer. Because rheumatic IrAEs are closely linked to the mechanisms of action of ICI on immune cells, we can expect many rheumatic IrAEs or exacerbations of rheumatic IrAEs considering the future worldwide prescription of ICI. In one study, about one-third of 30 patients (n=8) with known AID who received ipilimumab showed a flare of AID and another third (n=10) showed a new IrAE.[6] In another study of 52 patients with pre-existing AID who received anti-PD-1 therapy (52% with rheumatic diseases), 20 (38%) had a flare of the AID after the first anti-PD-1 dose (median time 38 days). Flares were mild and were managed with corticosteroids and steroid-sparing agents such as methotrexate. Flares occur mainly in patients with rheumatic disorders. Only two patients discontinued anti-PD-1 therapy because of exacerbation of the AID.[20]

CTLA-4 is a target of both autoimmunity and cancer. Drugs inhibiting this immune checkpoint have been developed for cancer (ipilimumab) and drugs activating this inhibitory signal have been developed for autoimmunity (abatacept) or transplantation (belatacept). Even if no signal for a possible increased risk of cancer has been described with abatacept, the development of RA or other types of IrAEs with ICI therapy is a strong reminder to rheumatologists that long-term studies need to be continued in real-life patients receiving immunosuppressive drugs in general, to be sure of no increased risk of cancer with time. In addition, the possible balance between cancer and autoimmunity is illustrated by a possible link between an IrAE and response to the immune treatment of the cancer. This suggestion was raised with the treatment of melanoma with interferon[21] and has been recently reemphasised in patients receiving pembrolizumab, for whom vitiligo occurrence, a clinically visible IrAE, could be associated with response to treatment.[22]

In our series, all patients were referred to a rheumatologist or an internist. However, the number of rheumatic IrAE cases could have been underestimated because mild arthralgia could have been managed with NSAIDs or corticosteroids prescribed by oncologists and then may not be referred to rheumatologists. The combined expertise of oncologists, immunologists and rheumatologists is crucial for successful management of these patients. In most cases, ICIs may be pursued with symptomatic treatment of the RA or PMR. As for ICI-induced colitis, prescription of anti-tumour necrosis factor antibodies or other DMARDs may be possible if needed with this multidisciplinary expertise.

In conclusion, patients with cancer treated with immunotherapy and who develop autoimmune or other reactions should be managed by multidisciplinary care units (MCU). Cancer immunotherapy is very promising and the number of available treatments is raising; thus, an implementation of MCU could be of great value for patients with cancer.

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CONCISE REPORT

Differences in the symptomatic phase preceding ACPA-positive and ACPA-negative RA: a longitudinal study in arthralgia during progression to clinical arthritis

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ABSTRACT

Objective Although anticitrullinated protein antibody (ACPA)-positive and ACPA-negative rheumatoid arthritis (RA) have different aetiopathology, the clinical presentation at the time of diagnosis is similar. This study evaluated whether there are phenotypic differences in the symptomatic pre-RA phase.

Methods Patients with arthralgia included in the Leiden clinically suspect arthralgia cohort who developed arthritis during follow-up were studied (n=67). Symptoms at symptom onset, symptoms and signs at presentation with arthralgia and time to arthritis development were compared between ACPA-positive and ACPA-negative patients.

Results In ACPA-negative patients (n=37), the location of initial symptoms less often included the lower extremities (22% vs 50%, p=0.014). At presentation with arthralgia, ACPA-positive patients had a longer symptom duration (median 22 vs 14 weeks, p=0.005), less tender joints (mean 5 vs 9, p=0.007) and less difficulty making a fist (11% vs 43%, p=0.004). However, after presentation with arthralgia, ACPA-positive patients developed arthritis more quickly (median 6 vs 18 weeks, p=0.015). A partial least squares regression analysis showed clustering of ACPA-positive and ACPA-negative patients based on the above-mentioned clinical variables.

Conclusion This study is the first showing that ACPA-positive and ACPA-negative patients have clinical differences in the symptomatic phase preceding clinical arthritis. This contributes to the notion that ACPA-positive and ACPA-negative RA develop differently.

INTRODUCTION

Anticitrullinated protein antibodies (ACPA)-positive and ACPA-negative rheumatoid arthritis (RA) are considered as different disease subsets with differences in aetiopathology because there are differences in underlying genetic risk factors and the best-known environmental risk factor, smoking, is confined to ACPA-positive RA.[1–4] Intriguingly, despite the presumed differences in underlying biological processes, the clinical presentation at the time of diagnosis is similar.[5–7]

Autoimmune processes can start months to years before the diagnosis of RA.[8 9] ACPA-positive RA has a phase in which autoantibodies with or without symptoms precede the phase of clinical arthritis, and recent evidence revealed that also ACPA-negative RA has a symptomatic pre-arthritis phase in which subclinical inflammation may be present.[10 11] Currently, it is unknown whether there are phenotypic differences between ACPA-positive and ACPA-negative RA in the symptomatic phase preceding clinical arthritis. Because of the differences in aetiopathology, we hypothesised that differences are present. To investigate this, we longitudinally studied patients who presented with arthralgia and progressed to clinical arthritis. Clinical data at the time of symptom onset, at the time of first presentation with arthralgia to the outpatient clinic and time to arthritis development were compared between ACPA-positive and ACPA-negative patients.

METHODS

Patients

The Leiden clinically suspect arthralgia cohort is a population-based inception cohort that started in April 2012 at the outpatient clinic of the Leiden University Medical Center.[12] Inclusion required the presence of recent onset (<1 year) arthralgia of small joints that was considered suspect to progress to RA according to the clinical expertise of the rheumatologist. Autoantibody status was generally unknown at first presentation as local and national guidelines for general practitioners (GP) discourage autoantibody testing, but instead encourage GPs to refer quickly.[13 14] Hence, inclusion, which generally coincided with the first visit to the outpatient clinic, was based on clinical information. At baseline, patients completed questionnaires; this concerned questions on initial symptoms that were present at symptom onset and questions on current symptoms. Rheumatologists completed a questionnaire on symptoms that they felt to be important for labelling a patient as having clinically suspect arthralgia and performed a physical examination. Laboratory tests were performed after inclusion and included determination of ACPA (EliA CCP, Phadia, Nieuwegein, The Netherlands, positive if ≥7 U/mL), IgM rheumatoid factor (RF) (positive if ≥3.5 IU/mL) and acute-phase reactants. Patients were followed until progression to clinical arthritis with a maximum of 2 years. The cohort has been described elsewhere in detail.[12] At the time of

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Table 1 Clinical characteristics of ACPA-negative and ACPA-positive patients in the symptomatic phase preceding clinical arthritis

	All patients		
	ACPA negative (n=37)	ACPA positive (n=30)	p Value
Symptoms at symptom onset			
Symptom onset			
Acute (<1 week)	8 (22)	8 (27)	0.53
Gradual	26 (70)	16 (55)	
Intermittent	3 (8)	5 (17)	
Symptoms started with*			
Pain	34 (92)	29 (97)	0.41
Stiffness	26 (70)	17 (57)	0.25
Loss of function	16 (43)	10 (33)	0.41
Localisation affected joints			
Small joints hand/feet	27 (73)	21 (70)	0.18
Large joints	5 (14)	1 (3)	
Both	5 (14)	8 (27)	
Localisation affected joints			
Upper extremities	29 (78)	15 (50)	0.014
Lower extremities	5 (14)	4 (13)	
Both	3 (8)	11 (37)	
Localisation affected joints			
Symmetric	22 (60)	22 (73)	0.23
Presentation with arthralgia			
Family history of RA	13 (35)	11 (37)	0.90
Symptoms determining inclusion in the cohort			
Inflammatory type of symptoms	14 (39)	18 (60)	0.089
Morning stiffness ≥60 min	9 (25)	2 (7)	
Both	13 (36)	10 (33)	
Physical examination			
68 TJC, mean±SD	9±8	5±3	0.007†
Difficulties making a fist	16 (43)	3 (11)	0.004†
Squeeze test			
Positive for both MTP and MCP joints	8 (22)	4 (14)	0.48
Positive for MCP joints only	11 (31)	7 (24)	
Positive for MTP joints only	4 (11)	2 (7)	
Negative for both	13 (36)	16 (55)	
HAQ score, mean±SD	0.8±0.6	0.7±0.6	0.57

All values are indicated as n (%), unless indicated otherwise.
Missings were as follows: symptom onset (1), symptoms determining inclusion in the cohort (1), difficulties making a fist (1), squeeze test (2), HAQ score (2).
*Multiple answers could be given, so the percentages can add up to >100%.
†Significant after correction for multiple testing.
ACPA, anticitrullinated protein antibodies; HAQ, health assessment questionnaire; MCP, metacarpophalangeal; MTP, metatarsophalangeal; RA, rheumatoid arthritis; TJC, tender joint count.

analysis (1 November 2016), 441 patients were included, of whom 74 had developed clinical arthritis. Seven of these patients participated in a placebo-controlled trial (NTR4853) and received either methotrexate or placebo; these patients were not studied here. Disease modifying antirheumatic drugs (DMARDs) (including steroids) were not prescribed in the phase of arthralgia outside this trial. Hence, 67 DMARD-naive arthralgia patients that progressed to arthritis were studied. The study was approved by the local medical ethical committee. All patients provided written informed consent.

Sensitivity analyses

Sensitivity analyses were performed in patients with RA, defined as fulfilling the 2010 criteria and/or DMARD initiation at the time of arthritis development. The latter criterion reflects the expert opinion on RA and was added because patients with seronegative arthritis require >10 involved joints to fulfil the 2010 criteria, which may have been hampered by the early initiation of DMARDs.[15 16]

Statistics

Student's t-test, χ^2 test and log-rank test were used when appropriate. The Benjamini-Hochberg method was used to correct for multiple testing.[17] A partial least squares (PLS) regression analysis was used to study whether certain clinical characteristics frequently occurred together in ACPA-positive or ACPA-negative patients. PLS clusters variables into latent factors. Individual patient scores on these factors were then plotted against each other to look for clustering. SPSS V.23.0 was used for all analyses.

RESULTS

Of the patients with arthralgia who developed clinical arthritis, 37 (55%) were ACPA negative and 30 (45%) were ACPA positive. The mean age in both groups was 45 years and the majority was female (73% and 77%, respectively).

Symptoms at symptom onset

Eighty-six per cent of ACPA-negative patients initially experienced symptoms at the upper extremities, while only 22% reported initial involvement of the lower extremities. In contrast, 50% of ACPA-positive patients reported initial involvement of the lower extremities (p=0.014, table 1).

Symptoms and signs at first presentation with arthralgia

At the time of first presentation with arthralgia, ACPA-positive patients had less tender joints (mean 5 vs 9, p=0.007, table 1), less difficulties making a fist (11% vs 43%, p=0.004, table 1) and a longer symptom duration than ACPA-negative patients (median of 22 compared with 14 weeks, p=0.005, figure 1). When splitting symptom duration in patient delay (symptom onset—first visit to the GP) and in GP delay (first visit to the GP—first visit to the rheumatologist), both patient delay (median 10 vs 7 weeks) and GP delay (median 6 vs 3 weeks) were longer in ACPA-positive patients.

Although this study focused on clinical characteristics, acute-phase reactants were routinely measured and were not different between ACPA-positive and ACPA-negative patients (C-reactive protein mean of 9 vs 8 mg/L and erythrocyte sedimentation rate mean of 16 vs 15 mm/h).

Time to arthritis development

Although ACPA-positive patients had a longer symptom duration when first presenting with arthralgia, they developed arthritis more quickly (median 6 weeks vs 18 weeks, p=0.015, figure 1).

Clustering of patients

A PLS regression analysis was performed to look for clustering of ACPA-positive and ACPA-negativepatients. All clinical variables included in table 1, age, gender, symptom duration and time to arthritis development, were entered. Two latent factors were identified that together explained 51.3% of the variance between ACPA-positive and ACPA-negative patients. Individual patient scores on these factors were plotted against each other

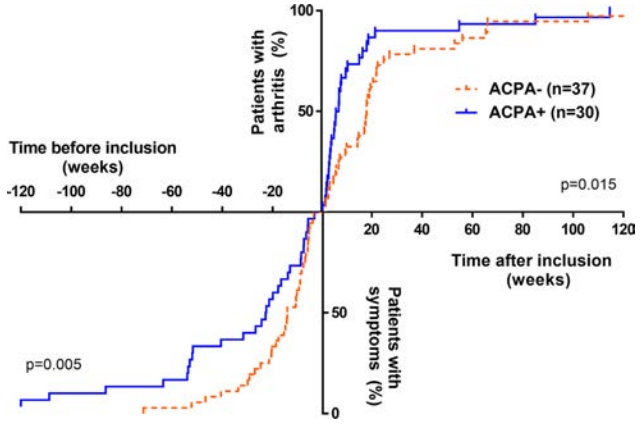

Figure 1 Time from symptom onset to presentation with arthralgia (left part) and from presentation with arthralgia to arthritis development (right part) in ACPA-positive and ACPA-negative patients. This graph shows that ACPA-negative patients have a shorter symptom duration at the time of first presentation with arthralgia, but that ACPA-positive patients progress to arthritis more quickly thereafter. Three data points were not shown (but were included in the analyses): two ACPA-positive patients had a symptom duration of ≥120 weeks and one ACPA-negative patient developed arthritis ≥120 weeks after inclusion in the cohort. Symptom duration was unknown in one patient. ACPA, anticitrullinated protein antibodies.

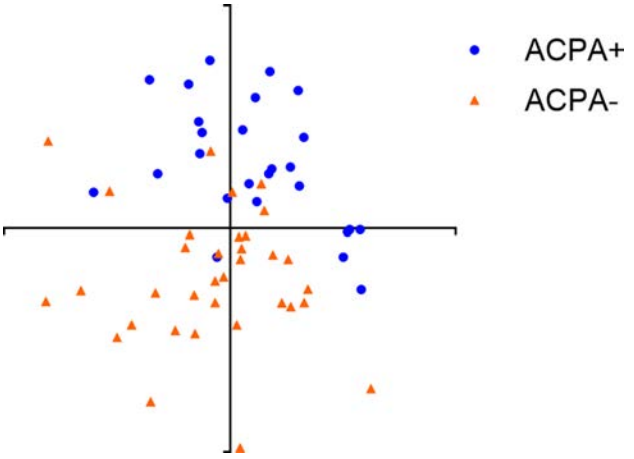

Figure 2 Clustering of anticitrullinated protein antibody (ACPA)-positive and ACPA-negative patients based on a partial least squares regression analysis that included only clinical information. Scores on latent factor 1 are plotted on the Y-axis, scores on latent factor 2 are plotted on the X-axis. Together these factors explain 51.3% of the variance in ACPA-positive and ACPA-negative patients. Importantly, results of laboratory tests were not included. This figure shows that especially the first latent factor partially clusters ACPA-positive and ACPA-negative patients. Important variables contributing to a higher score on factor 1 were initial symptoms in both upper and lower extremities, initial symptoms in both large and small joints and inflammatory type of symptoms. Variables contributing to a lower score on factor 1 were initial symptoms in the upper extremities only, morning stiffness ≥60 minutes as a reason for inclusion in the cohort, a shorter symptom duration, a higher TJC, problems making a fist and a positive squeeze test of the MTP joints. ACPA, anticitrullinated protein antibodies; MTP, metatarsophalangeal; PLS, partial least squares; TJC, tender joint count.

and showed clustering (figure 2). Variables contributing to this clustering included the same variables that were observed to be different between ACPA-positive and ACPA-negative patients in table 1 and figure 1 (identified by a variable importance projection >1, see online supplementary table 1). These findings indicate that there are indeed clinical differences between ACPA-positive and ACPA-negative patients in the phase preceding clinical arthritis.

Sensitivity analyses

At the visit at which clinical arthritis was identified, 59/67 (88%) started on DMARD therapy and/or fulfilled the 2010 criteria (2010 RA+/DMARD initiation− (n=3), 2010-RA-/DMARD-initiation+ (n=17), 2010 RA+/DMARD initiation+ (n=39)). Analyses were repeated in these patients and showed similar results (online supplementary table 2; supplementary figure 1). None of the patients classified with RA had a spontaneous resolution of arthritis.

Finally, as ACPA-negative patients can be RF positive, analyses were further stratified for RF. Although subgroups became small, this did not change the results (online supplementary table 3).

DISCUSSION

This study identified phenotypic differences between ACPA-positive and ACPA-negative patients in the symptomatic phase preceding arthritis development. Initial symptoms in ACPA-negative patients were less often located in the lower extremities. At first presentation with arthralgia, ACPA-positive patients had less tender joints, less difficulty making a fist and longer symptom duration compared with ACPA-negative patients. However, ACPA-positive patients progressed to arthritis more rapidly. This study is the first showing that, in addition to the differences in underlying risk factors, there are also clinical differences in very early symptomatic disease phases. This suggests that ACPA-positive and ACPA-negative RA are intrinsically different.

Previous studies among patients with RA (classified with the 1987 criteria) revealed no clinical differences between ACPA-positive and ACPA-negative patients.[5 7] This may be due to circularity as fulfilment of classification criteria requires certain clinical characteristics to be present, or to the disease stage, as a final common phenotype can have developed over time. A study on the symptomatic pre-arthritis phase does not have this drawback. Furthermore, whereas recall bias may be an issue when information on the earliest disease phases is collected at the time of diagnosis, most data in this study were collected prospectively. Only data on first symptoms were collected in retrospect, but symptom onset was recent.

In ACPA-negative patients, symptoms often started in the upper extremities only. A previous study that compared ACPA-negative and ACPA-positive RA at the time of diagnosis (hence studying different individuals) revealed the same.[5] Although it was then unclear if this finding was due to multiple testing, the present data on patients in a different disease phase support the validity of this finding. The observation that ACPA-negative patients had more difficulties making a fist prior to developing clinical arthritis is in line with these findings as well.

Both patient and GP delay were longer in ACPA-positive patients. This is in line with previous studies showing a longer delay in ACPA-positive RA.[6 18] This may be explained by a difference in symptom onset. It has been reported that autoantibody-positive RA has a more gradual onset and that initial symptoms more often 'come and go'.[6 19] This tendency was also present in the current data, although not statistically

significant. Interestingly, after presentation, ACPA-positive patients progressed to arthritis quicker and the total time period between symptom onset and arthritis development was not different. Together, these results suggest that ACPA-positive patients present in a later part of the symptomatic pre-arthritis phase.

This study only evaluated arthralgia patients who developed arthritis. A previous study compared patients with clinically suspect arthralgia who developed arthritis with those who did not develop arthritis to identify predictors,[20] which is a different study question than addressed here.

The sample size (n=67) may be considered as a limitation and may lead to false-negative results. In addition, we cannot exclude false-positive results as replication in independent datasets was not available. However, this is the first time that ACPA-positive and ACPA-negative patients were identified in a symptomatic pre-arthritis phase and were followed to arthritis development. Comparison of these disease phases reveals novel insights in the pathophysiology of RA development.

In conclusion, ACPA-positive and ACPA-negative patients have clinical differences in the symptomatic phase preceding arthritis development. This contributes to the notion that ACPA-positive and ACPA-negative RA are disease subsets that develop differently.

Contributors All authors contributed to the design of the study, the interpretation of the results and gave feedback on the manuscript. All authors approved the final version and agreed to be accountable for all aspects of the work. HWvS, RMtB and LEB contributed to the acquistion of data. LEB analysed the data. LEB and AvdH-vM drafted the manuscript.

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OPEN ACCESS

EXTENDED REPORT

H1N1 vaccination in Sjögren's syndrome triggers polyclonal B cell activation and promotes autoantibody production

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ABSTRACT

Objectives Vaccination of patients with rheumatic disease has been reported to result in lower antibody titres than in healthy individuals. However, studies primarily include patients on immunosuppressive therapy. Here, we investigated the immune response of treatment-naïve patients diagnosed with primary Sjögren's syndrome (pSS) to an H1N1 influenza vaccine.

Methods Patients with Sjögren's syndrome without immunomodulatory treatment and age-matched and gender-matched healthy controls were immunised with an H1N1 influenza vaccine and monitored for serological and cellular immune responses. Clinical symptoms were monitored with a standardised form. IgG class switch and plasma cell differentiation were induced *in vitro* in purified naïve B cells of untreated and hydroxychloroquine-treated patients and healthy controls. Gene expression was assessed by NanoString technology.

Results Surprisingly, treatment-naïve patients with Sjögren's syndrome developed higher H1N1 IgG titres of greater avidity than healthy controls on vaccination. Notably, off-target B cells were also triggered resulting in increased anti-EBV and autoantibody titres. Endosomal toll-like receptor activation of naïve B cells *in vitro* revealed a greater propensity of patient-derived cells to differentiate into plasmablasts and higher production of class switched IgG. The amplified plasma cell differentiation and class switch could be induced in cells from healthy donors by preincubation with type 1 interferon, but was abolished in hydroxychloroquine-treated patients and after in vitro exposure of naïve B cells to chloroquine.

Conclusions This comprehensive analysis of the immune response in autoimmune patients to exogenous stimulation identifies a mechanistic basis for the B cell hyperactivity in Sjögren's syndrome, and suggests that caution is warranted when considering vaccination in non-treated autoimmune patients.

INTRODUCTION

Infectious diseases are a major cause of morbidity and mortality in patients with systemic rheumatic diseases.[1 2] Vaccination is one of the most effective measures to prevent infections; however, safety and efficacy need to be considered in the context of a dysregulated immune system. Studies of individuals with autoimmune rheumatic diseases indicate that patients develop reduced protective antibody titres on immunisation.[3–5] However, many patients are treated with immunosuppressive drugs, which likely affect their response to vaccination. In addition, most reports focus on assessing vaccine efficacy by measuring seroconversion rates and production of neutralising antibodies, which leaves other potentially important aspects of the immune response unexplored. The response of an autoimmune-biased immune system to stimuli such as vaccination or infections therefore remains unclear.

Due to early reports of high mortality among younger individuals during the H1N1 influenza pandemic,[6] the Swedish government offered protective immunisations to all citizens. We used this opportunity to analyse the immune response to the vaccine of patients with primary Sjögren's syndrome (pSS) without immunomodulatory treatment.

Here, we report that untreated patients with pSS respond to vaccination with enhanced antibody responses and, importantly, rising autoantibody titres. We find that naïve B cells from untreated patients readily differentiate into class-switched antibody-producing cells on endosomal toll-like receptor (TLR) stimulation. Furthermore, this hyper-reactive state is linked to proinflammatory cytokine exposure and upregulation of several intracellular signalling pathways including those downstream of TLR7 and TLR9 in patients with primary Sjögren's syndrome.

METHODS

Detailed methods and study participant characteristics are provided in the online supplementary materials.

Participants and vaccination procedure

Female patients with pSS fulfilling the American-European consensus critera[7] and positive for anti-Ro/SSA and/or anti-La/SSB autoantibodies (n=14) and matched healthy controls (n=18) (supplementary table S1) were vaccinated twice with the squalene-adjuvanted inactivated split-virion H1N1 vaccine Pandemrix (GlaxoSmithKline, Brentford,

UK). Blood sampling and collection of clinical parameters was performed prior to, and 1 and 3 weeks after each vaccination.

In vitro class switch experiments were performed using blood samples from 14 untreated and 11 antimalarial drug-treated patients with Sjögren's syndrome and 16 matched healthy controls (supplementary table S2). Cytokine stimulation and *in vitro* chloroquine treatment experiments were performed using cells from buffy coats of healthy blood donors.

The local Ethics Committee Stockholm North approved the study and all participants gave written informed consent.

Statistical analysis

Student's t-test (normal distribution) or Mann-Whitney U-test (non-normal distribution) was used when comparing two groups, and Wilcoxon paired test when analysing paired data, all using Prism V.7 (GraphPad). Area under the curve (AUC) was calculated and analysed using R. Longitudinal variation of continuous parameters was analysed by quantile regression using Stata (StataCorp, College Station, Texas, USA).

RESULTS

Vaccination induces higher specific and non-specific antibody responses in untreated patients with pSS

To assess the impact of vaccination in autoimmune individuals without interference from immune-targeting therapies, we monitored untreated patients diagnosed with pSS during vaccination with an H1N1 influenza vaccine (Pandemrix) (figure 1A, supplementary table S1).[8–10 11] In contrast to previous reports,[5 12–14] we observed markedly higher levels of H1N1 influenza-specific IgG antibodies in patients, mainly of the IgG1 subclass, compared with controls. Furthermore, H1N1 antibodies developed by the patients had higher avidity than those of controls (figure 1B-D, supplementary figure S1A). H1N1-specific IgM and IgA titres did not differ between the two groups, and haemagglutinin antibody titres, used as a measure of vaccine-induced protection and previously reported to be lower in patients with rheumatic disease,[15] were comparable between the groups (supplementary figure S1B, C).

To further explore the impact of vaccine-induced immune activation on humoral responses in non-treated patients with pSS, we analysed the presence of antibodies to other influenza A and B strains. Interestingly, we observed that these antibody titres increased more in patients than in controls on A/H1N1 vaccination (supplementary figure S1D). While this may be due to similarities between the H1N1 vaccine influenza strain and previously encountered viruses, it is however also possible that vaccination reactivated the patients' memory B cells in an unspecific manner. We therefore investigated antibody levels to the non-influenza pathogen Epstein-Barr virus (EBV), to which immunity is common and which has been implicated in pSS pathogenesis. Notably, antibody titres to EBV increased in patients following vaccination, but not in controls (figure 1E). Next, we analysed whether vaccination had an impact also on autoreactive memory B cells and found that autoantibody titres to the Sjögren's syndrome-associated autoantigens Ro/SSA (Ro52 and Ro60) and La/SSB[16 17] indeed increased significantly during the course of vaccination (figure 1F). No new autoantibody specificities were noted (data not shown), and no expansion or dominance of a specific B cell clonotype was observed in patients compared with controls, as analysed by VH-spectratyping (supplementary figure S2). Consistent with the elevated specific IgG titres and polyclonal B cell activation, higher frequencies of circulating $CD19^{dim}CD138^{+}$ plasmablasts and IgG-secreting B cells were detected in patients following vaccination (figure 1G and H supplementary figure S3).

Previous studies have indicated that vaccination does not exacerbate disease in patients with autoimmune rheumatic diseases.[5 14] Considering a potentially stronger immune reaction in non-treated patients, we monitored common clinical signs of disease activity, such as fever, fatigue, myalgia and arthralgia, by a standardised form filled out by the participants at each visit throughout the study. These parameters are now part of the Sjögren's syndrome disease activity scores European League Against Rheumatism Sjögren's Syndrome Patient Reported Index (ESSPRI) and European League Against Rheumatism Sjögren's Syndrome Disease Activity Index (ESSDAI),[18 19] which were not yet established at the time of the study, but confirms their relevance. Notably, fever, fatigue, myalgia and arthralgia also constitute common adverse reactions to vaccination, and the frequencies of patients and controls reporting such symptoms followed similar trends during the vaccination period (figure 2A-D supplementary figure S4).

In all, our data demonstrate that immunisation with an H1N1 influenza vaccine not only induces higher specific antibody responses in untreated autoimmune patients with pSS, but also drives polyclonal B cell activation, including that of autoreactive cells, leading to an increase in autoantibody titres.

Differential peripheral responses to vaccination between patients with pSS and controls

To acquire a more comprehensive understanding of the response triggered by vaccination in untreated autoimmune patients beyond antibody production, we analysed circulating B cell and T cell populations as well as serum levels of cytokines. There were no major differences in lymphocyte populations between patients and controls before vaccination, except for a lower frequency of circulating memory B cells and an increased proportion of naïve B cells in patients, confirming previous studies.[20] Vaccination did not induce further differences between patients and controls in either B cell counts or frequencies of B cell subsets. However, B cells from patients expressed significantly more HLA-DR as a sign of activation 3 weeks after both the vaccination and the boost (figure 3A supplementary figure 5). We did not detect any notable difference in either T cell numbers or activation status between groups apart from a decreased CD62L expression on $CD4^{+}$ T cells after the initial vaccination (supplementary figure S6A-C). None of the hallmark T-helper lineage cytokines interferon-γ (IFN-γ), interleukin (IL)-4 or IL-17 were differently expressed (supplementary figure S6D).

Analysis of cytokine expression showed high basal serum levels of the proinflammatory cytokines IFN-α, BAFF and TNF-α in untreated patients with pSS (figure 3B), as previously reported.[9 21–23] These levels remained significantly elevated in patients throughout the study, or were even further induced. By contrast, the B cell activating cytokines IL-10, IL-6 and IL-7 were detected at comparable serum levels in patients and controls before vaccination, but were significantly more induced in patients on immunisation (figure 3C), suggesting that they might be responsible, at least in part, for the increased antibody production and B cell activation observed in patients after vaccination.[24–27]

Naïve B cells from patients with pSS more readily differentiate into $CD19^{dim}CD138^{+}$ plasmablasts after endosomal TLR stimulation

In view of the increase in IgG titres following vaccination, we investigated the possibility that B cells from untreated patients

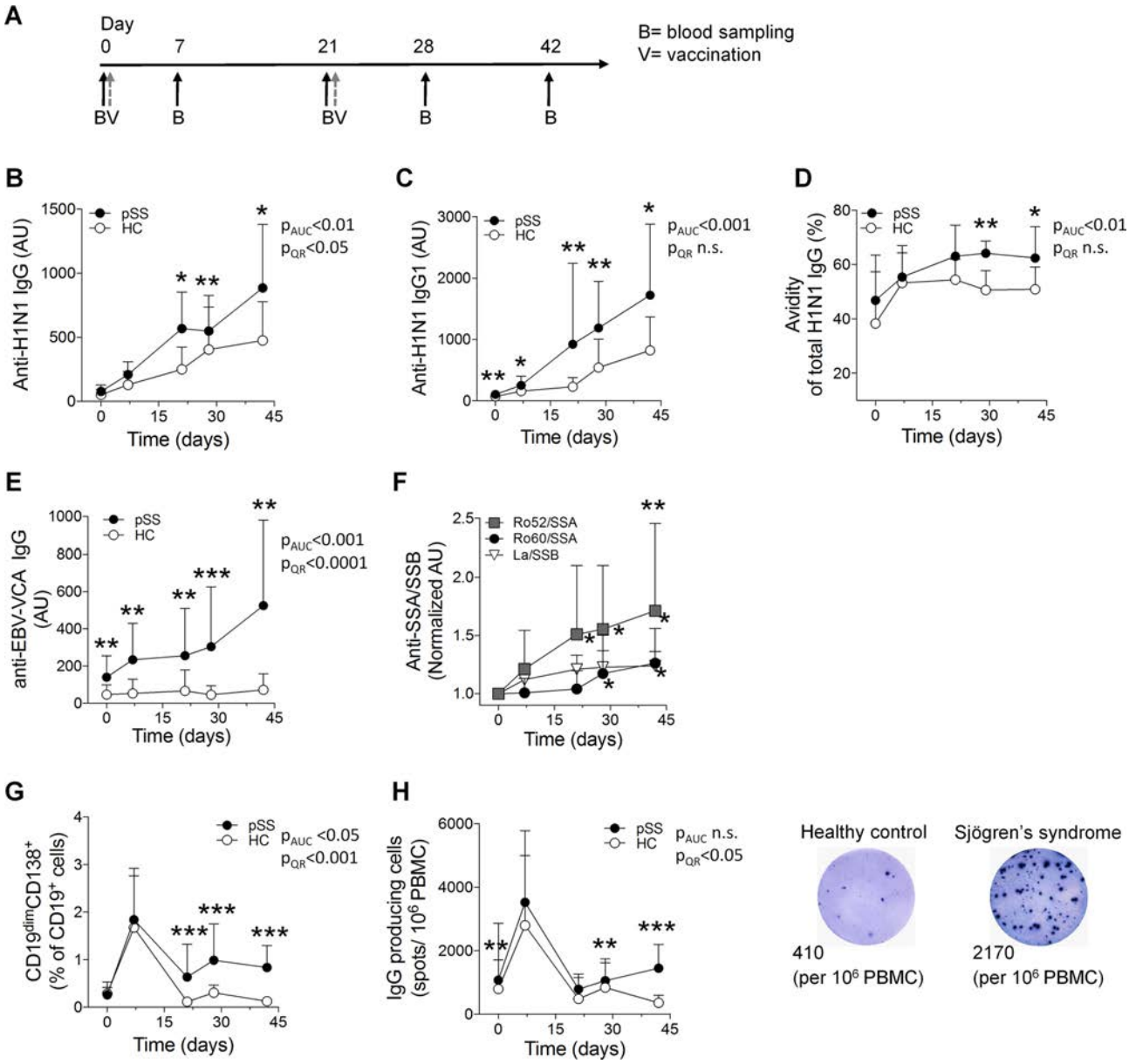

Figure 1 H1N1 vaccination induces higher specific IgG response and polyclonal activation of B cells in Sjögren's syndrome. (A) Untreated patients with primary Sjögren's syndrome (pSS, n=14) and healthy controls (HC, n=18) were subjected to H1N1 vaccination and boost, and followed by blood sampling five times during 42 days. (B) H1N1-specific IgG levels in pSS and HC measured by ELISA. (C) IgG1 subclass H1N1-specific antibodies in pSS and HC measured by ELISA. (D) Avidity of anti-H1N1-specific IgG in pSS and HC, measured by an ELISA-based 8 M urea competition assay. (E) Anti-EBV-VCA IgG levels in pSS and HC measured by ELISA. (F) Ro52/SSA, Ro60/SSA and La/SSB autoantibody levels in pSS measured by ELISA. (G) Live $CD14^-CD3^-CD19^{dim}CD138^+CD27^+$ plasmablasts in pSS and HC assessed by flow cytometry. (H) IgG producing cells detected by ELISPOT. Representative wells from day 42 are shown in the right panel. Numbers indicate spots/10^6 peripheral blood mononuclear cell (PBMC). Data are presented as mean±SD. AUC, area under the curve; QR, quantile regression. *p<0.05, **p<0.01 (Mann-Whitney U test, Student's t-test, Wilcoxon signed-rank test).

with pSS are more prone to differentiate into antibody-producing cells in response to immune triggering. Therefore, we analysed plasma cell differentiation and immunoglobulin (Ig) class switch *in vitro* of naïve $CD19^+IgD^+$ B cells isolated from untreated patients with pSS and healthy donors (supplementary table S2). Anti-CD40, BAFF as well as TLR7 (imiquimod) and TLR9 (CpG) agonists have previously been described to drive these processes[28–35] and were thus selected for *in vitro* differentiation assays. After an initial screening of IL-4, IL-10 and IL-21 (supplementary figure S7A), supplementation with IL-10 was chosen for all cultures as this cytokine promoted the most efficient plasma cell differentiation and class switch.[24] Interestingly, we observed that a higher number of $CD19^{dim}CD138^+$ plasmablasts developed in cultures of pSS patient-derived cells compared with controls on TLR7 and TLR9 stimulation, and higher levels of IgM and IgG were detected in supernatants (figure 4A, supplementary figure S7B). By contrast, anti-CD40 and BAFF stimulation did not differentially affect Ig class switch or plasma cell differentiation between patients and controls.

To understand why endosomal TLR ligands induced plasma cell differentiation and class switch more effectively in naïve B cells isolated from untreated patients with pSS, we investigated gene expression profiles before and after *in vitro* stimulation, using NanoString nCounter multiplex expression profiling (supplementary figure S8). For the analysis, genes were grouped into functional clusters based on GeneOntology and Ingenuity

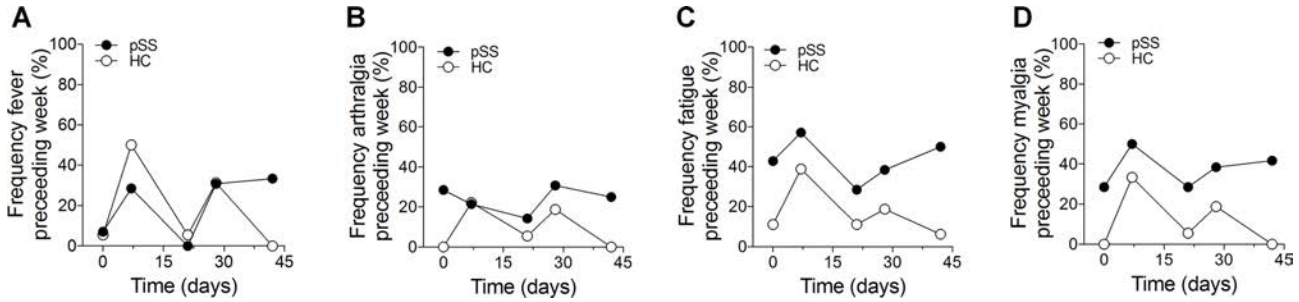

Figure 2 Disease activity on vaccination. Information on experienced symptoms of Sjögren's syndrome during the preceding week was collected. Significant differences were observed after the second vaccination. (A) Fever. (B) Fatigue. (C) Myalgia. (D) Arthralgia. Data are presented as percentage.

gene sets (supplementary table S3). Gene expression profiling revealed prominent differences between the naïve, unstimulated B cells derived from patients and controls: immune genes belonging to a broad spectrum of clusters, including the type I IFN pathway, antigen presentation, B cell development and endosomal TLR signalling, were markedly upregulated in B cells from patients (figure 4B,C, supplementary figure S9). After *in vitro* cell stimulation, the initial gene expression pattern differences diminished, except for gene sets pertaining to plasma cells, reflecting the enhanced differentiation process (supplementary figure S10, table S4).

Taken together, our findings show that naïve B cells from patients with pSS are in a state of hyper-responsiveness, characterised by the overexpression of a variety of immune-related genes, including those downstream of endosomal TLR signalling, and an enhanced capacity to undergo class switch and

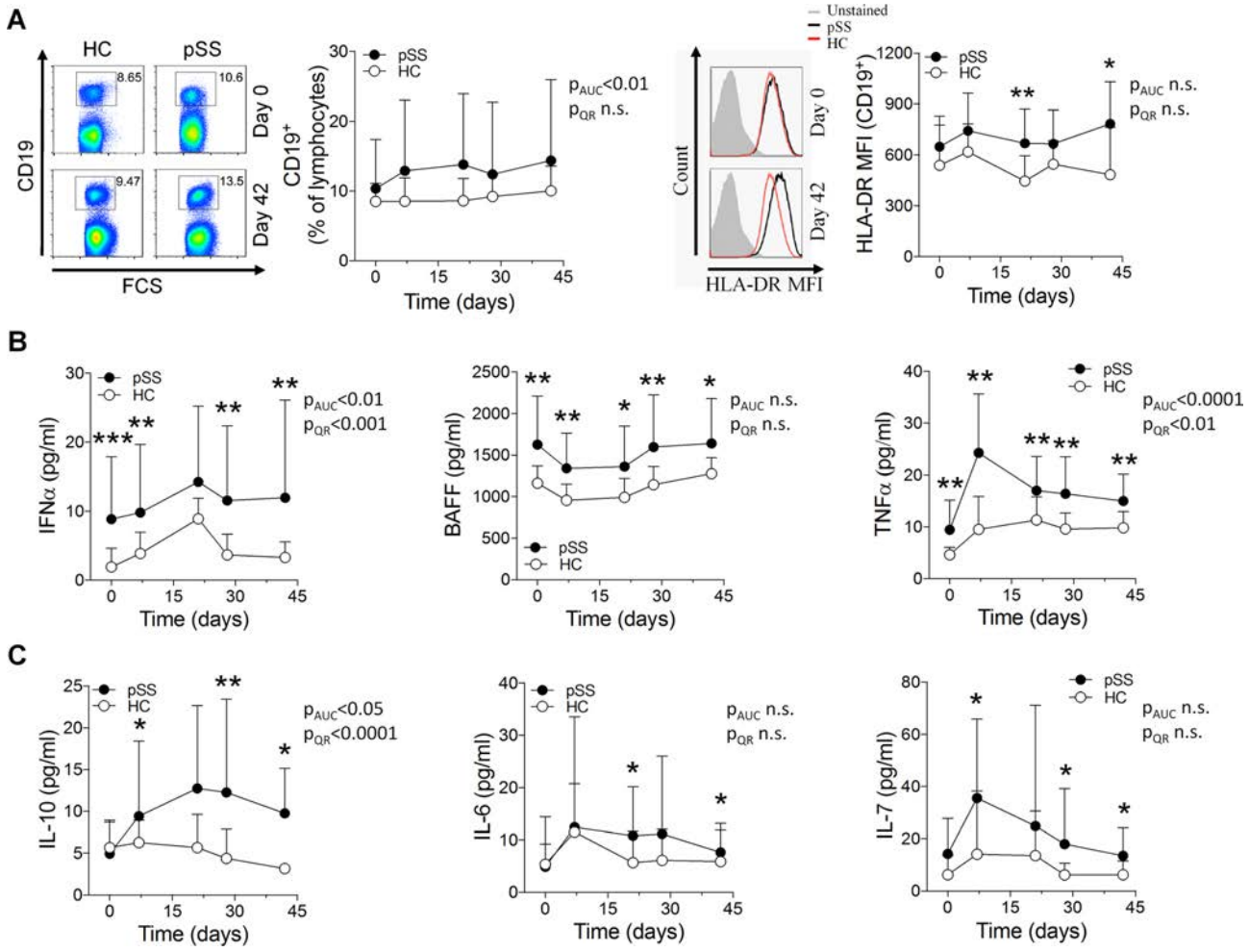

Figure 3 B cell activation and induction of proinflammatory cytokines on vaccination in patients with pSS. (A) Freshly isolated PBMC from patients with pSS and healthy controls were analysed by flow cytometry at each blood sampling time point during the vaccination protocol. Representative dot or histogram plots (left) and quantification graphs (right) are shown for each analysis. Numbers adjacent to outlined gates indicate percentage positive cells. (B, C) Cytokine concentrations in serum samples analysed by a Luminex assay (tumour necrosis factor-α (TNF-α), interleukin (IL)-10, IL-6, IL-7) or ELISA (interferon-α (IFN-α), B cell activating factor (BAFF)). Data are presented as mean±SD. AUC, area under the curve; QR, quantile regression. *p<0.05, **p<0.01, ***p<0.001 (Mann-Whitney U test).

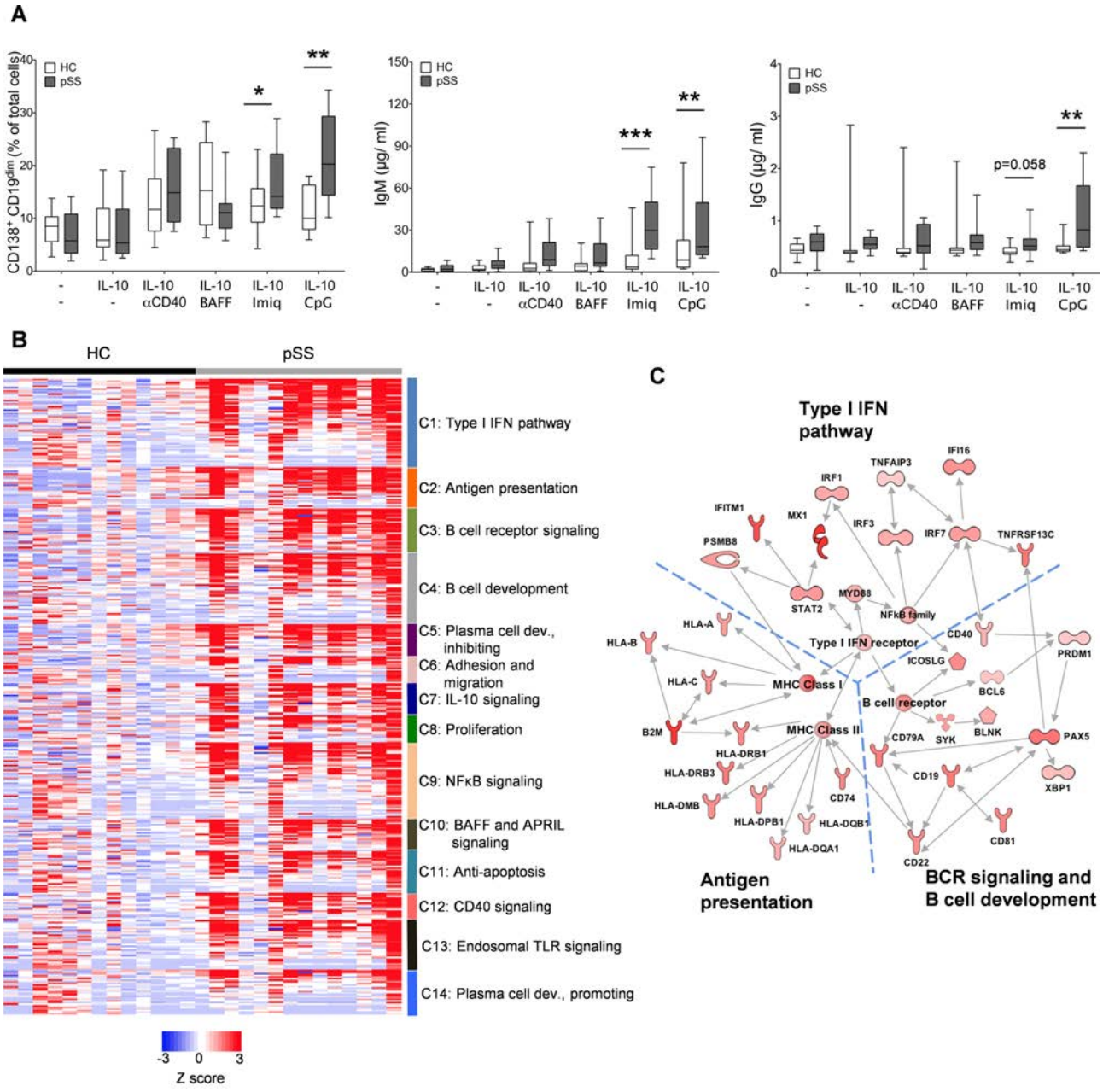

Figure 4 Toll-like receptor (TLR)-induced plasma cell differentiation and class switch are enhanced in naïve B cells from patients with primary Sjögren's syndrome (pSS). (A) fluorescence-activated cell sorter (FACS) sorted naïve $CD19^{+}IgD^{+}$ B cells from freshly isolated peripheral blood mononuclear cell of untreated patients with pSS (n=14) and healthy controls (HC) (n=16) were cultured for 8 days under plasmablast and class switch promoting conditions with anti-CD40, BAFF, CpG or imiquimod (Imiq). At day 8, the frequency of $CD19^{dim}CD138^{+}$ plasmablasts and IgM and IgG concentrations in supernatants were assessed by flow cytometry and ELISA. Data represent pooled data from 10 independent experiments. (B) mRNA was extracted from the naïve B cells at day 0 and gene expression analysed by NanoString. Heat map illustrates expression in patients with pSS and HC organised by Ingenuity pathways and Gene ontology analysis using average Z scores. The 14 most differentially regulated gene clusters are displayed in order of mean fold change of the top 10 upregulated genes in each cluster. (C) Network analysis of differentially expressed genes show that pSS-related genes cluster into the pathways type I interferon (IFN) pathway, antigen presentation and BCR signalling and B cell development. Symbols according to Ingenuity. Brighter red indicates higher upregulation in pSS compared with HC. Data are presented as mean±SD. *p<0.05, **p<0.01, ***p<0.001 (Mann-Whitney U test). IL, interleukin.

plasma cell differentiation when triggered by endosomal TLR ligands.

IFN-α sensitises B cells and promotes $CD19^{dim}CD138^{+}$ plasmablast differentiation on TLR stimulation

We next set out to understand the mechanisms underlying the hyper-responsiveness of B cells from untreated patients with pSS. Based on our observations that these patients had elevated serum levels of IFN-α (figure 3B) and that genes involved in downstream type I IFN pathways were upregulated in cells isolated from untreated patients (figure 4B), combined with the known role of IFN-α in promoting class switch,[36 37] we hypothesised that IFN-α might be responsible for the hyper-reactive state of B cells in untreated patients with pSS. Therefore, to

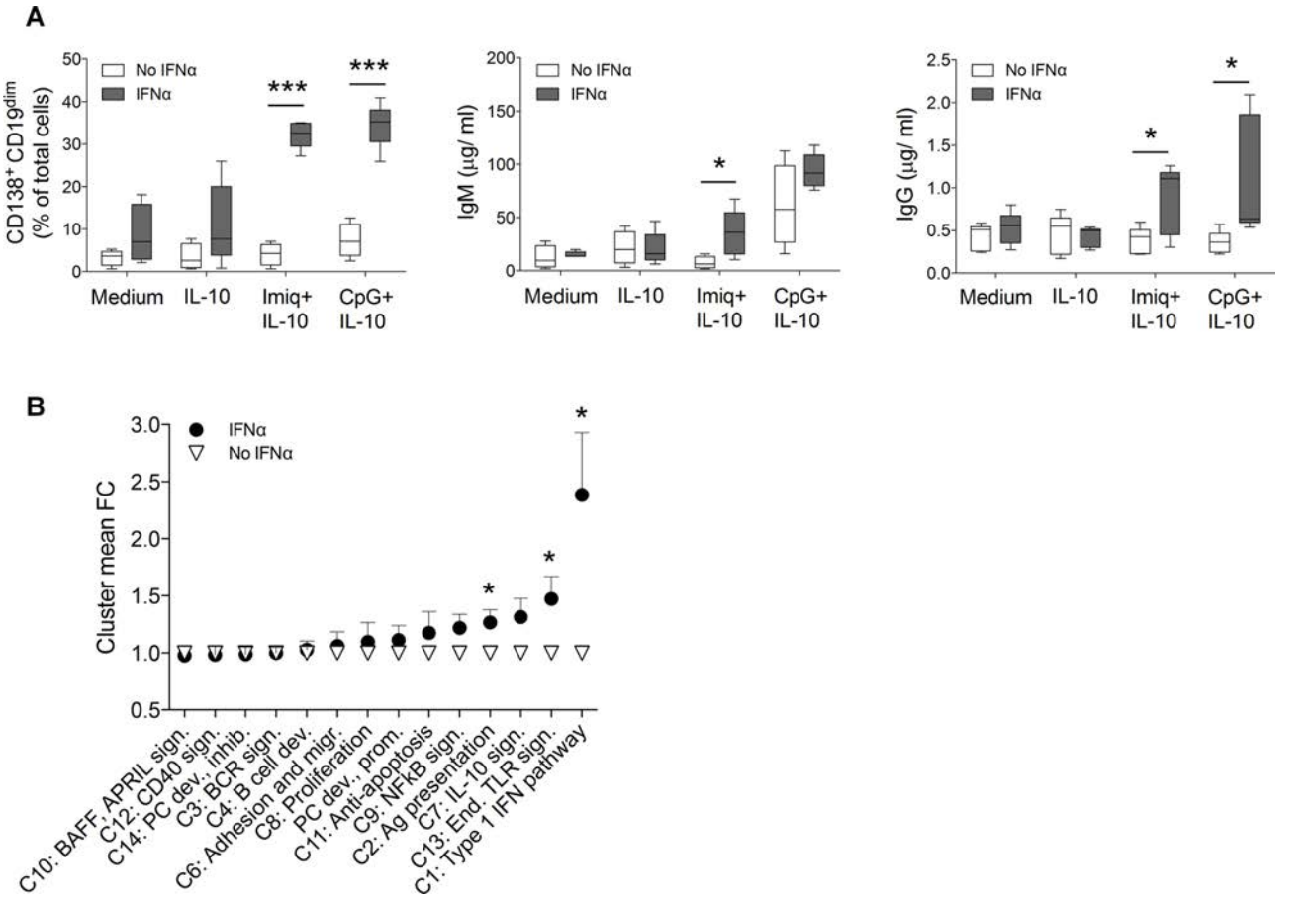

Figure 5 Interferon-α (IFN-α) preactivation recapitulates a Sjögren-like B cell phenotype. (A) fluorescence-activated cell sorter (FACS) sorted $CD19^+IgD^+$ cells from healthy donors (n=5) were cultured with IFN-α for 18 hours prior to stimulation with CpG+interleukin (IL)-10, imiquimod (Imiq)+IL-10, IL-10 alone or medium. Frequencies of $CD19^{dim}CD138^+$ plasmablasts, and total IgM and IgG concentrations in supernatants were analysed at day 8. Data represent pooled data from 3 independent experiments. (B) Gene expression of cells was measured at day 0 and 8 by NanoString. Graph depicts values of mean fold change of clusters 1–14 in cells cultured for 8 days and normalised to expression day 0. Data are presented as mean±SD. *p<0.05, **p<0.01, ***p<0.001 (Mann-Whitney U test).

directly assess whether IFN-α could sensitise B cells to subsequent endosomal TLR stimulation, we incubated naïve B cells isolated from healthy donors with IFN-α before culturing them under plasmablast-differentiating conditions. Strikingly, this was enough to recapitulate the enhanced differentiation and class-switch capacity that we had previously observed in B cells isolated from untreated patients with pSS (figure 5A). Gene expression analysis of cells primed with IFN-α revealed upregulation of genes related to type I IFN pathways and endosomal TLR signalling (figure 5B, supplementary table S4), which we had also found to be upregulated in cells from untreated patients with pSS.

Hydroxychloroquine treatment abolishes B cell hyper-responsiveness to TLR stimulation in patients with pSS

Initially discovered as an antimalarial drug, hydroxycholoroquine (HCQ) is commonly used for treatment of pSS and systemic lupus erythematosus (SLE).[38 39] HCQ acts by inhibiting endosomal TLR signalling, and was recently reported to decrease both IFN-α production in SLE[40 41] and the IFN signature in patients with pSS.[42] Since priming B cells with IFN-α resulted in enhanced B cell differentiation and class switch after TLR stimulation *in vitro*, we investigated whether HCQ treatment would decrease the B cell hyper-responsiveness in patients with pSS. So far, no direct effect of chloroquine on B cells has been established. We therefore first analysed the effect of chloroquine on B cell class switch. $CD19^+IgD^+$ B cells from healthy donors were treated with chloroquine *in vitro*, simultaneously as class switch was induced by TLR7 and TLR9 stimulation. Addition of chloroquine at pharmacologically relevant concentrations led to significantly less differentiation of $CD19^{dim}CD138^+$ cells, class switch and IgM and IgG production (figure 6A). Strikingly, we further found that naïve B cells isolated from HCQ-treated patients with pSS were significantly less prone to differentiate into $CD19^{dim}CD138^+$ plasmablasts and undergo class switch on TLR9 stimulation (CpG) than cells of untreated patients (figure 6B). A similar trend was observed for TLR7-stimulated cells. Analysis of gene expression revealed that HCQ treatment of patients with pSS abolished the upregulation of immune genes otherwise found in B cells from untreated patients (figure 6C,D, supplementary figure S11, table S4). This suggests that HCQ treatment may act directly on naïve B cells, and reverses the enhanced capacity of B cells from patients with pSS to differentiate into plasma cells on TLR stimulation *in vitro* by preventing these cells to become hyper-responsive in the first place.

Altogether, our findings demonstrate that B cells from healthy donors can become hyper-responsive to TLR stimulation on exposure to IFN-α and that, conversely, the increased reactive state of B cells from patients with pSS can be reversed by HCQ treatment.

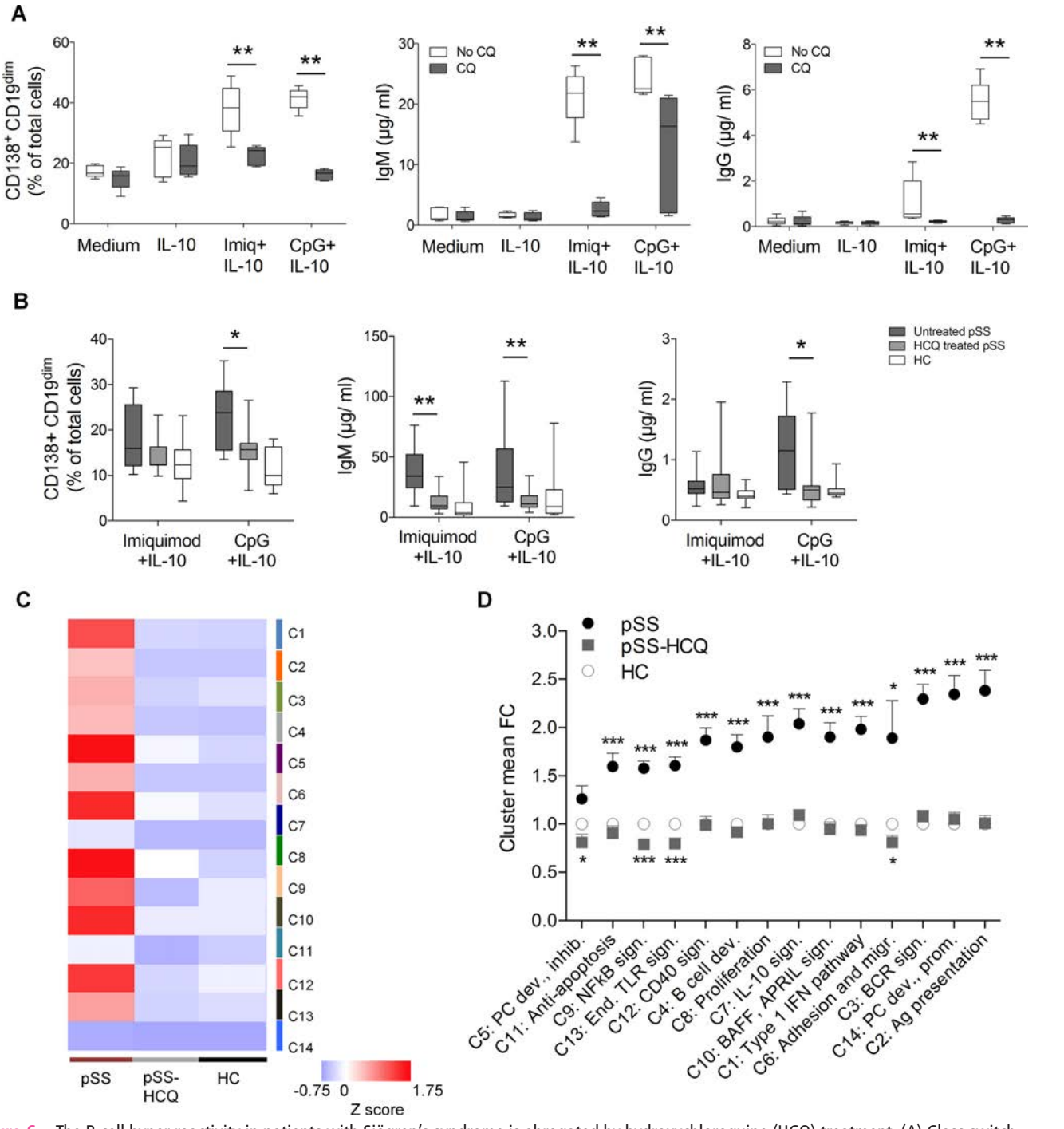

Figure 6 The B cell hyper-reactivity in patients with Sjögren's syndrome is abrogated by hydroxychloroquine (HCQ) treatment. (A) Class switch was induced by imiquimod (Imiq)+interleukin (IL)-10 or CpG+IL-10 in freshly prepared $CD19^+IgD^+$ B cells from buffy coats (n=5) with or without chloroquine in physiological concentrations (1 µg/mL). At day 8, cells were analysed for $CD138^+CD19^{dim}$ expression by flow cytometry, and IgM and IgG concentrations were determined in supernatants. Chloroquine significantly reduced class switch in both Imiq-treated and CpG-treated wells, without affecting cells in IL-10 and untreated wells. Data represent pooled data from 3 independent experiments. (B) FACS sorted $CD19^+IgD^+$ B cells from untreated (n=14) and HCQ or chloroquine treated (n=11) patients with pSS and healthy controls (HC) (n=16) were subjected to class switch induction *in vitro* by Imiq+IL-10 or CpG+IL-10. Data represent pooled data from 18 independent experiments. (C) Heat map of gene expression comparing naïve IgD^+ B cells from untreated and HCQ-treated patient with pSS and HC. (D) Mean fold change of clusters 1–14 in cells cultured for 8 days compared with day 0. Gene expression was measured by NanoString and normalised to expression day 0. Data are presented as mean±SD. *p<0.05, **p<0.01, ***p<0.001 (Mann-Whitney U test).

DISCUSSION

Assessing the efficacy of vaccines in patients with autoimmune rheumatic diseases is crucial to provide adequate recommendations for clinical practice. However, these types of studies may miss potential aspects of how a dysregulated immune system reacts to an immune trigger such as a vaccine, especially since they often include patients treated with immunomodulatory drugs. Here, we studied the immune response of treatment-naïve

autoimmune patients diagnosed with pSS to an H1N1 influenza vaccine. In contrast to previous studies reporting less vigorous immune responses to vaccination in patients with autoimmune rheumatic disease,[5 12 13] we found that untreated patients with pSS developed higher levels of vaccine-specific IgG antibodies than matched controls. Impaired responses to vaccination in autoimmune patients may therefore relate rather to ongoing immunosuppressive therapy than to the immune system being inherently less able to mount protective antibody responses.

Interestingly, we observed that untreated patients with pSS responded to immunisation with higher vaccine-specific antibody titres than matched controls, and with a general increase in non-specific antibody and autoantibody levels, and higher numbers of circulating $CD19^{dim}CD138^{+}$ plasmablasts. We further found that B cells isolated from untreated patients were more prone to differentiate into class-switched antibody-producing plasma cells than cells from controls when stimulated *in vitro* with TLR7 and TLR9 agonists, and that they overexpressed a broad panel of immune-related genes compared with controls already prior to stimulation including those in the signalling pathways downstream of TLR7 and TLR9. These findings suggest that B cells from untreated patients with pSS are in a state of hyper-responsiveness, in particular to endosomal TLR stimulation. Interestingly, a recent study showed that TLR7 was required for optimal antibody production after immunisation with the 2009 pandemic split vaccine (Sanofi Pasteur, Swiftwater, Pennsylvania, USA) in mice,[43] and that this was likely due to TLR7 recognition of viral RNA present in the split vaccine. It is therefore possible that RNA present in the split-virus Pandemrix vaccine used in our study may contribute to the increased plasma cell differentiation and antibody production detected in patients by stimulating B cell endosomal TLRs. We also observed an increased production of the B cell-promoting cytokine IL-10 following immunisation in patients with pSS compared with controls, suggesting that additional factors besides TLR stimulation of B cells may contribute to enhanced humoral responses in patients with pSS. Altogether, in view of our findings showing that B cells from untreated patients with pSS are especially hyper-responsive to endosomal TLR triggers, caution may be warranted when immunising these patients with vaccines containing TLR agonists—whether added as adjuvants or naturally present in whole or split-virus preparations. Of note, the same patients would presumably respond to vaccination and to natural infections with a broad increase in specific and non-specific antibody responses. An evolutionary advantage of the capacity to respond with high levels of specific antibodies may be the ability to survive infections, and could explain the persistence of autoimmune-associated alleles within our gene pool. The delicate balance is illustrated by the fact that despite the markedly increased B cell responses observed in patients, we noted no significant differences between patients and controls with regard to the recorded clinical parameters fever, fatigue, myalgia and arthralgia. These manifestations represent common symptoms of both Sjögren's syndrome and adverse reactions to vaccination,[8 10 44] and similar frequencies of affected individuals was observed after vaccination.

Further analysis of the mechanism underlying B cell hyper-responsiveness in untreated patients with pSS revealed a major role for type I IFN. Indeed, the phenotype could be recapitulated by priming B cells from healthy donors with IFN-α and was abolished by HCQ treatment in patients with pSS. HCQ has been reported to inhibit IFN-α production by plasmacytoid dendritic cells in SLE,[40] as well as decrease the IFN signature in patients with pSS,[42] and may therefore exert its therapeutic effect in pSS and SLE at least partly by suppressing TLR-induced IFN-α production and IFN-α-induced B-cell hyper-responsiveness. Such a mechanism may underlie the decrease in both IgM and IgG titres that were previously reported in patients with pSS receiving HCQ treatment.[38] Clinical trials of HCQ in pSS on the other hand have not met primary end points.[45 46] However, both inclusion criteria and primary outcomes analysed differed from the present study and they are therefore not directly comparable.

In all, our findings reveal that untreated patients with pSS respond to immunisation with increased antibody responses, that this effect is due to a hyper-responsiveness of B cells to endosomal TLR stimulation, and that this phenotype is, in turn, is related to the type I IFN milieu present in these patients.

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Contributors SB and MWH conceived the study and designed the experiments with input from VM, CT, LK and VKK; SB, SM, SP, MF-M, KAB and MWH performed the experiments, SB, LF, SP, JM, LW, CT, VM, VKK and MWH analysed the data, MK and GN managed study participant recruitment and clinical data analysis, SB, AA, VKK and MWH wrote the manuscript and all authors participated in revision until its final form.

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Competing interests None declared.

Patient consent Detail has been removed from this case description to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

Ethics approval Ethics Committee Stockholm North.

Provenance and peer review Not commissioned; externally peer reviewed.

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OPEN ACCESS

EXTENDED REPORT

Microarray analysis of bone marrow lesions in osteoarthritis demonstrates upregulation of genes implicated in osteochondral turnover, neurogenesis and inflammation

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ABSTRACT

Objective Bone marrow lesions (BMLs) are well described in osteoarthritis (OA) using MRI and are associated with pain, but little is known about their pathological characteristics and gene expression. We evaluated BMLs using novel tissue analysis tools to gain a deeper understanding of their cellular and molecular expression.

Methods We recruited 98 participants, 72 with advanced OA requiring total knee replacement (TKR), 12 with mild OA and 14 non-OA controls. Participants were assessed for pain (using Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)) and with a knee MRI (using MOAKS). Tissue was then harvested at TKR for BML analysis using histology and tissue microarray.

Results The mean (SD) WOMAC pain scores were significantly increased in advanced OA 59.4 (21.3) and mild OA 30.9 (20.3) compared with controls 0.5 (1.28) ($p<0.0001$). MOAKS showed all TKR tissue analysed had BMLs, and within these lesions, bone marrow volume was starkly reduced being replaced by dense fibrous connective tissue, new blood vessels, hyaline cartilage and fibrocartilage. Microarray comparing OA BML and normal bone found a significant difference in expression of 218 genes ($p<0.05$). The most upregulated genes included stathmin 2, thrombospondin 4, matrix metalloproteinase 13 and Wnt/Notch/catenin/chemokine signalling molecules that are known to constitute neuronal, osteogenic and chondrogenic pathways.

Conclusion Our study is the first to employ detailed histological analysis and microarray techniques to investigate knee OA BMLs. BMLs demonstrated areas of high metabolic activity expressing pain sensitisation, neuronal, extracellular matrix and proinflammatory signalling genes that may explain their strong association with pain.

INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis worldwide affecting more than 27 million adults in the USA alone[1] and is a major cause of pain and functional disability. OA prevalence is set to rise globally with ageing populations accompanied by the rising epidemic of obesity.[2] OA most commonly affects large weight-bearing joints, affecting the knees in up to 37% of adults over 60.[1] Pain is a major symptom for people with OA, with 16.7% of US adults aged 45 years and above reporting pain as a predominant problem.[1]

Pain in OA is thought to arise from several structures within the arthritic joint, including the synovium (from which prostaglandins, leukotrienes and inflammatory mediators are released), joint effusions, joint capsule involvement, tendon and muscle weakness that all contribute to pain and reduced function.[3] Synovitis is often observed by MRI in OA and strongly correlates with pain.[4] Cartilage degradation is one of the hallmarks of OA disease[5] and exposes the structures from which pain is most likely arising as cartilage is an avascular, aneural structure composed largely of extracellular matrix (ECM) embedded sparsely with chondrocytes. Recent interest has grown in the importance of bone marrow lesions (BMLs) in relation to pain in OA. Epidemiological studies have shown a strong correlation between BMLs observed by MRI and OA-related knee pain in several large cohorts,[6 7] with an OR of 3.2 for the association of BMLs with pain. The data outlined above demonstrate the multifactorial nature of OA and how pain mechanisms are supported by the biopsychosocial model of pain.

Recently, BMLs have been shown to be a very early biomarker of joint damage in OA[6 7] with descriptions of their histology and histomorphometry. However, no previous transcriptomic studies of BMLs in OA are described. In the current study, we describe novel findings demonstrating BMLs have features of angiogenesis, fibrosis, new cartilage formation and increased bone turnover with disruption of the physiological osteochondral interface. Whole transcriptomic analysis of BML regions found upregulated expression of genes involved in neurogenesis, pain sensitisation, chemokine and cytokine signalling as well as cartilage remodelling pathways.

MATERIALS AND METHODS

All study procedures were carried out after ethical approval was granted (Health Research Authority approval number 12/LO/1970 and clinical trials.gov identifier NCT02603939). Participants attending the South London Elective Orthopaedic

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Centre were recruited at assessment for total knee replacement (TKR), comprising the 'advanced OA group'. For the 'mild OA' group, participants were recruited from rheumatology clinics at St George's University Hospitals NHS Foundation Trust. For bone tissue controls, participants undergoing surgery following trauma, amputation or trochleoplasty were recruited (approval number 09/H0806/45) with no clinical or radiographic arthritis. Blood and urine samples were also obtained with full consent for biomarker studies.

Study criteria

Eligibility for participation included age of 35–90 years, presenting with pain and fulfilling ACR criteria for the diagnosis of knee OA.[8] Participants continued to experience pain despite treatment for OA.[9] All participants underwent baseline knee radiography to confirm knee OA with a Kellgren-Lawrence grade of greater than 2 in the affected tibio-femoral knee joint.[10]

Clinical data collection

All scores were collected for participants with advanced OA and mild OA. For controls, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was not collected as participants underwent different surgeries. The primary pain score was the WOMAC with subscales for pain, stiffness and function.[11] Participants were asked to score based on symptoms in the last 48 hours. Data were also collected for body mass index (BMI), Visual Analogue Scale pain rating 0–10[12] and the Hospital Anxiety and Depression Scale.[13]

Molecular methods

Total RNA was isolated from approximately 200 mg of bone tissue. Amplified labelled cRNA samples (600 ng) were hybridised to Agilent whole human genome 60 k microarray chips. Array signal intensities were analysed by the Agilent GeneSpring GX software. Significant differentially expressed entities between bone samples from healthy controls and OA participants were selected using a union of a Student's/moderated t-test corrected for multiple comparisons with the Bonferroni correction ($p<0.05$). Further methodical details are provided in the online supplementary methods. [14–17]

STATISTICAL ANALYSIS

Data were anonymised for all analyses independently by the research team who were not involved in diagnosing or treating the study participants. To detect significant differences between groups at $p<0.05$, recruitment of at least 80 subjects was required, and we achieved n=98 participants. GraphPad Prism V.7 was used for all analyses, and significance was set at $p<0.05$ for all analyses. For microarray statistical analysis, refer to online supplementary methods.

RESULTS

Demographic data showed that our participants were representative of a knee OA population. Knee OA participants who underwent TKR had a high BMI and high pain scores measured by WOMAC (table 1). The mean (SD) WOMAC pain scores were significantly increased in advanced OA 59.4 (21.3) and mild

Table 1 Demographics showing characteristics of study population key. Data presented as means and SD

		Advanced OA	Mild OA	Tissue control
Number*		72	12	10
Age range Mean (SD)		51–88 69.1 (7.7)	49–79 62.2 (8.5)	21–88 56.2 (27.7)
Gender Female N (%)		55 (76.4)	9 (75)	9 (90)
Body mass index Mean (SD)		32.5 (5.7)	28.8 (3.9)	N/A
WOMAC pain Mean (SD)		59.4 (21.3)	30.9 (20.3)	N/A
WOMAC stiffness Mean (SD)		62.8 (25.4)	33.0 (29.7)	N/A
WOMAC function Mean (SD)		59.8 (20.6)	34.0 (24.3)	N/A
NRS pain Mean (SD)		5.7 (2.3)	2.6 (2.4)	N/A
HADS Mean (SD)		12.6 (7.2)	9.6 (6.7)	N/A
MOAKS* N (%) BML	MOAKS=0 MOAKS=1 MOAKS=2 MOAKS=3	9 (14.1) 52 (81.3) 3 (4.6) 0 (0)	4 (57.1) 3 (42.9) 0 (0) 0 (0)	N/A
Synovitis/effusion N (%)	MOAKS=0 MOAKS=1 MOAKS=2 MOAKS=3	2 (3.1) 28 (43.8) 18 (28.1) 16 (25)	2 (28.6) 2 (28.6) 1 (14.2) 2 (28.6)	N/A
Cartilage damage N (%)	MOAKS=0 MOAKS=1 MOAKS=2 MOAKS=3	0 (0) 16 (25) 41 (64.1) 7 (10.9)	4 (57.1) 3 (42.9) 0 (0) 0 (0)	N/A
Clinical Management		Underwent knee replacement surgery	Medical management	Underwent other surgery

BML, bone marrow lesion; HADS, Hospital Anxiety and Depression Scale; MOAKS, MRI Knee Osteoarthritis Score; NRS, Numerical Rating Scale; OA, osteoarthritis; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

OA 30.9 (20.3) compared with controls 0.5 (1.28) (p<0.0001), showing the advanced OA group had significantly more severe pain and functional impairment.

A mixture of OA participants was identified, and they were classified as severe or mild based on MRI. In the advanced OA group, 81.3% of participants had up to 33% of the bone volume (MOAKS score 1) forming a BML in at least one of the 21 measured regions, in addition to significant levels of synovitis and cartilage damage (table 1). MRI scans found BML areas to be invariably associated with regions of established cartilage damage, particularly in medial tibial regions, which were the focus of our tissue and microarray to maintain consistency of anatomical tissue lesions analysed. We found that 37.5% of grade 1 and 2 BML were in the medial tibial compartment, with 12.5% in the lateral tibial compartment. The remainder were distributed in the femur, trochlea and patella. For microarray, 50% samples were localised in the medial tibial compartment, 35.7% were found in the lateral tibial compartment and 14.2% crossed both tibial compartments.

Trends for WOMAC pain with individual MOAKS modalities showed higher WOMAC pain scores were associated with significantly greater BMLs in the advanced OA versus mild OA groups (see online supplementary figure S1). There was also a trend of increasing WOMAC pain with worsening MOAKS-scored synovitis, although these correlations did not reach statistical significance.

Histological analysis showed most normal bone marrow was adipocytic with adipocytes being the primary bone lining cells (figure 1). The bone volume fraction was starkly reduced in BML areas, with marrow replaced by new blood vessels, dense fibrous connective tissue, hyaline cartilage and fibrocartilage. Areas of aggressive resorption were found at the periphery of BML zones alongside regions of cartilaginous aggregates found at least 2 mm deep to the articular surface embedded within the bone compartment. Regions of vascular proliferation with fibrocartilage were interspersed with areas of de novo cartilage formation. Other BML regions exhibited a cellular infiltrate working through the osteoid network. Histological quantification found the BML group had increased vascular proliferation, cellular infiltration and trabecular thickening when compared with the non-BML (NBML) group (p<0.05).

Whole transcriptomic analysis identified 218 entities to be significantly differentially expressed between the OA BML and control bone samples (p<0.05) (figures 2 and 3). The most highly upregulated genes were stathmin 2 (*STMN2*), ATP-binding cassette protein, thrombospondin 4 *(THBS4)*, matrix metalloproteinase 13 (*MMP-13*) and chromosome 21 open reading frame, which are genes involved in diverse functions including bone remodelling, pain sensitisation and matrix turnover (see Discussion). The most downregulated genes included haemoglobin, S100 calcium binding protein A12, hemogen, proplatelet basic protein ((chemokine C-X-C) motif ligand 7) and delta amino levulinate synthase 2 (table 2 see online supplementary table S1 for full list).

Among other significantly upregulated genes were the epidermal growth factor (EGF)-like domain (*EGFL6*), which is involved in cell adhesion, apoptosis and calcium binding; collagen type XVI (*COL16A1*) with functions in ECM organisation, cell adhesion and integrin-mediated signalling; and G protein coupled receptor (*GPR158*), which facilitates signal transduction and binds hormones/neurotransmitters and ATPase H+ transporting lysosomal (*ATP6V0D2*) gene expressed at axon termini and synaptic vesicles that is implicated in neuron projection. We also found upregulation of the DIRAS family, GTP-binding RAS-like 2 (*DIRAS2*) which is a Ras GTPase implicated in neurodegeneration. PC4 and SFRS1 interacting protein 1 (*PSIP1*) were also identified and are molecules involved in neuroepithelial stem cell differentiation, neurogenesis and apoptosis. Neuronal tyrosine phosphorylated phosphoinositide-3-kinase adaptor 2 (*NYAP2*) was also detected, which is a gene involved in neuronal development, interacting with WAVE1 proteins and is implicated in cytoskeletal modelling. We also found catenin (cadherin-associated protein) (*CTNND2*) upregulation, an adhesive junction associated protein implicated in bone, pain sensitisation, brain development and cancer formation.

Gene ontology analysis identified 166 of the 218 significantly differentially regulated entities to be associated with 59 canonical pathways. The angiogenic, Alzheimer disease-presenilin pathway, EGF/FGF/gonadotrophin signalling, inflammation mediated by chemokine and cytokine signalling with PDGF/Notch/vascular epidermal growth factor (VEGF) and Wnt signalling pathways were a few of which had the greatest number of entities related.

Quantitative polymerase chain reaction analysis confirmed *STMN2*, *MMP-13* and *THBS4* were significantly upregulated in BML regions compared with the control comparator group. *THBS4* and *STMN2* were the most highly upregulated genes between the BML and control bone groups (p<0.0001), reflecting comparable results to the microarray (figure 4). *MMP-13* and *STMN2* were upregulated within BML regions compared with NBML matched regions (p<0.0001). However *THBS4* was found to be most upregulated in the NBML compared with both BML and control groups. Serum STMN2 levels were not significantly increased in mild/ advanced OA groups compared with controls. Protein quantification of STMN2 in BML tissue found control bone to have higher presence of STMN2 compared with BML bone (p<0.0001). Functional significance of *MMP-13* protein activity, one of the highest array-expressed genes, found a significant increase in urine CTX-II levels, that is, cleavage products of type II collagen, in the advanced OA group compared with mild OA and control groups (p<0.001) (see online supplementary figure S2).

DISCUSSION

BMLs have been well described by MRI in knee OA,[6 7 18] but very little is known about their transcriptomic expression. To our knowledge, our study is the first to use a multimodal approach with MRI to locate knee OA BMLs, followed by detailed histological analysis and whole transcriptomic techniques for a multivariate interrogation of the changes seen within BMLs.

Bone marrow signal changes were first described on MRI by Wilson *et al* who used the term 'bone marrow oedema' to describe MRI findings in painful joints.[19] Studies so far have focused on acquiring data from patients undergoing joint surgery of the knee and hip. Zanetti *et al* determined histologically that BMLs contained normal fatty marrow with marrow necrosis, necrotic or remodelled trabeculae, oedema and bone marrow bleeding.[20] The same group matched MRI changes to BML abnormalities in participants undergoing TKR and found regions of normal tissue alongside bone marrow fibrosis, oedema and bleeding. In a hip and knee OA study, Hunter *et al* reported increased bone volume fraction but decreased tissue mineral density within BML using light microscopy.[21] Samples from the lesion area showed increased trabecular thickness, with granulation, oedema, necrosis, fibrinoid deposition and hyperplasia of blood vessels. Taljanovic reported one of the largest histological studies of hip OA BML, where regions of fibrosis and microfracture formation at different stages of

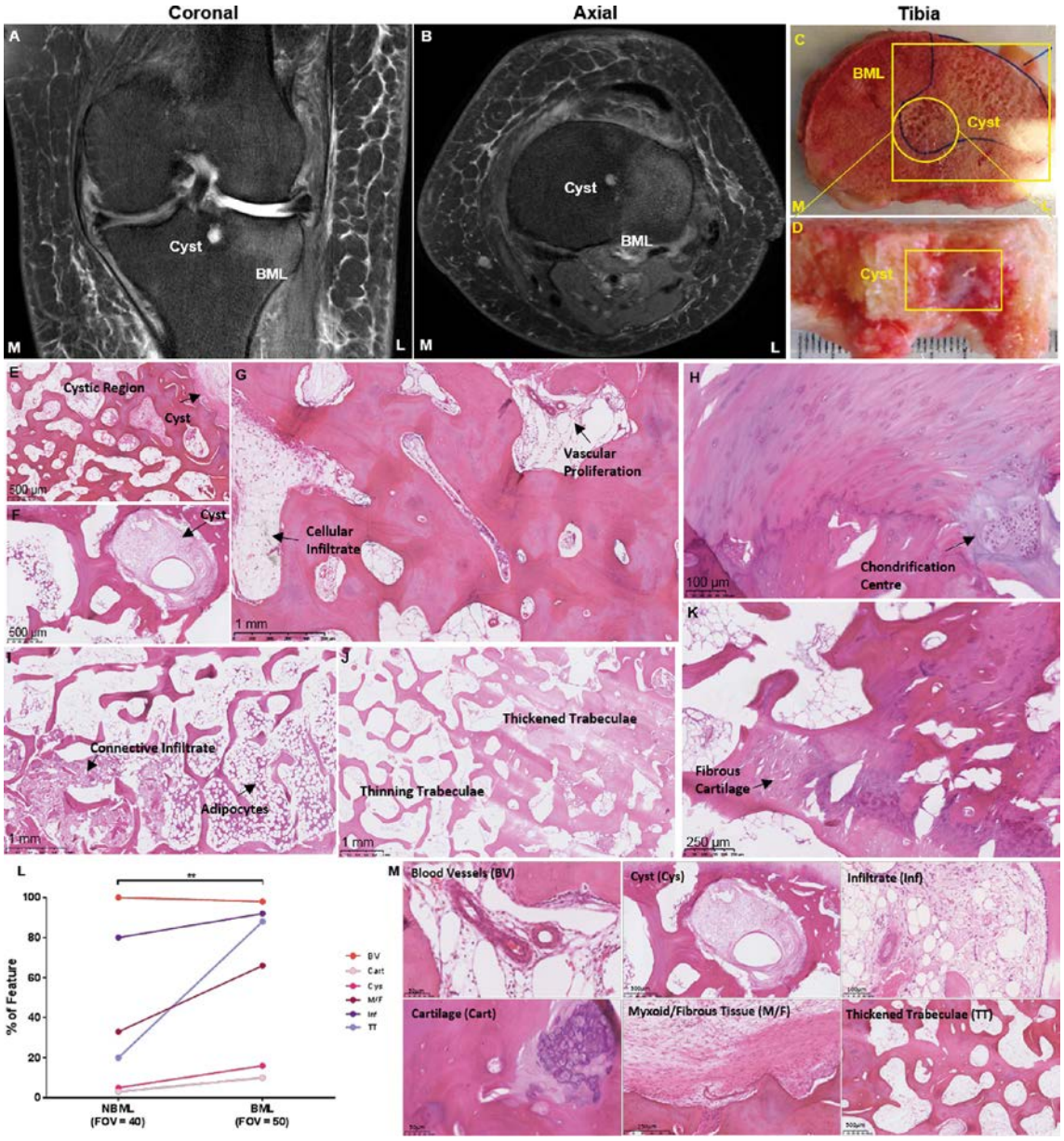


Figure 1 (A) Coronal plane of MRI scan visualising BML and associated cyst. (B) Axial plane of MRI scan presenting BML and associated cyst. (C) Macroscopic view of tibial BML and cystic area. (D) Image of cross section cut through BML and cyst localised by MRI revealing a gelatinous aggregate. (E) H&E staining of cystic region presenting cellular infiltrate in marrow spaces. (F) H&E staining of subchondral cyst forming. (G) H&E staining of BML region with vascular proliferation and cellular infiltration. (H) H&E staining of BML visualising a chondrification centre near the tidemark. (I) H&E staining of adipocyte in bone compartment with a soft tissue infiltrate working through osteoid network. (J) H&E staining of BML showing areas of thickened trabecular adjacent to thinning trabeculae. (K) H&E staining of BML demonstrating areas of fibrotic cartilage formation within the subchondral bone compartment. (L) Quantification of histology analysing 50 BML FOVs and 40 non-BML (NBML) FOVs for blood vessels (BV), cartilage within bone compartment (Cart), cysts (Cys), myxoid/fibrous tissue (M/F), cellular infiltrate (Inf) and trabecular thickening (TT) (n=4). A percentage for the presence of each histological feature was determined for each group. Significance was tested between the groups using Friedman test (*p<0.05). (M) Magnification of each histological change within the bone compartment: BV within subchondral bone, Cart within bone compartment with a chondrification centre, Cys within subchondral bone, M/F adjacent to subchondral bone, Inf within the osteoid network and TT. BML, bone marrow lesion; FOV, field of view.

healing were observed.[22] Leydet-Quilici *et al* also described oedema, necrosis and fibrosis within BML biopsies.[23] Using MRI, Roemer *et al* previously demonstrated that progression of disease and the development of BMLs correlated with an increased risk of cartilage loss within the same subregion and that regions without BMLs are associated with decreased risk of cartilage loss,[24] changes that our work supported. Carrino *et al*[25] reported 87% of subchondral cysts were associated with BML abnormalities, which our analysis confirmed on MRI and by histology. In comparison with other studies, our detailed MRI matching with histological techniques allowed improved visualisation of BMLs, with direct observation of areas appearing as BML-associated cystic structures on MRI and transcriptomic expression. We found higher WOMAC pain scores with greater MOAKS-measured cartilage damage, as suggested by previous studies.[7]

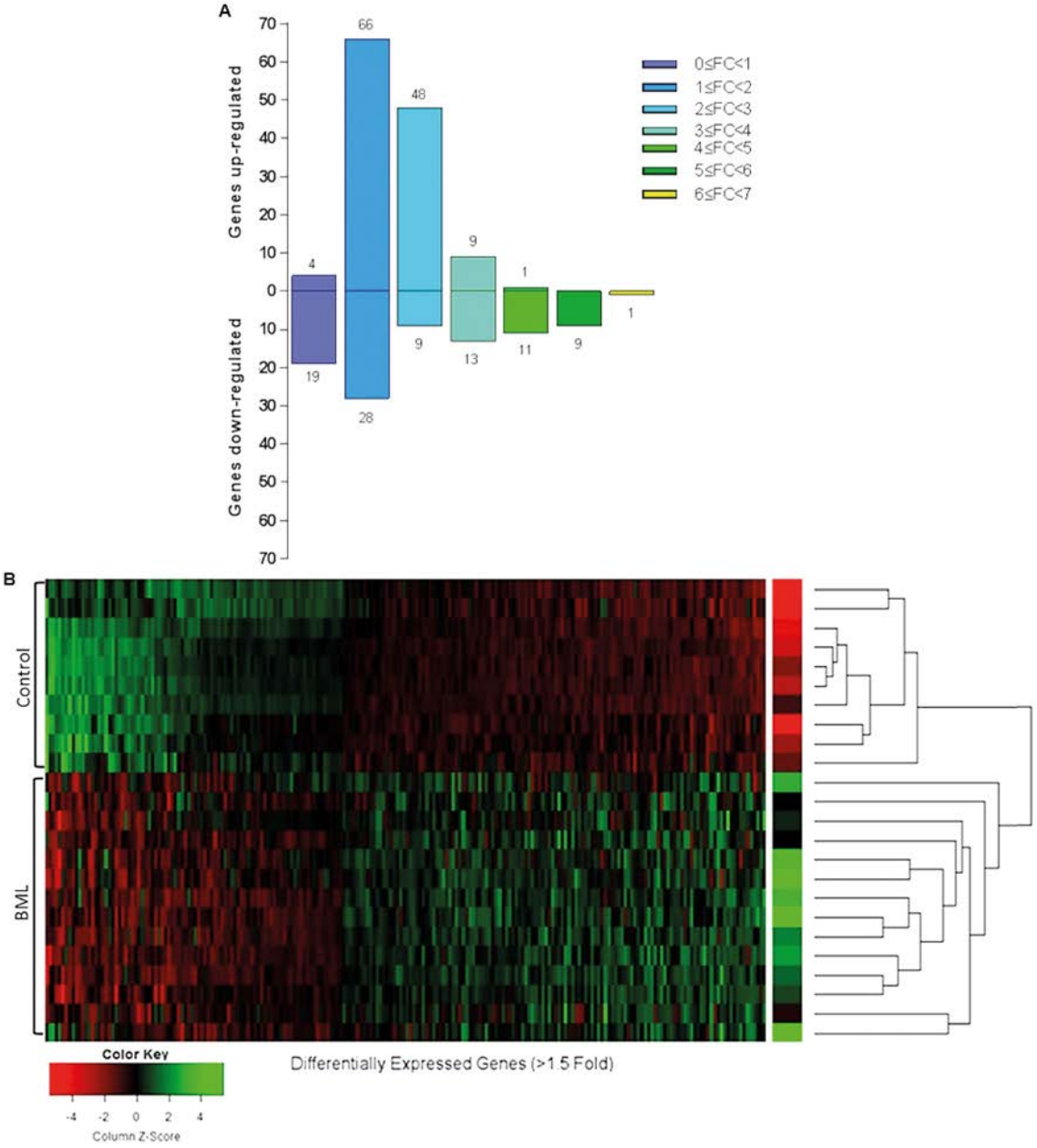


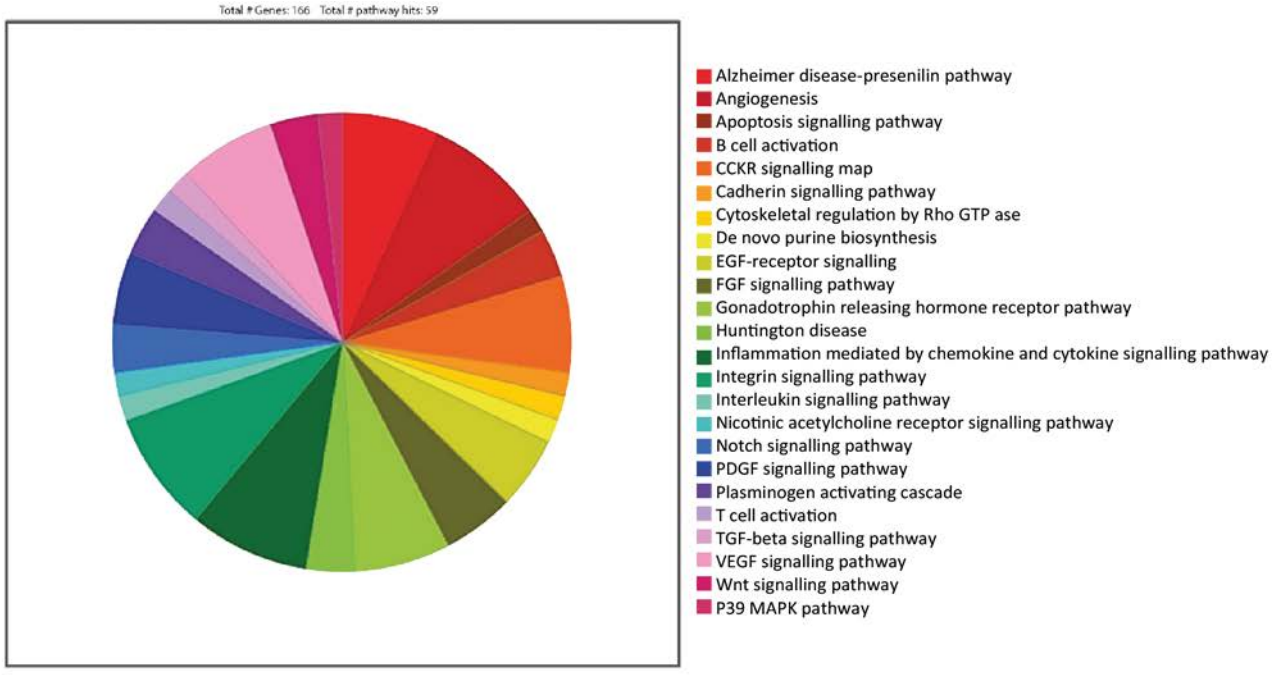
Figure 2 (A) Bar chart presenting the most significantly upregulated and downregulated entities by fold change (FC). One hundred twenty-eight entities were found to be upregulated and 90 were downregulated. The mean WOMAC pain score in the OA microarray group was 61.4, and all subjects in the OA array group had a MOAKS BML score of at least 1, with cartilage and synovitis scores of at least 2. (B) Pearson's correlation hierarchical clustering of 218 genes clearly segregating the OA BML group from the control group.

In our study, cystic BML areas were surrounded by regions of fibrosis, infiltration by inflammatory cells and vascular proliferation. Previous hypotheses that BMLs could be precystic but that not all BMLs become cystic is also supported by our histological findings, where we observed cystic structures within the areas defined as cysts using MRI, and also adjacent to areas of fibrocartilage, vascular proliferation, chondrogenesis and amorphous tissue deposition. We observed new cartilage forming deep within the subchondral bone compartment. The new cartilage tissue within the BML could be arising from mesenchymal stem cells (MSCs) in the marrow, which is seen by other groups.[26] Campbell *et al* reported an altered phenotype of MSCs in hip OA BMLs, showing BML-derived MSCs undergo osteochondral angiogenesis and have lower proliferation and mineralisation capacities.[27]

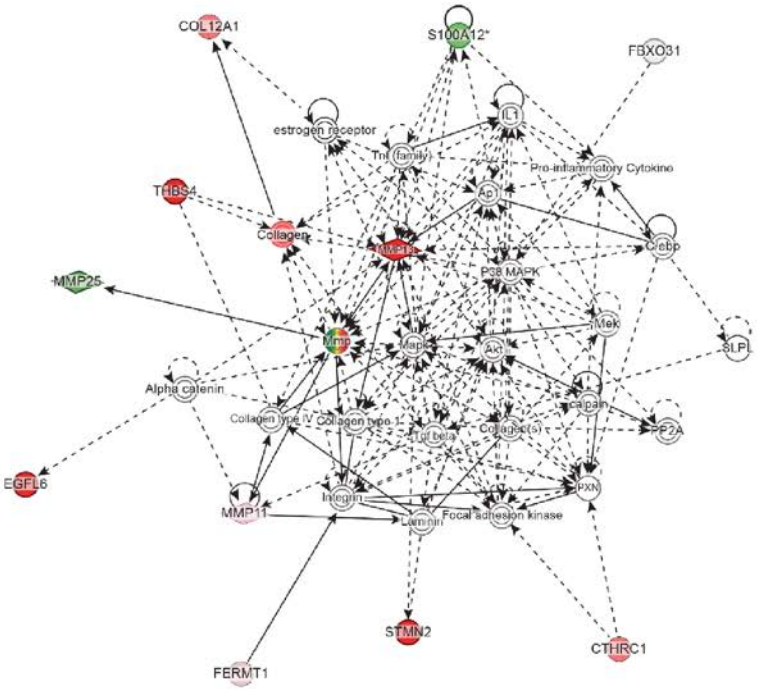
From our microarray, the highest upregulated gene was *STMN2*, a phosphoprotein involved in regulating microtubule function, responsiveness to nerve growth factor (NGF), neuronal growth and osteogenesis.[28] Upregulation of *STMN2* within BML could lead to new neuronal structures and expansion of the BML in OA, thereby causing pain.[29] Stathmin 2 protein expression was higher in normal than BML bone, which could reflect increased stathmin 2 turnover in OA BMLs.

We also identified neuronal markers including thrombospondin 4 (*THBS4*), implicated in the inflammatory response to Central Nervous system (CNS) injury, presynaptic hypersensitivity and neuropathic pain states.[30] In animal models of pain sensitisation, *THBS4* levels are increased locally in dorsal root ganglion neurons and contribute to pain behaviour, which can be inhibited by the calcium channel modulator gabapentin.[31]

A.



B.



Molecules in Network	Focus Molecules
Akt, Alpha catenin, Ap1, C/ebp, calpain, ↑COL12A1, Collagen, Collagen type 1, Collagen type IV, Collagen(s), ↑CTHRC1, ↑EGFL6, estrogen receptor, ↓FBXO31, ↑FERMT1, Focal adhesion kinase, IL1, Integrin, Laminin, Mapk, Mek, MMP, ↑MMP11, ↑MMP13, ↓MMP25, P38 MAPK, PP2A, Pro-inflammatory Cytokine, ↓PXN, ↓S100A12, ↓SLPL, ↑STMN2, Tgf beta, ↑THBS4, Tnf (family)	↑COL12A1, ↑CTHRC1, ↑EGFL6, ↓FBXO3, ↑FFRMT1, ↑MMP11, ↑MMP13, ↓MMP25, ↓PXN, ↓S100A12, ↓SLPL, ↑STMN2, ↑THBS4

Figure 3 (A) Gene ontology analysis of 218 differentially expressed entities found 166 genes associated with 59 canonical pathways. Pie chart of the 24 predominant pathways identified. The main significant correlation for WOMAC pain with gene correlation was for MMP-13 ($p<0.05$). (B) Network analysis was performed on the differentially expressed genes by ingenuity pathway analysis (IPA). MMP-13, matrix metalloproteinase 13; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Other upregulated genes involved in neuronal morphogenesis included *ATP6V0D2*, *PSIP1*, *NYAP2*, FERM and PDZ containing 4 (*FRMPD4*), implicated in CNS development and pain states.[32 33] ECM genes were also represented in the array, including *MMP-13* and collagens, *COL16A1*, fibronectins and growth factors, which are known to be bound within the ECM.[34]

Table 2 Summary of the top differentially expressed entities between the OA BML and non-OA control groups using whole transcriptomic analysis

Accession no	Symbol	Entity name	↑↓	Abs FC	Log FC	P Value*	P Value†
NM_007029	*STMN2*	Stathmin 2	Up	19.30	4.27	3.67×10^{-6}	1.6×10^{-6}
NM_001163942	*ABCB5*	ATP-binding cassette, sub-family B (MDR/TAP), member 5	Up	12.11	3.60	2.06×10^{-6}	8.86×10^{-7}
NM_003248	*THBS4*	Thrombospondin 4	Up	11.53	3.53	1.31×10^{-4}	7.35×10^{-5}
NM_002427	*MMP13*	Matrix Metallopeptidase 13 (collagenase 3)	Up	11.18	3.48	2.78×10^{-5}	1.41×10^{-5}
NR_037585	*C21orf37*	Chromosome 21 open reading frame 37	Up	9.32	3.22	3.64×10^{-6}	1.65×10^{-6}
NM_001167890	*EGFL6*	EGF-like-domain, multiple 6	Up	9.07	3.18	2.69×10^{-5}	1.38×10^{-5}
NM_001856	*COL16A1*	Collagen, type XVI, alpha 1	Up	8.25	3.04	1.8×10^{-5}	9.08×10^{-6}
NM_020752	*GPR158*	G protein-coupled receptor 158	Up	8.21	3.04	1.13×10^{-4}	6.35×10^{-5}
NM_012093	*AK5*	Adenylate kinase 5	Up	8.01	3.00	5.77×10^{-6}	2.73×10^{-6}
NM_174858	*AK5*	Adenylate kinase 5	Up	8.01	3.00	3.33×10^{-5}	1.74×10^{-5}
NM_152565	*ATP6V0D2*	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d2	Up	7.89	2.98	4.11×10^{-6}	1.91×10^{-6}
	ALU2	Alu 2 Element	Up	7.44	2.89	1.32×10^{-6}	5.82×10^{-7}
NM_017594	*DIRAS2*	DIRAS family, GTP-binding RAS-like 2	Up	7.14	2.84	2.8×10^{-6}	1.29×10^{-6}
XR_245643	*LOC101929504*	Uncharacterized LOC101929504	Up	7.02	2.81	3.79×10^{-5}	2.02×10^{-5}
NM_021233	*DNASE2B*	Deoxyribonuclease II beta	Up	7.02	2.81	1.55×10^{-5}	7.86×10^{-6}
NM_014980	*STXBP5L*	Syntaxin binding protein 5-like	Up	6.72	2.75	2.68×10^{-6}	1.24×10^{-6}
NM_004789	*LHX2*	LIM homeobox 2	Up	6.71	2.75	7.61×10^{-5}	4.23×10^{-5}
NM_021144	*PSIP1*	PC4 and SFRS1 interacting protein 1	Up	6.57	2.72	3.62×10^{-6}	1.71×10^{-6}
NM_020864	*NYAP2*	Neuronal tyrosine-phosphorylated phosphoinositide-3-kinase adaptor 2	Up	6.48	2.70	2.53×10^{-5}	1.33×10^{-5}
NM_001332	*CTNND2*	Catenin (cadherin-associated protein), delta 2	Up	6.36	2.67	6.52×10^{-6}	3.19×10^{-6}
NM_032532	*FNDC1*	Fibronectin type III domain containing 1	Up	6.09	2.61	7×10^{-5}	3.91×10^{-5}
NM_001426	*EN1*	Engrailed homeobox 1	Up	5.75	2.52	1.21×10^{-6}	5.56×10^{-7}
NR_027054	*MIR31HG*	MIR31 host gene (non-protein coding)	Up	5.64	2.50	1.21×10^{-6}	1.03×10^{-4}
	XLOC_006820		Up	5.48	2.45	9.05×10^{-6}	4.6×10^{-6}
NM_014728	*FRMPD4*	FERM and PDZ domain containing 4	Up	5.34	2.42	3.09×10^{-5}	1.68×10^{-5}
TCONS_00014487	*LOC101929450*	Uncharacterized LOC101929450	Up	5.33	2.41	1.31×10^{-5}	6.78×10^{-6}
NM_022970	*FGFR2*	Fibroblast growth factor receptor 2	Up	5.30	2.41	9.69×10^{-6}	4.97×10^{-6}
NM_012152	*LPAR3*	Lysophosphatidic acid receptor 3	Up	5.27	2.40	3.65×10^{-5}	2×10^{-5}
NM_004370	*COL12A1*	Collagen, type XII, alpha 1	Up	5.27	2.40	1.32×10^{-6}	6.2×10^{-7}
BC043571	*LOC613266*	Uncharacterized LOC613266	Up	5.09	2.35	1.2×10^{-7}	5.25×10^{-8}
NM_000170	*GLDC*	Glycine dehydrogenase (decarboxylating)	Up	5.00	2.32	6.11×10^{-5}	3.46×10^{-5}
NM_031913	*ESYT3*	Extended synaptotagmin-like protein 3	Up	5.00	2.32	3.61×10^{-5}	1.99×10^{-5}
	ALU1	Alu 1 Element	Down	−5.02	−2.33	3.17×10^{-7}	1.44×10^{-7}
NM_025260	*C6orf25*	Chromosome 6 open reading frame 25	Down	−5.82	−2.54	5.35×10^{-6}	2.62×10^{-6}
NM_080429	*AQP10*	Aquaporin 10	Down	−6.92	−2.79	6.26×10^{-7}	2.62×10^{-6}
NM_005306	*FFAR2*	Free fatty acid receptor 2	Down	−7.29	−2.87	5.63×10^{-5}	3.06×10^{-5}
AB305916	*TRBV28*	T Cell Receptor Beta Variable 28	Down	−7.50	−2.91	3.35×10^{-6}	1.55×10^{-6}
NM_000517	*HBA2*	Hemoglobin, alpha 2	Down	−7.64	−2.93	7.61×10^{-7}	3.25×10^{-7}
	XLOC_014512		Down	−7.99	−3.00	2.74×10^{-7}	1.1×10^{-7}
NM_000517	*HBA2*	Hemoglobin, alpha 2	Down	−8.20	−3.04	5.59×10^{-7}	2.33×10^{-7}
NM_016509	*CLEC1B*	C-type lectin domain family 1, member B	Down	−8.24	−3.04	1.03×10^{-4}	2.33×10^{-7}
NM_002620	*PF4V1*	Platelet factor 4 variant 1	Down	−9.31	−3.22	2.34×10^{-6}	1.04×10^{-6}
NM_022468	*MMP25*	Matrix Metallopeptidase 25	Down	−9.33	−3.22	4.32×10^{-5}	2.28×10^{-5}
NR_120522	*LOC102724484*	Uncharacterized LOC102724484	Down	−10.04	−3.33	1.01×10^{-4}	5.6×10^{-5}

Continued

Table 2 Continued

Accession no	Symbol	Entity name	↑↓	Abs FC	Log FC	P Value*	P Value†
NM_001136503	*SMIM24*	Small integral membrane protein 24	Down	−10.29	−3.36	1.38×10^{-5}	6.73×10^{-6}
NM_030773	*TUBB1*	Tubulin, beta 1 class VI	Down	−12.37	−3.63	5.86×10^{-7}	2.34×10^{-7}
	HSJ1167H4		Down	−13.17	−3.72	3.71×10^{-6}	1.65×10^{-6}
NR_001552	*TTTY16*	Testis-specific transcript, Y-linked 16 (non-protein coding)	Down	−13.65	−3.77	6.28×10^{-5}	3.34×10^{-5}
NR_047499	*LINC00570*	Long intergenic non-protein coding RNA 570	Down	−14.00	−3.81	1.03×10^{-4}	8.67×10^{-5}
NM_144673	*CMTM2*	CKLF-like MARVEL transmembrane domain containing 2	Down	−14.25	−3.83	2.71×10^{-5}	1.36×10^{-5}
NM_001557	*CXCR2*	Chemokine (C-X-C motif) receptor 2	Down	−14.93	−3.90	9.27×10^{-6}	4.34×10^{-6}
NM_000519	*HBD*	Hemoglobin, delta	Down	−15.75	−3.98	7.89×10^{-8}	2.74×10^{-8}
NM_002100	*GYPB*	Glycophorin B (MNS blood group)	Down	−16.15	−4.01	1.03×10^{-4}	1.43×10^{-4}
XM_005261527	*SEC14L3*	SEC14-like 3 (S. cerevisiae)	Down	−16.65	−4.06	2.98×10^{-5}	1.5×10^{-5}
AK128128	*FLJ46249*		Down	−16.90	−4.08	6.19×10^{-5}	3.27×10^{-5}
NM_016509	*CLEC1B*	C-type lectin domain family 1, member B	Down	−17.06	−4.09	1.34×10^{-5}	6.39×10^{-6}
NM_016509	*CLEC1B*	C-type lectin domain family 1, member B	Down	−17.67	−4.14	4.83×10^{-6}	2.15×10^{-6}
NM_002049	*GATA1*	GATA binding protein 1 (globin transcription factor 1)	Down	−19.55	−4.29	7.87×10^{-5}	4.21×10^{-5}
NM_005764	*PDZK1IP1*	PDZK1 interacting protein 1	Down	−20.36	−4.35	7.59×10^{-6}	3.47×10^{-6}
NM_006163	*NFE2*	Nuclear factor, erythroid 2	Down	−22.54	−4.49	3.22×10^{-5}	1.62×10^{-5}
	XLOC_013489		Down	−23.69	−4.57	2.85×10^{-5}	1.42×10^{-5}
NM_002619	*PF4*	Platelet factor 4	Down	−31.42	−4.97	1.26×10^{-7}	4.32×10^{-8}
	XLOC_000346		Down	−31.94	−5.00	1.26×10^{-7}	2.56×10^{-5}
NM_000032	*ALAS2*	Aminolevulinate, delta-, synthase 2	Down	−33.49	−5.07	1.93×10^{-5}	9.3×10^{-6}
NM_005980	*S100P*	S100 calcium binding protein P	Down	−33.56	−5.07	1.11×10^{-4}	6.06×10^{-5}
NM_005331	*HBQ1*	Hemoglobin, theta 1	Down	−34.07	-5.09	3.58×10^{-6}	1.53×10^{-6}
NM_002704	*PPBP*	Pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)	Down	−39.94	−5.32	4.11×10^{-8}	1.3×10^{-8}
NM_000517	*HBA2*	Hemoglobin, alpha 2	Down	−41.07	−5.36	2.47×10^{-7}	8.77×10^{-8}
NM_001003938	*HBM*	Hemoglobin, mu	Down	−45.11	−5.50	7.66×10^{-5}	4.05×10^{-5}
NM_018437	*HEMGN*	Hemogen	Down	−53.12	−5.73	1.89×10^{-6}	7.66×10^{-7}
NM_005621	*S100A12*	S100 calcium binding protein A12	Down	−56.95	−5.83	7.25×10^{-5}	3.81×10^{-5}
NM_005621	*S100A12*	S100 calcium binding protein A12	Down	−58.82	−5.88	$4.6 \times 10-^{5}$	2.34×10^{-5}
NM_000559	*HBG1*	Hemoglobin, gamma A	Down	−88.82	−6.47	1.94×10^{-6}	7.82×10^{-7}

Symbol, Entity Symbol. ↑↓, Regulation. Abs FC, Absolute Fold Change. Log FC, Log transformed Fold Change.
*Adjusted Student T-test P value for microarray corrected for multiple testing by the Bonferroni FWER method.
†Adjusted Moderated T-test P value for microarray corrected for multiple testing by the Bonferroni FWER method.

Our data demonstrate that BMLs are regions of high metabolic activity with increased cell turnover, bone remodelling, neuronal and inflammatory gene signatures. Gene ontological analysis revealed canonical pathways involved in chemokine, integrin and cytokine signalling. We found neurodevelopment and pain pathway signalling represented by the Alzheimer's, Notch, catenin, Wnt pathways alongside VEGF and angiogenic pathway expression. Work by Hopwood *et al*[35] and Chou *et al*[36] analysing the gene expression profile of OA bone also found expression of bone remodelling signalling pathways including Wnt, transforming growth factor and bone morphogenic protein and bone remodelling molecules such as periostin and leptin. Kusumbe *et al* described how growth of blood vessels in bone and osteogenesis are coupled, proposing that type H endothelial cells mediate local growth of the vasculature and provide specific signals for perivascular osteoprogenitors.[37] The same group reported that endothelial Notch activity promotes angiogenesis and osteogenesis in bone.[38] We also demonstrated *OMD* in our BML tissue: Ninomiya *et al* showed that osteoclast activity induces *OMD* expression in bone, suggesting BMLs represent areas of active bone remodelling.[39]

The expression of both osteogenic and angiogenic genes along with the tissue changes we identified may suggest that vascular proliferation and bone formation are likely to be coupled in BML formation. Since blood vessels are formed within neurovascular bundles, it is likely that increased neuronal pathway gene expression including *STMN2*, *THBS4*, *PSIP1*, *NYAP2* and catenin, which were among some of the most highly expressed genes from our BML analysis, are implicated in neural pathway development, new nerve formation and pain mediation in BML tissue.

Our array also identified molecules within the Wnt signalling pathway, including catenin. Other studies have demonstrated a critical role for Wnt signalling in the production and persistence of neuropathic pain after nerve injury and bone cancer.[40] Rodent models show that in nerve injury and bone cancer pain models, respectively, Wnt signalling is activated, which may contribute to pain by regulating pro-inflammatory cytokines interleukin-18 and tumour necrosis factor-alpha, as well as NR2B and subsequent

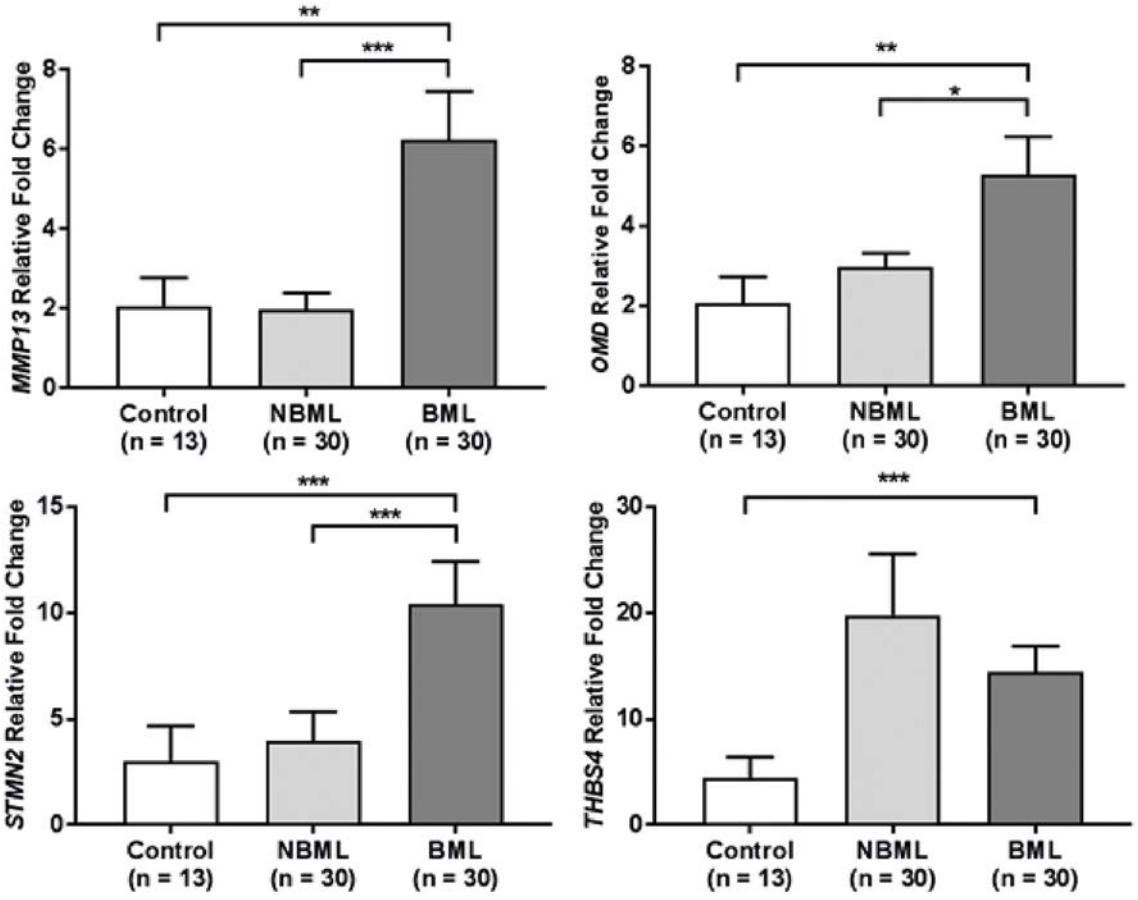


Figure 4 qPCR validation for stathmin 2 (*STMN2*), thrombospondin 4 (*THBS4*), matrix metalloproteinase 13 (*MMP-13*) and osteomodulin (*OMD*) of OA BML compared non-BML tissue and control bone. *STMN2*, *THBS4* and *MMP-13* were selected as they were among the most upregulated genes from the microarray. Osteomodulin was selected as a bone-specific marker as it is involved in bone homeostasis (**p<0.005, ***p<0.0005). BML, bone marrow lesion; NBML, non-bone marrow lesion; OA, osteoarthritis.

Ca2+-dependent signals in the dorsal horn. We found a high representation of the inflammatory chemokines and cytokine signalling; other groups have also identified chemokines in OA pain, for example, *CCR2* was recently reported to mediate pain in a murine model of OA.[41] Our data suggest that chemokine pathway molecules could be pain sensitisers in BMLs. Walsh *et al* showed that OA neurovascular changes at the osteochondral junction, including vessels and both sensory and sympathetic nerves breaching the tidemark, could possibly be a source of joint pain.[42] The genes we have identified in our BML transcriptome support the hypothesis of neurovascular gene upregulation in BML tissue.

One of our most highly expressed genes was *MMP-13*, an enzyme expressed in cartilage, involved in regulating ECM turnover and cartilage destruction in OA.[43] Our data showed that type II collagen degradation products were increased in urine from our advanced OA population. The de novo cartilage formation observed within BMLs, coupled with the increased transcriptomic expression of *MMP-13* observed using microarray and the detection of MMP-13 cleavage products, could suggest recapitulation of the embryonic bone development phenotype within OA BML regions.

Limitations of our study included the sample size for microarray, which although on a standard format of 24 samples, will benefit from larger studies. Future work for protein evaluation of the genes identified is needed, investigating which cells within BMLs are responsible for producing the genes identified and how BMLs develop with respect to the pathological changes identified in OA over time. Although we did not identify NGF, we found genes in neurotrophin pathways, including stathmin 2, which increases responsiveness to NGF,[28] syntaxin, which regulates brain-derived neurotrophic factor[44] and pituitary adenylate cyclase-activation polypeptide, implicated in neuronal development.[45]

In conclusion, our work demonstrates that BMLs are regions of high metabolic activity, with expression of genes involved in neuronal development, pain, ECM turnover, cartilage/bone formation and angiogenesis. Our findings contribute to understanding of OA pathogenesis and could help lead to the development of new diagnostic tools and future therapies for this most common arthritic disease.

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Contributors NS wrote the study protocol and associated documents, coordinated the implementation of the study, collated and managed the study data, conducted data analysis and drafted the manuscript. LA, KL, GW, CH, PM and FAH supported AK and NS in the study design, data collection study implementation and analysis. VE and CH interpreted the MRI knee scores for data analysis.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval This study was approved and implemented in accordance with Good Clinical Practice guidelines. All participants gave written informed consent.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Our microarray data is available publicly online.

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EXTENDED REPORT

Cross-phenotype association mapping of the MHC identifies genetic variants that differentiate psoriatic arthritis from psoriasis

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ABSTRACT

Objectives Psoriatic arthritis (PsA) is a chronic inflammatory arthritis, with a strong heritable component, affecting patients with psoriasis. Here we attempt to identify genetic variants within the major histocompatibility complex (MHC) that differentiate patients with PsA from patients with cutaneous psoriasis alone (PsC).

Methods 2808 patients with PsC, 1945 patients with PsA and 8920 population controls were genotyped. We imputed SNPs, amino acids and classical HLA alleles across the MHC and tested for association with PsA compared to population controls and the PsC patient group. In addition we investigated the impact of the age of disease onset on associations.

Results HLA-C*06:02 was protective of PsA compared to PsC ($p=9.57\times10^{-66}$, OR 0.37). The HLA-C*06:02 risk allele was associated with a younger age of psoriasis onset in all patients ($p=1.01\times10^{-59}$). After controlling for the age of psoriasis onset no association of PsA to HLA-C*06:02 (p=0.07) was observed; instead, the most significant association was to amino acid at position 97 of HLA-B ($p=1.54\times10^{-9}$) where the presence of asparagine or serine residue increased PsA risk. Asparagine at position 97 of HLA-B defines the HLA-B*27 alleles.

Conclusions By controlling for the age of psoriasis onset, we show, for the first time, that *HLA-C*06:02* is not associated with PsA and that amino acid position 97 of HLA-B differentiates PsA from PsC. This amino acid also represents the largest genetic effect for ankylosing spondylitis, thereby refining the genetic overlap of these two spondyloarthropathies. Correcting for bias has important implications for cross-phenotype genetic studies.

BACKGROUND

Psoriatic arthritis (PsA) is a chronic inflammatory arthropathy characterised by spondylitis, enthesitis and arthritis. It is associated with the presence of psoriasis, with a prevalence of up to 14% in this patient group.[1] The presence of PsA has a substantial impact on a patient's quality of life, which has been shown to be lower than that of patients with psoriasis alone, partly attributable to increased rates of comorbidities such as cardiovascular disease.[2 3] The identification of patients with psoriasis at high risk of developing PsA has the potential for significant benefit in patient health as it would allow early intervention to reduce disability and result in an improved outcome for the patient.[4]

Both psoriasis and PsA have a substantial genetic component that influences an individual's susceptibility; indeed there are now 63 confirmed risk loci for psoriasis in populations of European origin.[5] The identification of risk loci that are specific for the development of PsA in patients with psoriasis has been more challenging, but evidence is now emerging of loci associated at genome-wide significance thresholds with PsA and not PsC, including loci at 5q31, *IL23R*, *PTPN22*, *TNFAIP3* and *HLA-B*.[6–9] Genes within the major histocompatibility complex (MHC), in particular HLA class I genes, have been consistently shown to contribute to the susceptibility of both PsC and PsA, with independent associations to *HLA-C*, *HLA-B* and *HLA-A*.[6 9 10] Of these, the largest effect is observed with the *HLA-C*06:02* allele, where carriage is associated with increased risk and lower age of disease onset of psoriasis.[11] Interestingly a paradoxical association of *HLA-C*06:02* has been reported whereby it is a risk factor for PsA compared with controls, but conversely carriage is protective of PsA within psoriasis.[12–14] Finally, previous studies have consistently identified the *HLA-B*27* and *HLA-B*39* alleles as associated with PsA but not PsC, while a more recent analysis of the HLA region based on amino acids rather than genetic haplotypes has reported that an amino acid at position 45 of the mature HLA-B protein is associated with PsA in psoriasis.[9]

The MHC is a particularly challenging region of the genome to map due to the presence of multiple independent associations and extensive linkage disequilibrium between genetic variants. Given the complexity of fine-mapping genetic associations in the MHC, in this study, we attempt to independently validate the previously reported association to the amino acid at position 45 of HLA-B. Here we fine-map genetic associations that differentiate PsA

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from PsC in large sample collections using imputed single nucleotide polymorphisms (SNPs), classical HLA alleles and amino acid residues.

METHODS

PsA cohort

A total of 2217 patients with PsA were recruited from rheumatology centres in the UK, Ireland and Australia, as previously described.[6] PsA classification was defined as the presence of both psoriasis and inflammatory arthritis, regardless of rheumatoid factor status, and all had peripheral arthritis. The majority of patients satisfied the CASPAR (ClASsification criteria for Psoriatic ARthritis) classification system,[15] although some were collected prior to the introduction of this classification system and all patients were diagnosed by a rheumatologist. All patients provided written informed consent (UK PsA National Repository MREC 99/8/84).

Psoriasis cohort

We had access to data on 1306 psoriasis patient samples obtained through the Biomarkers of Systemic Treatment Outcomes in Psoriasis study (BSTOP). Patients with severe psoriasis who had also consented to the British Association of Dermatologists Biologics Interventions Registry (a UK pharmacovigilance registry, BADBIR.org.uk) were recruited to BSTOP between October 2011 and October 2015 from 60 secondary and tertiary care outpatient dermatology departments throughout the UK, including centres in London, Manchester, Nottingham and Liverpool. All patients provided written informed consent (BSTOP ethics reference 11/H0802/7). In addition we had access to data on 2622 patients with psoriasis from the Wellcome Trust Case Control Consortium 2 (WTCCC2) study.[16] Samples from each of these collections were only included in the analysis if they had no previous diagnosis of PsA; we refer to this sample group as cutaneous-only psoriasis (PsC). Classification of PsC in the BSTOP cohort is based on information collected at multiple follow-up consultancies, one every 6 months in the first 3 years and then annually, where an active enquiry of rheumatologist-diagnosed PsA is made. Individuals from the WTCCC2 cohort were excluded based on a known diagnosis of PsA using information provided by sample contributors.

Population control cohort

A total of 9006 population controls were obtained through the 1958 British birth cohort and the UK Blood Service control group. In addition control data were available from 478 individuals from Ireland.

Genotyping and quality control

PsA and control population samples were genotyped using the Illumina Immunochip array as previously described, and details are provided in the online supplementary text.[6] Psoriasis samples were genotyped using the Illumina HumanOmniExpressExome-8v1-2_A array performed at King's College London. Automated genotype reclustering was performed followed by extensive manual review of genotype clusters based on GenTrain score, cluster separation, allele frequency and call rate. Data for the additional psoriasis samples from the WTCCC2 psoriasis GWAS were generated using the Illumina Human660-Quad genotyping array as previously described.[16]

Statistical quality control

Statistical quality control (QC) was performed conforming to established standards in each data set independently. The Immunochip data set (PsA and control samples) was filtered as previously described and details are provided in the online supplementary text.[6] Statistical QC of the BSTOP data set consisted of the exclusion of samples with a call rate <0.99 and with discrepant sex based on inferred and labelled sex, exclusion of duplicate and related samples using identity-by-state analysis on a set of 75 784 linkage disequilibrium (LD)-pruned SNPs with minor allele freqeuncy (MAF) >0.1 in KING (V.1.4), and exclusion of outliers based on ancestry via principal component analysis (PCA) on the LD-pruned SNPs (also using KING). SNPs were excluded with a call rate <0.99 or Hardy-Weinberg deviation of $p<7.5\times10^{-8}$. Both data sets were aligned to the forward strand of the haplotype reference consortium (HRC) reference panel (HRC.r1-1.GRCh37) using the HRC checking tool (http://www.well.ox.ac.uk/~wrayner/tools/). QC of the WTCCC2 data set has been described previously[16]; in addition to this we excluded known PsA samples from the data set, leaving a total of 1784 PsC samples. The data sets were merged and intersecting SNPs were retained. Identity by descent (IBD) was performed on the combined genotype data to identify any overlapping samples.

Imputation of MHC markers

Imputation of HLA alleles, amino acids and SNPs within the HLA region (chr6:29–34, hg19) was performed with the SNP2HLA software package (V.1.0.3) using the T1DGC reference panel.[17] Analysis was performed using the imputed dosage on all variants with an information score ≥0.9 and an MAF ≥0.1.

Statistical analysis

Analysis of all markers was performed using logistic regression assuming an additive effect based on the carriage of alleles. Population structure was controlled for by including the top two principal components as covariates calculated using an intersection of non-HLA SNPs in the combined data set (online supplementary figure S1). For multiallelic sites, such as amino acids, we identified the most common residue or allele in the control population to be selected as the reference and excluded from the model. The p value for each marker was derived from an omnibus test performed with a log-likelihood ratio test of the null and fitted models. Forward stepwise logistic regression was used to identify independent effects where the top marker, ranked by the log-likelihood p value, was included as a covariate by addition to the null model. This was repeated until no further marker reached a predefined significance threshold based on the Bonferroni-corrected type I error rate for the number of markers in the data set. Interactions between the *HLA-B*27* allele and non-HLA SNPs were tested in the PsA and control Immunochip data set by fitting an interaction term in the logistic model with *HLA-B*27* fitted as a dominant term and the SNP as an additive term.[18] We tested interactions with rs30187 (*ERAP1*), a previously reported interaction in ankylosing spondylitis (AS), and also to rs12044149 (*IL23R*), rs715285 (5q31), rs2476601 (*PTPN22*) and rs9321623 (*TNFAIP3*), which have previously been reported as differentiating PsA from PsC.[6 8 18 19] Association of genetic markers with age of psoriasis onset, as a continuous variable, was tested using linear regression, and a difference in the median age of onset between groups was tested using a Wilcoxon test.

RESULTS

After QC the study data set comprised 1945 PsA cases, 2808 PsC cases and 8920 control samples for 6833 SNPs, 334 amino acids, 71 classical HLA alleles at two-digit resolution and 87 classical HLA alleles at four-digit resolution. A Bonferroni-corrected threshold for p values of 6.8×10^{-6} based on a total of 7325 markers was used to determine significant associations.

The paradoxical association of *HLA-C*06:02* and PsA

First, we compared the imputed dosages for all MHC markers for each of the disease groups, PsC and PsA, with the population control group. As expected we replicated the three previously reported independent associations to the class I genes *HLA-C* (*HLA-C*06:02*), *HLA-B* (amino acid position 67) and *HLA-A* (*HLA-A*02:01* or the highly correlated amino acid at position 95, r^2 valine=0.99) for each of the diseases (online supplementary figures S2 and S3). We then directly compared PsA with PsC (PsC labelled as the reference group) and observed the most significant association was to the *HLA-C*06:02* allele ($p=9.57\times10^{-66}$), where the presence of the allele was protective of PsA compared with PsC (OR 0.37, 95% CI 0.33 to 0.41) (figure 1A); the result was in contrast to the previous comparison against controls where the allele was a risk factor for PsA ($p=7.44\times10^{-48}$, OR 2.13, 95% CI 1.92 to 2.35). The association remained significant after conditioning on the previously reported HLA-B amino acid position 45 ($p=6.88\times10^{-27}$).

*HLA-C*06:02* is associated with the age of onset of psoriasis

Given the previously reported association of *HLA-C*06:02* with age of psoriasis onset, we investigated the potential for confounding of the statistical analysis due to selection bias in a data set of 2050 case-only samples with relevant phenotype data (PsA=981, PsC=1069). We found significant association of *HLA-C*06:02* allele dose with a younger age of psoriasis onset in all samples ($p=1.01\times10^{-59}$; online supplementary figure S4a) and that carriage of the risk allele resulted in a difference in the median age of psoriasis onset of approximately 14 years (online supplementary figure S4b). We found a significant difference ($p=1.85\times10^{-71}$) in the median age of psoriasis onset between the PsC (19 years, IQR 15 years) and the PsA groups (34 years, IQR 27 years) (online supplementary figure S5). This result illustrates the potential for confounding when investigating features known to be associated with age of onset as is the case for *HLA-C*06:02*.

Psoriasis age of onset confounds HLA analyses

All association analyses comparing PsA with PsC were repeated while conditioning on age of psoriasis onset as a covariate (figure 1B). Within this subgroup of samples, *HLA-C*06:02* is significantly associated with a protective effect on PsA ($p=4.17\times10^{-15}$, OR 0.52, 95% CI 0.44 to 0.61); however, when conditioning on age of psoriasis onset, there was no evidence of association between *HLA-C*06:02* and PsA (p=0.07), suggesting the previously observed protective effect was the result of confounding due to the different age of psoriasis onset in the disease subgroup strata.

Amino acid position 97 of HLA-B differentiates PsA from PsC

The most significant association with PsA compared with PsC after correcting for age of psoriasis onset was to an amino acid at position 97 of HLA-B ($p=1.54\times10^{-9}$), where the presence of an asparagine (OR 2.46, 95% CI 1.78 to 3.42) or serine (OR 1.45, 95% CI 1.22 to 1.74) residue increased the risk of PsA (table 1). An asparagine residue at position 97 of HLA-B is predominantly found on *HLA-B*27* alleles, and *HLA-B*27:05* is the most associated HLA allele after correcting for age of psoriasis onset ($p=3.53\times10^{-7}$, OR 2.34, 95% CI 1.69 to 3.25); in addition, a serine residue is found on multiple HLA alleles including *HLA-B*07* ($p=1.9\times10^{-3}$) and *HLA-B*08* (p=0.05). However neither of these two alleles were independently associated with PsA when conditioning on amino acid position 97 (p>0.05), while amino acid 97 remained associated independently of either of these two HLA alleles and was independently associated when adjusting for *HLA-B*27*, indicating that amino acid 97 is the primary driver of the associations observed with these HLA-B alleles. This amino acid is an important risk factor for AS; comparison of effect estimates shows that an asparagine residue increases risk for both diseases, although with a substantially larger effect estimate in AS (OR 16.51, 95% CI 15.43 to 17.69) than PsA (OR 2.46, 95% CI 1.78 to 3.42) (figure 2).[20] In contrast, the presence of a serine residue is associated with risk of PsA (OR 1.45, 95% CI 1.22 to 1.74) while reported to have a protective effect for AS (OR 0.86, 95% CI 0.81 to 0.91).

p Value and ORs are determined with multivariate logistic regression.

We found significant association to the previously reported amino acid at position 45 of HLA-B ($p=3.5\times10^{-4}$; online supplementary table S1); however, this was not significant after adjusting for amino acid position 97 (p=0.16). No further associations exceeded the significance threshold when conditioning on amino acid 97 (figure 1C). We found no evidence to support the previously reported interaction between *HLA-B*27* and *ERAP1* observed in AS or with any of the other previously reported PsA differentiating loci (p value >0.05).

DISCUSSION

Through detailed analysis of the MHC region using data from patients with PsA, PsC and population controls, we show that, first, previous reports of a protective effect of *HLA-C*06:02* with PsA are due to confounding by differences in the age of onset of psoriasis due to the strong association of *HLA-C*06:02* with a younger age of psoriasis onset; second, *HLA-C*06:02* and *HLA-A*02:01* are primarily associated with psoriasis and confer no additional risk of PsA; and, third, that when age of psoriasis onset is accounted for, the primary association conferring additional risk for PsA in patients with psoriasis is to the presence of asparagine (*HLA-B*27*) or serine (*HLA-B*07* and *HLA-B*08*) residues at amino acid position 97 of HLA-B.

Understanding the genetic factors that differentiate PsA from PsC is important both for screening patients at risk for psoriasis and for understanding the disease mechanisms involved. In terms of screening, given that psoriasis often predates PsA, factors that identify a group of patients with psoriasis at higher risk of developing PsA could potentially allow the introduction of preventative strategies in the future. Indeed, the application of genetic risk scores in high-risk groups where disease prevalence is much higher than the general population has been shown to greatly increase the diagnostic benefit of genetic risk factors.[21] At a practical level, however, while genotyping costs have improved, analysis and interpretation of HLA data from genotyping arrays remain time-consuming and challenging, and it is still not clear how much more information is provided over and above classical HLA typing methods. Thus, if HLA screening were shown to be useful in prospective studies of patients with psoriasis, HLA typing for *HLA-B*27* may remain the preferred option.

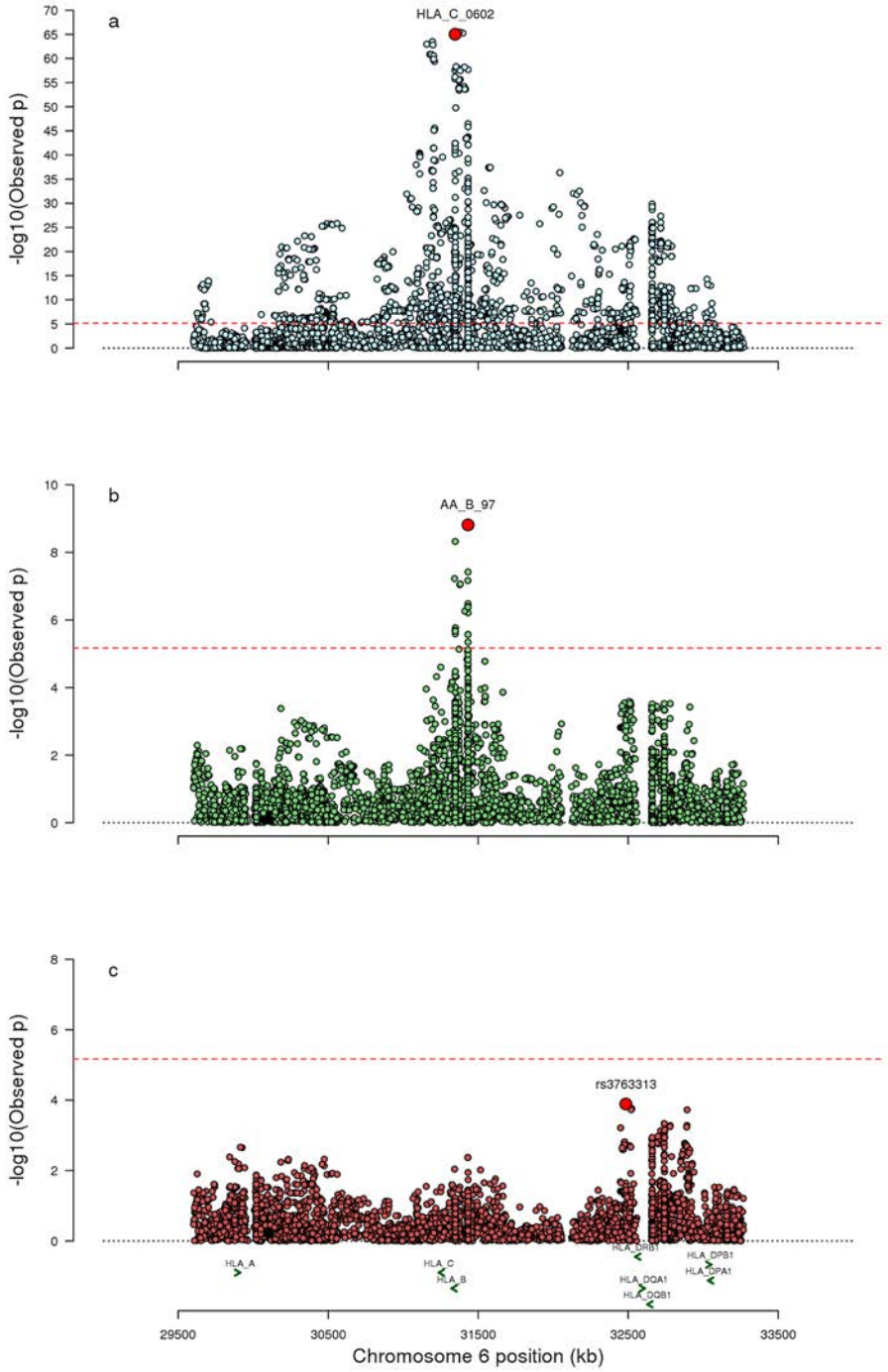

Figure 1 Association results for (A) PsA compared with PsC, (B) PsA compared with PsC controlling for age of psoriasis onset and (C) PsA compared with PsC controlling for age of psoriasis onset and association at amino acid position 97 at HLA-B. Red horizontal line indicates significance threshold; y-axis is $-\log_{10}$ of the omnibus test p value, and the x-axis indicates chromosomal base position and gene locations. PsA, psoriatic arthritis.

Okada *et al* report residues at the amino acid position 45 of HLA-B as the key risk factor for PsA in psoriasis; however, the current study does not support this after correcting for the age of psoriasis onset (p value=3.5×10^{-4}). PsA is a clinically heterogeneous disease and one possible explanation of this discrepancy is differing proportions of clinical subgroups between the studies. For example *HLA-B**27* has been reported to be associated with axial disease within a well-phenotyped PsA patient cohort[22]; therefore, the current study may be enriched for axial disease. This highlights the need for accurate clinical phenotyping of PsA cases, and one of the major limitations of the current study was that this could not be investigated further due to the lack of information about the presence of axial disease in all of our patients with PsA.

Amino acid position 97 of HLA-B represents the largest genetic effect reported in AS[20]; our analysis has confirmed the genetic overlap of PsA with AS. It could be argued that PsA may simply be an overlap of AS and psoriasis, but clinical, radiographic and genetic differences have been observed. For example, methotrexate is more effective in PsA than AS; classical pencil-in-cup

Table 1 Summary statistics for residues of amino acid at position 97 of HLA-B and association with psoriatic arthritis compared with cutaneous psoriasis alone

Residue	Amino acid	Frequency	p Value	OR	95% CI
R	Arginine	0.4619	Ref	Ref	Ref
S	Serine	0.2785	3.58E-05	1.45	1.22:1.74
T	Threonine	0.1003	0.716	0.959	0.76:1.20
V	Valine	0.0751	0.913	0.988	0.78:1.24
N	Asparagine	0.0474	5.76E-08	2.46	1.78:3.42
W	Tryptophan	0.0384	0.283	0.795	0.52:1.20

deformities, osteolysis and juxta-articular new bone formation in hands and feet are more common in PsA, and genetic variants have been identified that are associated with one disease but not the other; for example, PsA variants at the *IL23R* are distinct from those reported for AS.[6] Amino acid residues at position 97 are the most important risk factor for both diseases, and our results highlight both overlapping and differential associations. The asparagine residue is associated with increased risk in both diseases; however, the serine residue has a differential association representing increased risk for PsA while being protective of AS. The position is located within the peptide binding groove of the HLA-B molecule and highlights the importance of antigen presentation in disease aetiology. We were unable to replicate the interaction of *HLA-B*27* and *ERAP1* observed in AS.[18] This may be due to insufficient power of the current study to detect an interaction due to the lower effect sizes in PsA or could indicate differing disease mechanisms.

Our study highlights the importance of accounting for confounding in genetic studies, particularly when associated loci are correlated with timing of disease onset. We believe the confounding observed in this study is due to ascertainment bias where cases of type I psoriasis, age of onset <40 years, are preferentially included in genetic studies and such selection does not occur in PsA collections. The issue of selection bias is increasingly being recognised in the statistical methodology literature.[23 24] In particular, index event bias describes how conditioning on an outcome, for example psoriasis, can induce correlation between risk factors leading to spurious associations.

In conclusion, we show that *HLA-C*06:02* is primarily associated with psoriasis with no effect, either risk or protective, on PsA, while HLA-B amino acid 97, the same variant that represents the major AS risk factor, is the most important risk factor for PsA.

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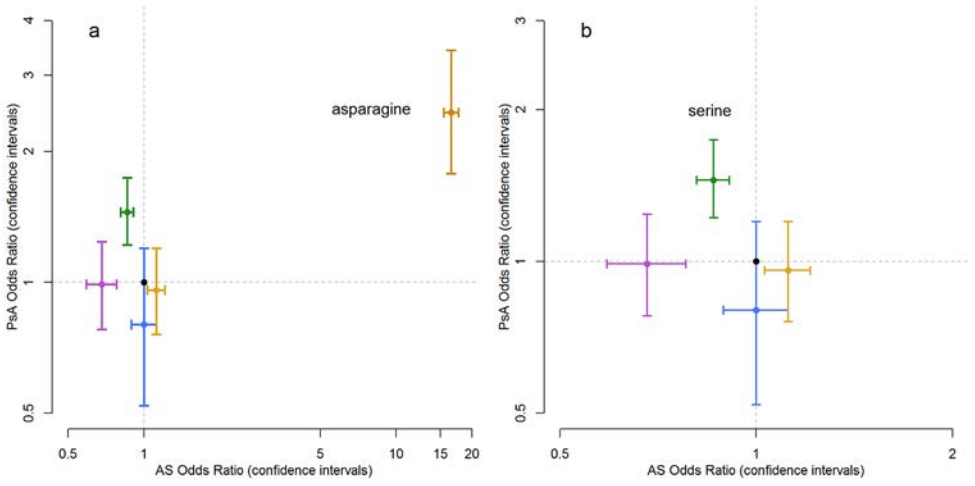

Figure 2 Comparison of effect estimates for residues at amino acid position 97 of HLA-B for PsA and AS showing (A) the asparagine residue is a risk factor for both diseases and (B) the differential effects at the serine residue, which is a risk factor for PsA but protective for AS. AS, ankylosing spondylitis; PsA, psoriatic arthritis.

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Contributors AB and JB devised the study concept and design. JB, JA, FJN and EB performed statistical analysis. JB and AB wrote the manuscript. ND and MAS collected and performed quality control on the BTOP data. PH, HM-O, PSH, MF, AWR, DJK, EK, JP, RM, MAB, CHS, JNB, NM, OF, RBW and AB contributed samples and data. All authors contributed to and approved the manuscript.

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CONCISE REPORT

Germinal centres in diagnostic labial gland biopsies of patients with primary Sjögren's syndrome are not predictive for parotid MALT lymphoma development

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ABSTRACT

Objective Patients with primary Sjögren's syndrome (pSS) have an increased risk of developing non-Hodgkin's lymphoma (NHL), particularly parotid gland mucosa-associated lymphoid tissue (MALT) lymphomas. Presence of germinal centres (GCs) in labial gland biopsies has been suggested as predictive factor for NHL. We assessed whether presence of GCs is increased in labial gland biopsies from patients with pSS who developed parotid MALT lymphoma, the dominant NHL-subtype in pSS, compared with patients with pSS who did not develop lymphoma.

Methods Eleven labial gland biopsies from patients with pSS that were taken prior to parotid MALT lymphoma development were compared with biopsies of 22 matched pSS controls (1:2) who did not develop lymphoma. Biopsies were evaluated for GCs (H&E and Bcl6).

Results Labial gland biopsies of pSS MALT lymphoma patients, revealed GCs in 2/11 (18%) H&E sections and 3/11 (27%) Bcl6 stained sections. In controls, GCs were present in 4/22 (18%) of H&E sections and 5/22 (23%) of Bcl6 stained sections.

Conclusion Presence of GCs in labial gland biopsies does not differ between patients with pSS that develop parotid MALT lymphoma and patients with pSS who do not develop lymphoma. The presence of GCs in labial gland biopsies is therefore not a predictive factor for pSS-associated parotid MALT lymphomas.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease, in which salivary and lacrimal glands are affected by a chronic inflammatory process, which leads to dryness of mouth and eyes.[1] Histopathologically, this inflammatory process is characterised by a periductal lymphoid infiltrate in the glandular parenchyma.[2] In roughly one quarter of the patients with pSS, germinal centres (GCs) can be found within these lymphoid infiltrates reflecting the B-cell hyperactivity that characterises the disease.[3 4] Although the clinical significance of these GCs remains to be elucidated, the presence of GCs in the glandular tissue of patients with pSS is generally associated with more severe clinical disease as reflected by a higher focus score (FS), increased presence of anti-SSA/Ro (52 kD + 60 kD) and anti-SSB/La autoantibodies and elevated levels of proinflammatory cytokines in the blood.[3]

A serious complication of pSS is the 5%–10% lifetime risk of developing non-Hodgkin's B-cell lymphomas (NHL).[5] The most common subtype NHL in pSS is the mucosa-associated lymphoid tissue (MALT) lymphoma.[5–7] These MALT lymphomas preferentially arise in the parotid glands and account for >60% of the lymphomas arising in patients with pSS.[6–8] Which patients with pSS will develop NHL is largely unknown, but several predictors have been identified including disease activity, persistent glandular enlargement, lymphadenopathy, palpable purpura, anti-Ro/anti-La antibodies, rheumatoid factor, lymphopaenia, declined C3 or C4 levels, cryoglobulinaemia and an FS ≥3 in the labial gland biopsy.[9–11] Presence of GCs in diagnostic labial gland biopsies has also been proposed as a predictive factor for the development of NHL. However, in the study underlying this assumption, all subtypes of NHL were taken into account, including NHL subtypes not typically associated with pSS, such as follicular lymphoma and T-cell lymphoma.[12] For this reason, we explored the predictive role of GCs in labial gland biopsies from patients with pSS for parotid gland MALT lymphomas.

MATERIALS AND METHODS

Patients

From 56 patients with pSS diagnosed with parotid MALT lymphoma, we were able to acquire labial gland biopsies of 11 patients taken at diagnosis of pSS, before (median 4.0, IQR 1.5–6.1 years) lymphoma diagnosis (table 1). Labial gland biopsies from 22 pSS patients with an NHL free follow-up (median 12.0, IQR 6.3–16.8 years) served as controls (see online supplementary table S1). Matching of control pSS patients (1:2) was based on age at diagnosis of pSS and the presence of SSA autoantibodies. Patients were frequency-matched within three age groups: patients diagnosed with pSS at an age of ≤40, between 40 and 60 and ≥60 years. All patients were clinically diagnosed as pSS and retrospectively fulfilled the ACR-EULAR(American College of Rheumatology - European League Against Rheumatism) classification) criteria[13] at time of diagnosis. Of the 33 included patients, 32 also fulfilled the AECG-criteria at time of diagnosis. Of one pSS patient this is uncertain due to missing sialometry and ocular examination.

Histopathological assessment of diagnostic salivary gland biopsies

Diagnostic labial salivary gland biopsies were formalin fixed, paraffin embedded and sectioned at 3 µm thickness. Serial sections were stained

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Table 1 Patient characteristics and histopathology results of patients with pSS (n=11) developing a parotid MALT lymphoma

Patient	Gender	Age pSS (year)	Δ* lymph pSS (year)	Ann Arbor Musshoff	pSS biopsy	FS	CD45 (%)	GC H&E	GC Bcl6	LEL H&E	Anti-SSA	Anti-SSB
1	F	37	3.5	2	Labial	4.0	27.6		–	+	+	+
2	F	60	13.7	1	Labial	0.8	5.3	–	–	–	+	–
3	F	32	4.0	1	Labial	1.1	12.2	–	–	–	+	+
4	F	28	0.2*	1	Labial	0	7.4	–	–	–	+	+
5	F	63	6.1	2	Labial	4.7	38.8	+	+	+	+	–
6	F	47	3.2	2	Labial	1.8	23.4	–	–	+	+	+
7	F	45	0.3*	1	Labial	2.0	34.1	–	+	+	+	+
8	F	67	4.6	1	Labial	0	5.3	–	–	–	+	+
9	F	31	4.0	2	Labial	1.7	20.3	–	–	+	+	+
10	F	51	13.3	3	Labial	2.7	18.2	+	+	+	+	–
11	F	61	1.5	1	Labial	4.0	21.6	–	–	+	+	–

*Biopsy taken shortly before lymphoma diagnosis. +present. -not present.
Δ*Lymph pSS: time between diagnosis of pSS and parotid MALT lymphoma, Ann Arbor Mushoff: (1) localised disease: lymphoma located in one or more salivary glands, (2) locally disseminated: lymphoma localised in one or more salivary glands with one or more enlarged regional lymph nodes (>1 cm), (3) disseminated disease: localisation of lymphoma in one or more salivary glands, with one or more enlarged regional lymph nodes (>1 cm) and/or bone marrow, spleen, liver or other extra nodal site than the salivary gland, or localisation of lymphoma in multiple extra nodal sites.[20]
Bcl6, B cell lymphoma 6; FS, focus score; GC, germinal centre; LEL, lymphoepithelial lesions; MALT, mucosa-associated lymphoid tissue.

with H&E, and immunohistochemically for B-cell lymphoma six protein (Bcl6, clone GI191E/A8, Ventana, Illkirch, France) and CD45 (clone 2B11+PD7/26, Ventana, Illkirch, France). Staining was performed on a Ventana Benchmark platform as previously described.[14] In H&E stained sections, FS, lymphoepithelial lesions (LELs) and GCs were assessed. FS was based on the number of clusters of ≥50 lymphocytes (foci)/4 mm^2 parenchyma. In case of multiple large confluent foci, an arbitrary FS of 12 was used.[15] LELs were defined as a striated duct with lymphocytes within its basement membrane. GCs were defined as a clearly visible lighter area in a lymphocytic infiltrate containing cells usually present in classical GCs: follicular dendritic cells (FDCs), centrocytes, centroblasts and macrophages. Since detection of GCs is difficult in H&E stained sections, and small GCs may be overlooked,[16] we also evaluated GCs in Bcl6 stained sections. Bcl6 is a transcription factor highly expressed by all GC B-cells. A cluster of ≥5 adjacent $Bcl6^+$ cells within a focus was classified as a GC.

Besides FS, we also measured the extent of glandular inflammation as proposed.[2] This was assessed using CD45 staining. CD45 is expressed by all lymphoid and non-lymphoid cells of hematopoietic origin, allowing easy quantification of the relative area of the infiltrate. CD45 expression was measured using ImageScope V.12.0 (Aperio Technologies). Slides were blinded and independently scored by a trained resident (EH) and a dedicated head and neck pathologist (BvdV).

Statistical analysis

Mann-Whitney U test and Fisher's exact test were used accordingly to test differences between groups (IBM-SPSS Statistics V.23).

RESULTS

Analysis of H&E stained sections from diagnostic labial gland biopsies, taken prior to parotid MALT lymphoma development, revealed presence of GCs in 2/11 (18%) patients (table 2, online supplementary figure 1). Staining for Bcl6 revealed an extra (small) GC in a biopsy of one additional patient (figure 1, table 2). Thus, in patients with pSS who developed parotid MALT lymphoma, GCs were present in 3/11 (27%) prelymphoma labial gland biopsies. In the patients with pSS that did not develop parotid MALT lymphomas (nor any other type of NHL), GCs were detected in 4/22 (18%) diagnostic labial gland biopsies in H&E stained sections and in 5/22 (23%) of Bcl6 stained sections (table 2). This proportion was comparable with that seen in patients with pSS who did develop parotid MALT lymphoma.

Since FS ≥3 has been suggested as predictive factor for NHL development,[9] we compared FS and relative area of $CD45^+$ infiltrate in prelymphoma labial gland biopsies and biopsies from control pSS patients. FS did not differ between both groups (Mann-Whitney U test, p=0.204). The percentage of biopsies with FS ≥3 was even higher in the control group (36% vs 27%). The relative area of $CD45^+$ lymphocytic infiltrate, however, tended to be higher in the prelymphoma labial gland biopsies than in the controls (table 2, online supplementary figure 1).

DISCUSSION

This study shows that the presence of GCs does not differ between diagnostic labial gland biopsies from patients with pSS who did develop parotid MALT lymphoma and patients with pSS who did not develop such lymphoma. In H&E stained sections, we observed an identical percentage of GCs in both categories of patients (18%). With a more sensitive and specific method to identify GCs, viz. staining for the GC B-cell associated transcription factor Bcl6,[16] a slightly higher incidence of GCs was seen in both groups: 27% for patients with prelymphoma and 23% for non-lymphoma pSS patients. Although the two groups of patients with pSS are rather small, the percentages of GCs are similar to those reported for labial gland biopsies among the general pSS population.[3] Based on a large number of studies, Risselada *et al* reported that the mean weighted percentage of GCs in labial gland biopsies of patients with pSS was 25.1%±5.0% (range 18.3%–33%) in H&E stained sections. Since there was no difference in the occurrence of GCs in labial gland biopsies of patients with pSS prior to parotid MALT lymphoma development and the matched pSS controls as well as with the general pSS population, we conclude that presence of GCs in labial biopsies is not likely predictive for parotid MALT lymphoma development.

Other studies that examined the predictive value of GCs in NHL development did not restrict themselves to MALT

Table 2 Patient characteristics and histological results of diagnostic labial gland biopsies from pSS patients developing parotid MALT lymphomas and control labial gland biopsies

Variable	Labial biopsies prior to parotid MALT lymphoma (n=11)	Labial biopsies from patients with pSS without lymphoma (n=22)	p Value Mann-Whitney U test (MWU) or Fisher's exact test (FT)
Female n (%)	11/11 (100)	20/22 (91)	0.542 (FT)
Age (year), mean (SD)	47.5 (14.0)	48.7 (17.2)	0.638 (MWU)
Anti-SSA positive, n (%)	11/11 (100)	22/22 (100)	-
Anti-SSB positive, n (%)	7/11 (64)	13/22 (59)	1.000 (FT)
Anti-RF positive, n (%)	11/11 (100)	19/22 (86)	0.534 (FT)
Anti-ANA positive, n (%)	11/11 (100)	21/22 (96)	1.000 (FT)
Δ* pSS-lymph (year), median (IQR)	4.0 (1.5–6.1)	–	–
Δ* pSS-FU (year), median (IQR)	–	12.0 (6.3–16.8)	–
FS, median (IQR)	1.8 (0.8–4.0)	2.7 (1.4–3.5)	0.204 (MWU)
FS ≥3, n (%)	3/11 (27)	8/22 (36)	1.000 (FT)
Area CD45 (%), median (IQR)	20.3 (7.4–27.7)	12.7 (9.4–19.1)	0.143 (MWU)
LELs based on H&E, n (%)	7/11 (64)	13/22 (59)	1.000 (FT)
GC based on H&E, n (%)	2/11 (18)	4/22 (18)	1.000 (FT)
GC based on Bcl6, n (%)	3/11 (27)	5/22 (23)	1.000 (FT)

Δ*Lymph-pSS: time between diagnosis of pSS and parotid MALT lymphoma.
Δ*pSS-FU: time between diagnosis of pSS and last follow-up.
FU, follow up; Bcl6, B-cell lymphoma 6 protein; FS, focus score; GC, germinal centre; FT, Fisher's exact test; LEL, lymphoepithelial lesions; MALT, mucosa-associated lymphoid tissue; MWU, Mann-Whitney U test; pSS, primary Sjögren's syndrome; RF, rheumatoid factor.

lymphoma.[9 12 17] In a retrospective analysis of prelymphoma labial gland biopsies from 13 pSS patients with unspecified NHL lymphomas, Risselada *et al*[9] found that in H&E stained sections, GCs were present in only three (23%) of the patients. Johnsen et al[17] showed that in similarly stained labial gland biopsies of pSS NHL patients, 4 out of 12 biopsies (33%) exhibited GCs. The matched control group of pSS patients without malignant lymphoma development showed an even higher percentage of 46% (13/28) GCs in the biopsies. However, in Johnsen's study, biopsies were taken prior to NHL development and simultaneously or even after NHL development.

In contrast to our findings and the aforementioned reports, two earlier studies (Theander *et al*[12] and Bombardieri *et al*[18]) indicated an increased incidence of GCs in diagnostic biopsies preceding NHL development. Theander *et al* observed that six out of seven patients had GCs in diagnostic labial salivary gland biopsies, prior to NHL development. Besides differences in patient cohorts, the most likely explanation for the apparent discrepancy between Theander's study and our findings might be the selection of patients with pSS that developed NHL. While Theander *et al* took all NHLs into account, we restricted ourselves to NHLs that are typically associated with pSS, namely parotid MALT lymphomas. Remarkably, only one out of seven pSS lymphomas in Theander's retrospective study represented a salivary gland (parotid) MALT lymphoma, making comparison with our study difficult. Bombardieri *et al*[18] found 'GC-like structures' in six out of eight (75%) of labial gland biopsies from pSS and patients with secondary Sjögren's syndrome preceding parotid MALT lymphoma. However, in this study, GC-like structures were determined by the presence of T-cells, B-cells and $CD21^+$ FDC networks. Although $CD21^+$ FDC networks are a prerequisite for GC development, their presence does not imply that GCs are indeed present. This may lead to a significant overestimation of the number of GCs in the tissue compared with Bcl6 staining.[16 19]

In conclusion, there are no indications that the occurrence of GCs in diagnostic labial gland biopsies is increased in patients with pSS who developed parotid MALT lymphoma. Thus, in our opinion, labial salivary gland GCs of patients with pSS are not likely a predictive factor for parotid MALT lymphoma development. Nevertheless, their presence might be of clinical relevance for stratification of pSS patients regarding treatment options. For this reason, uniform histopathological criteria for the assessment of GCs are eagerly awaited.

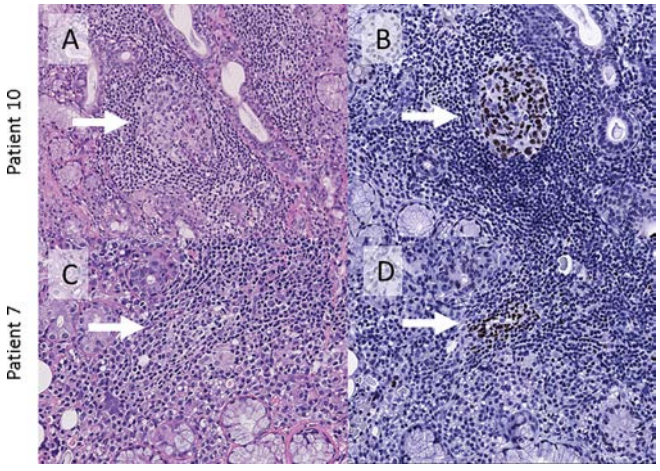

Figure 1 GCs in diagnostic labial salivary gland biopsies of patients with pSS who developed a parotid MALT lymphoma later on. (A) Clearly visible GC in a periductal focus of the labial gland , H&E stain. (B) Bcl6 staining of serial section, showing the same GC. (C) Suspicious GC in a periductal focus of the labial gland, H&E stain. (D) Bcl6 staining of a serial section shows a small GC. Arrows point to GCs. GCs, geminal centres; pSS, primary Sjögren's syndrome.

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Contributors Study concept and design: EAH, FGMK, BvdV, HB and AV. Patient recruitment: HB and EAH. Patient biopsy sampling: FKLS. Data collection: EAH and BvdV. Data analysis and interpretation: EAH, FGMK, BvdV, AV, FKLS and HB. The first manuscript was written by EAH and FGMK. All authors critically reviewed the manuscript and approved the final version to be published.

Competing interests None declared.

Ethics approval METc (University Groningen). Study registration number: 2014/211.

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Correction: How common is clinically inactive disease in a prospective cohort of patients with juvenile idiopathic arthritis? The importance of definition

Dore M, Marlow C, Cares WM, *et al*. How common is clinically inactive disease in a prospective cohort of patients with juvenile idiopathic arthritis? The importance of definition. *Ann of Rheum Dis* 2017;76:1381–8.

Figure 1 was corrected online but the incorrect version appeared in the August print issue.

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Regulatory role of the JAK STAT kinase signalling system on the IL-23/IL-17 cytokine axis in psoriatic arthritis

We read with great interest the article by Gao *et al*.[1] They have reported the regulatory role of the JAK/STAT kinase system on the inflammatory/proliferative cascades for pannus formation in psoriatic arthritis (PsA) such as on fibroblast like synovial cells (FLS) biology and on secretion of inflammatory cytokines (interleukin (IL) 6, IL-8, monocyte chemoattractant protein (MCP)-1) by these FLS. Tofacitinib targets JAK1 and JAK2 with IC50 values in the same order of magnitude as that of JAK3.[2] They have also provided the mechanisms of actions of tofacitinib by demonstrating that tofacitinib significantly decreased pSTAT3, pSTAT1, NFκBp65 in PsAFLS and inhibits the cellular and molecular events of pannus formation. However, Gao *et al* did not address a critical issue whether JAK/STAT signalling system regulates the IL-23/IL-17 cytokine axis in PsA. Here we are sharing an alternative mechanism for the role of JAK/STAT kinase system in the pathogenesis of PsA.

Aberrant activation of IL-23/IL-17 cytokine axis is a dominant pathology in PsA.[3 4] JAK2 is recruited to IL-23 receptor, so it is expected that JAK/STAT-mediated signalling system is important in PsA. We hypothesised- (i) JAK/STAT signalling system regulates the Th17 cells in PsA and (ii) that tofacitinib which inhibits Jak-2 likely targets the Th17 cells by inhibiting the IL-23-induced JAK/STAT signalling system.

METHODS

Mononuclear cells of peripheral blood (PBMC) and synovial fluid (SFMC) from patients with PsA (n=15) and PBMC from age/sex matched normal individuals (n=15) were collected. All patients had an active disease and were not on disease modifying anti-rheumatic drugs (DMARDS) or biologics. Recombinant IL-23 (rIL-23) (40 ng/mL) induced activated IL-17+ T cells were generated and evaluated as per our earlier reports.[3] Cells were cultured with and without tofacitinib (50 nM). Western

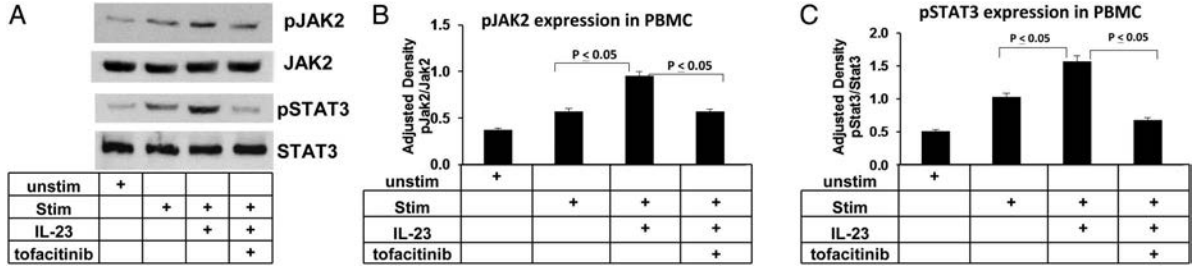

Figure 1 Interleukin (IL) 23 induces phosphorylation of JAK2 and STAT3 in mononuclear cells of peripheral blood (PBMC) of psoriatic arthritis (PsA) and that can be inhibited by tofacitinib. Results of the (A–C) demonstrate that IL-23 induced phosphorylation of JAK2 and STAT3 ($p<0.05$, t-test). Tofacitinib significantly inhibited activation of these signalling proteins ($p<0.05$, t-test). Experiments were done in triplicate and the results were expressed in mean±SD of the adjusted density.

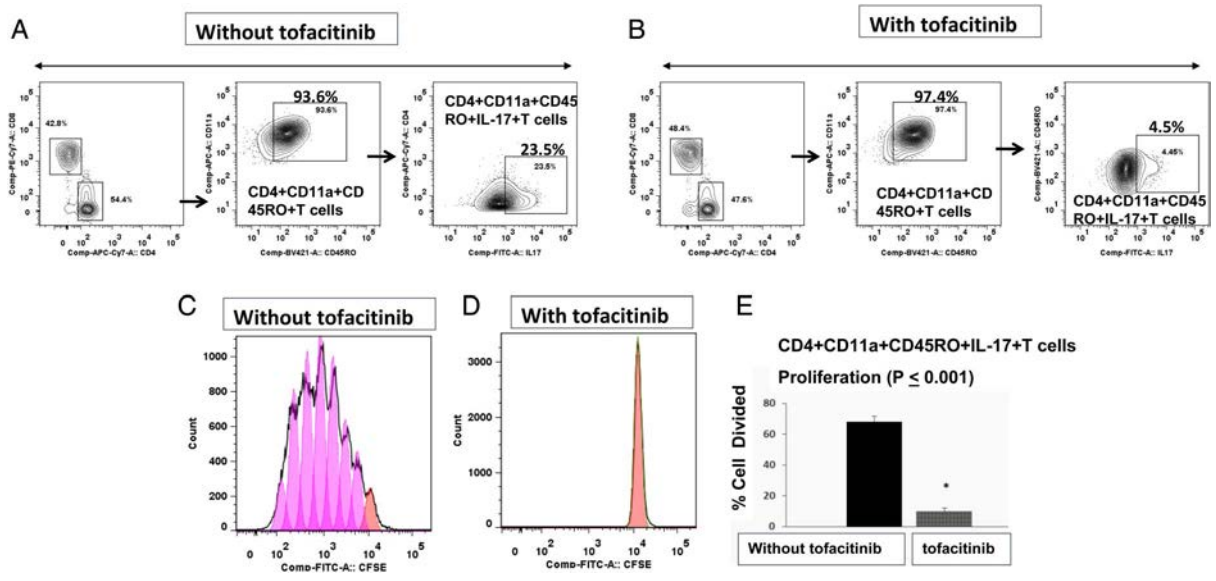

Figure 2 (A, B) Hi-D FACS studies of the mononuclear cells of peripheral blood from patients with psoriatic arthritis demonstrated that- (i) rIL-23 induced marked upregulation of IL-17 in the CD4+ memory T cells (CD11a+CD45RO+) (A) and that (ii) *rIL-23 induced IL-17 expression could be markedly inhibited by* tofacitinib ($p<0.001$, t-test) (B). Representative FACS plots are shown here and results are described in the text. (C–E) To determine the effect of the JAK-STAT kinase system on the proliferation of CD4+CD11a+CD45RO+IL-17+ T cells we performed the CFSE assay specifically on this T cell subpopulation. The bar diagram demonstrates that rIL-23 induced marked proliferation of the CD4+CD11a+CD45RO+IL-17 + T cells and tofacitinib significantly inhibited proliferation of these pathological cells ($p<0.001$, t-test) (E). Representative FACS plots demonstrate less number of generations and less numbers of CD4+CD11a+CD45RO+IL-17+ T cells on day 5 in PBMC cultured with tofacitinib (D) compared with cells cultured without tofacitinib (C). CFSE, carboxyfluorescein succinimidyl ester; IL, interleukin; rIL, recombinant IL.

blot studies were performed to indentify Jak2/p-Jak2 and stat3/p-stat3 in the sorted activated CD3+ T cells. Hi-D fluorescence-activated cell sorting (FACS) studies were performed to identify the activated memory CD4+ CD11a+CD45RO+IL-17+ T cells and CD8+CD11a+CD45RO+IL-17+ T cells in SFMC/PBMC of PsA and PBMC of normal individuals.

RESULTS

In both PsA and controls sorted activated CD3+ T cells in the presence of IL-23 demonstrated activation of Jak2 and STAT3. Further, we noticed tofacitinib markedly inhibited phosphorylation of Jak2 and STAT-3, the signalling proteins induced by IL-23 (figure 1A–C).

Hi-D FACS analyses of the activated CD3+T cells in patients with PsA demonstrated that IL-23 induced marked upregulation of IL-17 in the memory T cells (CD11a+CD45RO+) (figure 2A). We noticed that SFMC and PBMC treated with rIL-23 in patients with PsA had 30±4.5% and 18±3.8% activated memory CD4+IL-17+ T cells, respectively, compared with 5±0.7% in healthy persons ($p<0.001\%$). Further, we noticed that CD4+CD11a+CD45RO+IL-17+ T cells were 5±2% ($p<0.001\%$) in cells treated with Tofacitinib (figure 2B). Tofacitinib also significantly inhibited proliferation of these CD4+CD11a+CD45RO+IL-17+ T cells ($p<0.001\%$) (figure 2E).

CONCLUSION

Th17 cells play a critical role in the pathogenesis of PsA.[3 4] Here, we observed that the generation of these pathological CD4+CD11a+CD45RO+IL-17+ T cells and their proliferation are regulated by the JAK-STAT signalling system. A plausible mechanism of action of tofacitinib likely to be inhibition of the IL-23/IL-17 cytokine axis by inhibiting the IL-23-induced JAK-STAT signalling system.

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Contributors SKR: Reviewed and analysed data, helped to prepare the manuscript. CA: Performed experiments, analysed data and helped to prepare the manuscript. SPR: Designed the study, reviewed and analysed data, helped to prepare the manuscript.

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Response to: 'Regulatory role of the JAK STAT kinase signalling system on the IL-23/IL-17 cytokine axis in psoriatic arthritis' by Raychaudhuri *et al*

In their correspondence, Raychaudhuri *et al*[1] describe an alternative mechanism for the role of Janus Kinase and Signal Transducer and Activator of Transcription (JAK/STAT) signalling in the pathogenesis of psoriatic arthritis (PsA). The interleukin (IL)-23/IL-17 cytokine axis is a well-documented and prominent pathway in the understanding of the pathogenesis of PsA, indicated by the approval of the monoclonal antibody (mAb) ustekinumab, a fully human Ig G1k mAb against the common subunit p40 of IL-12 and IL-23, for the treatment of psoriasis and PsA by the European Medicines Agency and Food and Drug Administration.[2] In addition, IL-17 itself is also a direct therapeutic target for the treatment of PsA and psoriasis, with IL-17A mAb secukinumab now approved also for PsA and psoriasis.[3 4] We agree that the tofacitinib may function by inhibiting JAK2/STAT3, leading to an inhibition of the functional effects of IL-23 on memory T cells, with an ultimate consequence of reduced IL-17 production, as they suggest. Although there are no studies demonstrating the direct effect of tofacitinib on IL-23 secretion from dendritic cells (DCs), a recent study in psoriasis supports this data, having observed that expression of IL-12B, the IL-12/IL-23 p40 subunit and IL-17 were decreased following tofacitinib treatment, which was paralleled by improvements in clinical and histological features of psoriasis.[5]

However, it is important to note that in our study, we use an ex vivo synovial tissue model, which maintains synovial architecture and cell–cell contact. Our model uses undigested tissue, still structurally intact, which is obtained at keyhole arthroscopy and cultured immediately.[6] Consequently, immune/stromal cells within the PsA ex vivo biopsies are highly active and spontaneously release proinflammatory mediators such as cytokines, chemokines and growth factors, closely reflecting the inflamed PsA synovium in vivo. Raychaudhuri *et al* investigate the role of tofacitinib on a specific subset of lymphocytes in peripheral cells of patients with PsA. However, the pathogenesis of PsA involves a complex interaction of multiple innate and adaptive cell types, and it is likely that the exact mechanistic function of tofacitinib is not restricted to a single cell type within the synovium, and that the anti-inflammatory effects of tofacitinib on PsA synovial tissue that we have previously described are due to a complex interplay between the numerous cell types within the joint.

In our study, we demonstrate the inhibitory effect of tofacitinib on the proinflammatory mechanisms using PsA explants and PsAFLS, including migration, invasion, matrigel network formation, matrix metalloproteinase (MMP)/cytokine secretion and key signalling pathways such as nuclear factor kappa B (NFkB). While the precise mechanisms by which tofacitinib inhibits these key destructive processes in PsA is still unknown, it is likely that the IL-23/IL-17 cytokine axis may in part mediate some of these effects. Mechanisms may also involve inhibition of key cytokines including IL-6 resulting in negative feedback inhibition or through the observed inhibition of NFkB, which is known to mediate proliferative and invasive mechanisms in other cell types such as cancer cells.[7]

Raychaudhuri *et al* have previously demonstrated the existence of functional IL-17 receptors in synovial fibroblasts of patients with PsA and have further exhibited the proinflammatory effects of IL-17 in the joint pathology of PsA via induction of IL-6, IL-8 and MMP-3 on exposure to IL-17 in cultured Fibroblast-like synoviocytes (FLS) from patients with PsA.[8] These studies are in line with previous studies by our group which have also established that IL-17 itself can promote synovial inflammation and can drive matrix and cartilage degradation by inducing the production of MMPs.[9] These studies combined with the present data provided by the authors suggest evidence that tofacitinib may have an effect on IL-17-producing cells within the PsA synovial tissue, which can then negatively feedback on PsAFLS to inhibit MMP production and subsequent cartilage degradation in the joint.

Therefore, while we agree that tofacitinib may function to inhibit the IL-23/IL-17 cytokine axis in PsA, it is likely that tofacitinib also operates in a manner that is independent of both IL-23 and IL-17, depending on the cell type and inflammatory milieu. Further studies in a multicellular ex vivo system are needed to fully delineate the mechanistic role of tofacitinib in PsA. It would be interesting for the authors to investigate the direct effect of tofacitinib on IL-23 secretion from DC derived from the PsA inflammatory environment, either from synovial fluid or in synovial tissue. Furthermore, co-culturing PsA DC with T cells in the presence of tofacitinib and measuring specific T-cell subset cytokine secretion would provide strong evidence of JAK/STAT involvement in the IL-23/IL-17 cytokine axis in PsA.

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Commentary on the recent international multicentre study (EUVAS) on antineutrophil cytoplasmic antibodies

Antineutrophil cytoplasmic antibodies (ANCAs)-associated vasculitis (AAV) is a group of potentially life-threatening conditions that require early and accurate diagnosis, most often based on a combination of clinical and serological features.[1] Most of the therapeutic modalities have immune-suppressing activity and can result in serious consequences, especially if applied to patients without AAV, such as those with infectious diseases, which may have clinical features that mimic AAV. Because an early and accurate diagnosis of AAV is a clinical imperative, both the differential diagnosis and the working diagnosis leading to urgent initial treatment are critical steps in the clinical care pathway. Even then, refinement of the diagnosis might be required during the clinical course after the initial choice of therapeutic strategies.

In this context and with interest we read the recent article by Damoiseaux *et al*[2] summarising the results from a large international multicentre study on ANCA. The findings seemed to indicate that (1) most anti-proteinase 3 (PR3) and anti-myeloperoxidase (MPO) solid phase immunoassays outperform the ANCA indirect immunofluorescence (IIF) test and (2) there is significant variability and lack of interlaboratory commutability between two IIF methods for the detection of ANCA. These findings led to the conclusion that only solid phase assays are needed for the screening of ANCA, especially because IIF ANCA results were not commutable between laboratories. Based on this observation, it is very encouraging that novel solid phase immunoassays for the detection of anti-PR3 and anti-MPO antibodies have evolved over the past years and reached a very high degree of performance. However, we suggest that before wide adoption of a new testing algorithm, it would be of high relevance to perform studies with more than two IIF ANCA tests and especially include assays that are most commonly used in diagnostic laboratories. This information can be easily obtained from proficiency testing reports such as through College of American Pathologists (CAP), United Kingdom National External Quality Assessment Service (UK NEQAS) or similar organisations in other jurisdictions. This is of special importance since one of the assays used was a 'home-made' assay. The data generated during the European Vasculitis Study (EUVAS) are of high value and we encourage the authors to further expand the study by analysing the data from different perspectives to provide more insights. Such additional analyses might include the assessment of likelihood ratios as a function of autoantibody levels and the potential value of combining results from different tests,[3] especially high-performing IIF ANCA assays with high-performing solid phase tests. Such combined results might offer increased likelihood and ORs for AAV as demonstrated recently for antinuclear antibody (ANA)-associated rheumatic diseases[3] and for ANCA testing.[4] In addition, multivariate analyses are needed to assess different combinations of test results which might prove extremely useful in patients with low pretest probability of disease. The authors also discuss the potential need for gating strategies for ANCA testing,[5] mostly to reduce testing requests in the setting of low pretest probability. Although we agree with this challenge, we want to point out that this can be a double-edged sword since in an emergency or intensive care setting, the immediate detection and diagnosis of AAV is an imperative and can be life-saving. In those cases, IIF and solid phase assays might provide an increased likelihood ratio for AAV. On the same note, patients who are double negative for ANCA by IIF and solid phase assay would represent a very low likelihood of suffering from AAV.

Finally, as pointed out by Damoiseaux *et al*, ANCA are not only used for the diagnosis of AAV but also used for other conditions, such as inflammatory bowel disease,[6–8] autoimmune hepatitis,[9] and primary sclerosing cholangitis.[7] Accordingly, it is important that laboratories clearly differentiate between test requisitions for AAV versus these other conditions.

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Antineutrophil cytoplasmic antibodies: reporting and diagnostic strategies

It was with great interest that we read the correspondence of Mahler and Fritzler[1] on our recent European Vasculitis Study Group (EUVAS) study describing the performance of immunoassays for antineutrophil cytoplasmic antibodies (ANCA) in patients with ANCA-associated vasculitides (AAV).[2] In their letter, Mahler and Fritzler raise some interesting points, mainly related to (i) test result interpretation and (ii) diagnostic strategies. Besides, they pointed out that in the EUVAS study only two indirect immunofluorescence (IIF) assays were included, a commercial assay from Inova Diagnostics and a 'home-made' assay. They suggested to perform studies with more than two IIF ANCA tests and especially to include assays that are most commonly used in diagnostic laboratories. In a concomitant publication, we presented data on two additional commercial IIF assays, one from Euroimmun and one from Medipan.[3] Accordingly, we postulate that we included the most commonly used IIF assays. For example, IIF ANCA assays from Inova and Euroimmun are used by, respectively, 50% and 23% of the participants of the UK National External Quality Assessment Service (NEQAS) ANCA scheme (report September 2016). Our studies consistently showed that test characteristics of IIF were highly variable between assays. We envisaged that this variability was dependent on the substrates used and methods applied for ANCA IIF testing, that is, the use of only ethanol-fixed neutrophils versus the combination of ethanol-fixed and formalin-fixed neutrophils and HEp2 cells.[3] Moreover, we also found that the overall performance of high-quality immunoassays was at least as good as the performance of IIF methods, even when applied on modern automated systems. These observations lead us to conclude that the current international guidelines on ANCA testing[4] should be revised. A large Russian vasculitis centre has already abandoned IIF for ANCA testing several years ago[5] and in Japan, immunoassays are used for the diagnosis of AAV without IIF in most cases (Y Arimura, personal communication).

As we foresee that proteinase-3 (PR3)-ANCA and myeloperoxidase (MPO)-ANCA will be increasingly used to screen for ANCA, it is important to fully understand the clinical value inherent in the test results generated by such assays. Usually, PR3-ANCA and MPO-ANCA are interpreted as positive or negative. However, a lot of information is lost when such dichotomous interpretation is used. In a local study, we previously showed that the likelihood for disease increases with increasing ANCA levels and that the use of likelihood ratios can improve the clinical usefulness.[6] Using the large EUVAS dataset, we have performed in-depth studies on test result interval-specific likelihood ratios for each of the assays included in the study. As we determined the test result intervals based on predefined specificities, we maximally harmonised test result interpretation between assays. These results have been submitted for publication (Bossuyt *et al*, submitted for publication). We highly appreciate the genuine interest of Mahler and Fritzler in this approach and hope that the information—when available—will be widely adopted by manufacturers and users of the assays.

Mahler and Fritzler also encouraged us to further expand the study by analysing the potential value of combining results from different tests, in particular IIF and immunoassays. Also this suggestion is relevant as it has previously been shown that combing tests may indeed increase the clinical utility.[7] Here again, we have studied the value of combining different tests in detail using the EUVAS dataset and the results have been submitted for publication (Bossuyt *et al* submitted). Combining different tests can indeed increase the clinical utility, but the extent of the increase depends on the quality of the assay and the combination of assays. For example (based on data presented in ref. 2), in a simplified analysis using the single cut-off point proposed by the manufacturer, the area under the curve (AUC) of the Inova QuantaFlash PR3-ANCA and MPO-ANCA assay for AAV was 0.925 (95% CI 0.909 to 0.940). The AUC of combining QuantaFlash with the best performing IIF ANCA assay included in the EUVAS study (an assay from Inova which combines ethanol and formalin fixation with antinuclear antibody (ANA) detection on HEp2 cells) was not significantly different from the AUC of performing only QuantaFlash (p=0.088). In contrast, the AUC of combining QuantaFlash with an immunoassay for MPO-ANCA and PR3-ANCA from Euroimmun on all samples was significantly different from the AUC of QuantaFlash alone (p=0.01). The likelihood ratios for the different strategies (combinations) are given in table 1. This simplified approach, which does not take into account antibody levels, indicates the potential value of combining different tests. Moreover, these results suggest that combining two different immunoassays might be preferred to combining immunoassay with IIF. This can be even better appreciated by visual analysis of the results, as presented in figure 1. In this figure, the individual test results for patients with AAV and controls are shown for the combination of QuantaFlash with IIF and for the combination of QuantaFlash with immunoassays from Euroimmun. It can be seen that controls that are single positive by either of two immunoassays generally have low antibody levels. By contrast, controls that are single positive by IIF might have high IIF antibody levels. This, together with the fact that there are three times more controls that are single positive by IIF than by the two immunoassays, argues for combining two high-quality immunoassays rather than for combining immunoassay with IIF. Our data also show that combining different tests is mainly useful in case of low antibody levels by immunoassay (associated with a low likelihood ratio for disease) and much less useful for high antibody levels, as such results are associated with a high likelihood ratio for disease. This again illustrates the need for improved interpretation of test results that takes into account antibody levels.

Mahler and Fritzler[1] suggested to combine immunoassays with IIF by referring to ANAs testing, in which combining IIF with immunoassays adds value.[8] In ANA testing, IIF can pick up antibodies to relevant antigens that are not picked up by immunoassays. Such antibodies can be of high titre and are found in patients with systemic lupus erythematosus or systemic sclerosis.[9] However, in AAV, PR3 and MPO are the main autoantigens and there is no need for IIF to detect antibodies to autoantigens other than MPO and PR3. Moreover, in patients with AAV, there is high concordance of antibody detection between immunoassays and between immunoassays and IIF. Seronegative patients are usually negative by immunoassays and by IIF. Pertinent to this, it should be pointed out that ANCA testing is only an adjunct for the diagnosis of AAV: clinicopathological features determine the diagnosis.

The EUVAS study focused on AAV and did not address ANCA testing for gastrointestinal diseases. As previously suggested by us[10 11] and by Mahler and Fritzler,[1] laboratories should differentiate between test requisitions for AAV versus other inflammatory conditions such as inflammatory bowel disease or autoimmune hepatitis. However, the clinical relevance of ANCA testing in non-AAV conditions is limited, as illustrated by the fact that ANCA test results are not incorporated in the respective diagnostic criteria.[12–14]

Table 1 Likelihood ratios (with 95% CIs) for the cut-off point proposed by the manufacturer and for a combination of tests are given

	AAV (n)	Control (n)	Likelihood ratio	95% CI
QuantaFlash (–)	29	893	0.12	0.08 to 0.17
QuantaFlash (+)	222	31	26	18 to 37
Euroimmun (–)	27	894	0.11	0.08 to 0.16
Euroimmun (+)	224	30	27	19 to 39
QuantaFlash (–) IIF (–)	23	854	0.10	0.07 to 0.15
QuantaFlash (–) IIF (+)	6	39	0.57	0.24 to 1.32
QuantaFlash (+) IIF (–)	5	13	1.42	0.51 to 3.93
QuantaFlash (+) IIF (+)	217	18	44	28 to 70
QuantaFlash (–) Euroimmun (–)	23	880	0.09	0.06 to 0.14
QuantaFlash (–) Euroimmun (+)	6	13	1.70	0.65 to 4.42
QuantaFlash (+) Euroimmun (–)	4	14	1.05	0.35 to 3.17
QuantaFlash (+) Euroimmun (+)	218	17	47	29 to 75
Total	251	924		

The number of patients and controls with a particular test result or combination of test result are given as well. The highest level of reactivity from the PR3-ANCA and MPO-ANCA determinations was selected for analysis. Data are from ref. 2.
The AUC of the Inova QuantaFlash PR3-ANCA and MPO-ANCA assay for AAV was 0.925 (95% CI 0.909 to 0.940). The AUC of combining QuantaFlash with an IIF ANCA assay was 0.94 (95% CI 0.925 to 0.953), which was not significantly different from the AUC of performing only QuantaFlash (p=0.088) (method of Hanley and McNeil, MedCalc). The AUC of combining QuantaFlash with an immunoassay for MPO- and PR3-ANCA from Euroimmun on all samples was 0.943 (95% CI 0.928 to 0.955), which was significantly different from the AUC of QuantaFlash alone (p=0.01) (method of Hanley and McNeil, MedCalc).
AAV, ANCA-associated vasculitides; ANCA, antineutrophil cytoplasmic antibodies; MPO, myeloperoxidase.

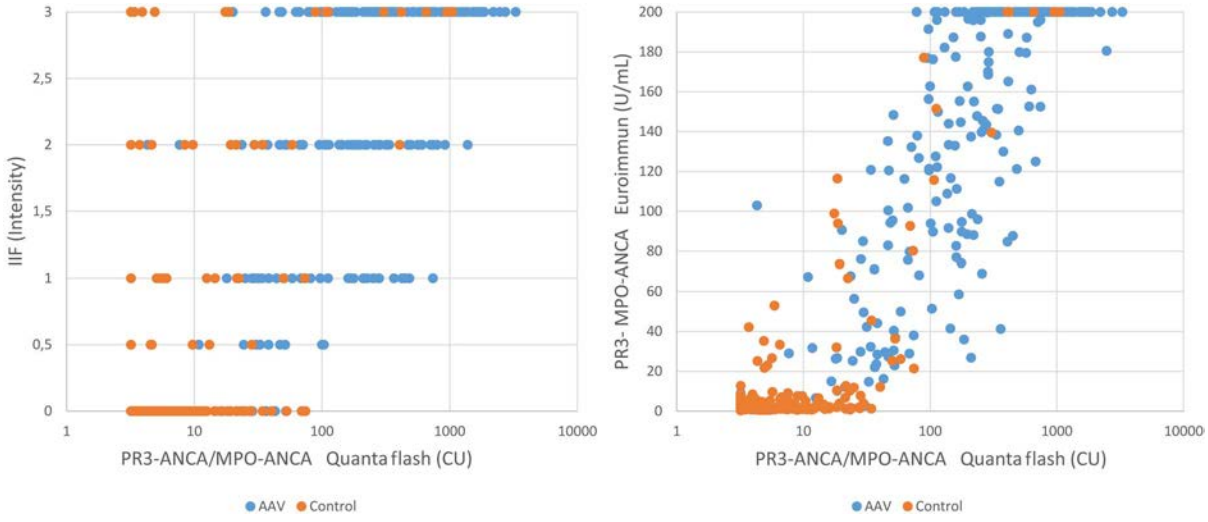

Figure 1 Test results for antineutrophil cytoplasmic antibodies (ANCA) by QuantaFlash (Inova) and by ELISA (Euroimmun). The highest level of reactivity from the PR3-ANCA and myeloperoxidase (MPO)-ANCA determinations was selected for analysis. Cut-off point proposed by the manufacturer is 20 U/mL of CU for both assays. Data are from ref. 2.

Taken together, the data of the EUVAS study[2] and additional data on test interpretation and testing strategies discussed above are a basis for a new international consensus on ANCA testing, which is currently in preparation. A strategy primarily based on antigen-specific assays seems to be supported by clinical practice in some laboratories,[2 5] but we consider it mandatory that such strategy is validated in a prospective study, potentially including a wider array of ANCA tests.

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Automated squeeze test (Gaenslen's manoeuvre) to identify patients with arthralgia suspicious for progression to RA: improving time delay to rheumatology consultation

We read with interest the article by van Steenbergen *et al*[1] and the response by Mankia *et al*.[2] In the former, a definition of suspicious arthralgia was proposed and in the latter a new approach to identifying individuals at risk of progression to rheumatoid arthritis (RA) was discussed. We agree with Mankia *et al* that general practitioners (GPs) are the first contact for those patients at risk. In other countries a delay in referring patients with RA has been seen, and we have similarly detected a delay of 28.2 (SD 46.9) months.[3] Thus, we are interested in clinical signs that allow GPs to identify patients with arthralgia suspicious for progression to RA—particularly, use of the squeeze test (ST) (also known as Gaenslen's compression test).[4] Previously, we found that the ST was useful in identifying RA progression in patients with undifferentiated arthritis in a year of follow-up.[5]

Because of the importance of physical examination and also medical education, we devised a study protocol. In the first phase, we noted the variations in the ST results by certified rheumatologists, and found important differences.[6] This supports the observations of van Steenbergen *et al*[7] about the importance of clinical expertise and its reliability for diagnosing patients with arthritis. In the second phase, having found differences in performance of the test, we constructed an automated device to evaluate the ST performance. We carried out a study in patients with established RA and in healthy individuals. The median squeeze force necessary to evoke pain in the RA group was 3.07 (IQR 2.4) kg and 2.78 (IQR 3.8) kg in the right and left hand, respectively; and in the healthy group these values were 4.2 (IQR 9.5) kg and 4.6 (9.7) kg.[8]

Mankia *et al*, in their study,[2] sought to reduce the impact of clinical inexperience by using the anti-cyclic citrullinated peptide test for the detection of individuals at risk of RA. However, the cost–benefit of this test is controversial and several causes contribute to the dearth of such specialised studies—for example, inadequate clinical expertise of the first contact physician, inadequate number of rheumatologists in a given population, long waiting times for evaluation or even economic factors. The automated test could reduce this gap. The force of the ST which is applied to distinguish between a healthy individual, and a patient with active disease is already established. The strength of the squeeze needed to screen the patient with arthralgia which it is suspected will progress to RA is in the process of determination. The objective of all investigations is to develop an easy to use, cheap tool that can identify RA in patients at an early stage.

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The squeeze test of MCP joints: a scarcity of scientific data, especially from primary care

We thank Vega-Moralis *et al*[1] for their interest in the EULAR definition of arthralgia suspicious for progression to rheumatoid arthritis (RA).[2] The definition is composed of seven parameters and the authors are particularly interested in the parameter 'presence of a positive squeeze test of metacarpal joints'. They suggest that the squeeze test can be used in primary care to promote referral to secondary care, and they have developed an automated test.[1]

First of all, we want to underline that the EULAR definition of arthralgia suspicious for progression to RA is developed for use in secondary care.[2] If the definition is also valuable for use in primary care needs to be determined. In addition, although the squeeze test is commonly used in daily practice, it is striking that there is not much scientific data on this test. A recent study performed in secondary care showed that the squeeze test of the MCP joints had a specificity >80%, but a low sensitivity.[3] To our knowledge, the performance of this test has never been studied in primary care. Although intuitively the squeeze test is believed valuable in decisions on referral, presently this is not supported by scientific data. There is also no evidence than an automated squeeze test performs better than manual squeezing of MCP joints. Although clinical evaluation is the basis of medicine, from many simple tests the test characteristics are insufficiently known. The squeeze test is an example that requires further studies, especially when the test is used in primary care.

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Imminent rheumatoid arthritis can be identified in primary care

We thank Vega-Morales *et al*[1] for their interest in our proposed approach for identifying individuals at risk of rheumatoid arthritis (RA) in a primary care setting.[2 3] The authors agree that primary care is usually the first point of contact for patients with RA when they initially develop symptoms. For this reason, they agree that general practitioners (GPs) are well placed to be involved in screening strategies to identify individuals at risk of progressing to RA.[1]

Vega-Morales *et al* advocate the squeeze test (Gaenslen's compression test) as a screening tool to aid GPs in identifying at-risk individuals for onward referral to a rheumatologist. The rationale for the squeeze test is that compression of the metacarpophalangeal (MCP) joints evokes pain in an individual with active synovitis. We agree that the squeeze test is useful for identifying early arthritis,[4] but its sensitivity is limited and it may need to be combined with other screening tests in order to be sufficiently discriminatory.[5] Nonetheless, it is cheap, quick and easy to perform and therefore an appropriate test for the GP who suspects a patient may have developed RA. Automation of the squeeze test, as proposed by Vega-Morales *et al*,[1] may indeed have a role in this setting. Whether the squeeze test is sensitive enough to identify subclinical synovitis detected by ultrasound will be an important question to address.

Despite its use in early arthritis, we would argue that the squeeze test is inappropriate for identifying at-risk individuals, including those with imminent, but not yet established, synovitis. This important group, who have risk factors for RA (including systemic autoimmunity) but crucially do not have arthritis, must be identified *before synovitis develops* in order to benefit from potential preventative intervention. In line with this, the EULAR taskforce definition for arthralgia suspicious for progression to RA is aimed at identifying homogeneous patient groups that are at risk of developing arthritis before they progress to RA.[6]

Testing for anti-cyclic citrullinated peptide (CCP) antibodies in individuals who present to their GP with a new musculoskeletal complaint can effectively identify individuals at high risk of developing RA, before the onset of synovitis, and without the need for specialist assessment.[3] In those that have a positive anti-CCP test, further risk assessment using clinical, serological and imaging tests can then quantify the risk of progression to arthritis more accurately.[7] Thus, at-risk individuals may be followed in longitudinal studies and further stratified for risk appropriate intervention. The cost-effectiveness of this approach will need to be determined, but must be considered in the correct context; it may be a key advance towards preventing a disease that is associated with considerable morbidity, treatment and societal costs.[8]

Vega-Morales *et al*'s response highlights the changing paradigm of early RA. The concept of early disease is changing from early arthritis to pre-arthritis while the target changes from early remission to prevention. As such, novel screening approaches are required. These must effectively identify at-risk individuals prior to the onset of synovitis for bone fide prevention to be feasible.

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